non-participant 'controls' were chosen from differently pooled database, which could include baseline imbalances between groups and hindsight bias (Davis *et al*, 1985; Braunholtz *et al*, 2001; Peppercorn *et al*, 2004). In this study, we compared the characteristics and outcomes of those who met the eligibility criteria but declined to participate in randomised trials, and instead chose to receive standard therapy. We thus aimed at excluding confounding factors as much as possible.

On the other hand, physician triage is pointed out to be one of the barriers to cancer clinical trial accrual (Lara *et al*, 2001; Corrie *et al*, 2003; Go *et al*, 2006; Ho *et al*, 2006). We excluded the barrier by making it a rule to offer clinical trials to every patient with advanced NSCLC who satisfied the eligibility criteria.

The response rate, MST, 1-year and 2-year survival rates were all similar in both groups. We have to admit that response evaluation might not be as strict in off-protocol therapy. However, the hazard ratio for the OS was very close to 1. Although the confidence interval of 0.73 to 1.28 could not rule out the existence of clinically important difference in the treatment effect, it could not by any means be taken as a clinically relevant prognostic factor. We thus believe this confidence interval of the adjusted hazard ratio, 0.73–1.28, was narrow enough to justify the conclusion that the clinical outcomes of trial participants and non-participants were not different in our study. The differences in the number of cycles of chemotherapy given to participants and non-participants may suggest the so-called protocol effect (Braunholtz *et al*, 2001; Peppercorn *et al*, 2004), in which explicit careful description of treatment regimens could lead to improvement of outcomes. On the other hand, there clearly existed no 'care effect' representing the differences in incidental aspects of treatment or care between participants and non-participants, which the protocol may require, such as extra follow-up or extra nursing care (Braunholtz *et al*, 2001; Peppercorn *et al*, 2004). In our cases, the same treatment teams took charge of and followed both groups of patients in the same manner, and found no differences in the post-treatment characteristics or follow-up periods. Thus, our first finding was that the clinical trials themselves seemed to have no influence on the outcomes or pattern of care of the patients.

The second finding was that we could not find any demographic characteristics to influence the patients' willingness to participate in clinical trials. Taken together with the first finding, both the characteristics and outcomes of the non-participants were very similar to the participants. This would imply that the participants ably represented the whole patient population of the disease status who met the eligibility criteria, and that conclusions from the clinical trials could be generalised.

Our study, however, could only show the similarity in the prognosis of the participants and non-participants, and, unlike an earlier report (Link *et al*, 1986), not that of the treatment effect itself. This could not be evaluated because there were no significant differences in the clinical effect between the arms in both Trial 1 and Trial 2. If newer, much more effective experimental treatment were presented in the trials, the outcome could be better in trial participants, which was the case in the adjuvant chemotherapy trial for osteosarcoma (Link *et al*, 1986). In that report, eligible patients who declined randomisation, but were given adjuvant chemotherapy, also had better outcomes. Therefore, a very effective treatment could lead to a better outcome both on and off trial. Ideally, strict comparison of the effects of the study participation itself would require randomised design of the trial participation (Braunholtz *et al*, 2001; Peppercorn *et al*, 2004), which is almost impossible to conduct.

Thirdly, the declining rate seemed to be influenced by the trial design. Trial 1 was the comparison of four similar platinum-doublet regimens. On the other hand, Trial 2 was the comparison of two arms with sequentially different types of chemotherapy. In general, people might have the impression that injection therapy would be more effective, and less convenient, than oral administration. It is easy to understand that more patients felt difficulty in accepting the randomisation of different types of therapy, such as Trial 2 (Schmoor *et al*, 1996; Jenkins and Fallowfield, 2000).

The declining rate also seemed to be greatly affected by the attending physician. The attending physician with longer experience as a thoracic oncologist tended to have lower rate of declination. Even though we do not have records on who actually informed the participants regarding the trial, residents or trainees under Physician A seemed to have had more chance to lead the consultation, which might have affected the rate of declination. Trust in the doctor is one of the most important reasons for agreeing to enter an RCT, whereas it has also been cited as the main reason for declining to participate (Jenkins and Fallowfield, 2000; Ellis *et al*, 2001; Stryker *et al*, 2006). Patients prefer the doctor to make the treatment decisions rather than to be randomised. A recent report emphasises the influence of physicians' clinical communication on patients' decision-making on participation in clinical trials (Albrecht *et al*, 2008). Improving communication and more interventions by clinical research coordinators and other medical staff members in all eligible patients may improve the accrual rate (Fallowfield *et al*, 1998; Wright *et al*, 2004; Stryker *et al*, 2006).

Finally, it was interesting to find that 8% of those who declined the RCTs participated in early-phase trials during follow-up. It is possible that the lack of effective therapies had changed their recognition of clinical trials. However, it might support the psychological states of patients as reported in earlier studies (Jenkins and Fallowfield, 2000; Ellis *et al*, 2001; Wright *et al*, 2004); patients expect experimental therapies to give them improved effectiveness but with fear of uncertainty. They are reported to have negative opinions regarding the principle of randomisation. Better understanding of the patients' decision-making process and the factors influencing their psychological states may lead to improvement in RCT accrual.

Our study has several limitations. One is that it was conducted at a single academic institution; the situation might well have been different in others or when the research was performed on a multi-institution basis. The second is that we analysed data from only two trials and could not definitely conclude that a trial design would affect the patient accrual. Third, we have no data on the reasons for patient participation. That information would be definitely useful for analysing factors for consent or declining to participate, and would help to improve the accrual rate. Further research is required.

In conclusion, there was no evidence of any difference in the response rates and survival times between participants and non-participants. The declining rate of clinical trials was influenced by the referring physicians and trial designs. Further analysis of the decision-making process of those offered trials is warranted, for it may improve patient accrual to RCTs.

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Multimodality Therapy for Patients With Invasive Thymoma Disseminated Into the Pleural Cavity: The Potential Role of Extrapleural Pneumonectomy

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Background. The optimal treatment method for thymoma with pleural dissemination remains controversial. We analyzed our experience with a multimodality approach and evaluated the role of extrapleural pneumonectomy (EPP) in the treatment of disseminated thymoma.

Methods. Multimodality therapy was used to treat 11 consecutive patients with invasive thymoma disseminated into the pleural cavity. Disease was stage IVa in 9 and stage IVb disease with lymph node metastasis in 2. Our treatment strategy for those patients was induction chemotherapy with cisplatin, doxorubicin, and methylprednisolone (CAMP therapy), followed by thymectomy combined with resection of the visible disseminated nodules and postoperative radiotherapy. EPP was applied for 4 patients who had chemoresistant tumors or pleural refractory recurrence.

Results. Eight patients underwent induction chemotherapy. The response rate to CAMP was 85%. Thymectomy with or without the resection of disseminated pleural tumors was performed in 7 patients and EPP in 3. Postoperative radiotherapy was administered in 6. All patients except 1 with EPP had recurrence: pleural recurrence in 7, lung in 1, and multiple organs in 2. Nine patients were retreated with chemotherapy, radiotherapy, pulmonary metastasectomy, or pleurectomy. One underwent EPP for pleural recurrence. Consequently, among the 7 patients without EPP, only 1 was alive without disease and 4 were alive with pleural recurrence. In contrast, 3 of the 4 patients with EPP had no local failure and were alive without recurrence.

Conclusions. In multimodality therapy for thymoma with pleural dissemination, EPP offers good local control and may lead to cure.

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Thymoma is an epithelial tumor originating from the thymus that grows relatively slowly and generally responds to surgical resection, chemotherapy, and radiotherapy [1]. For patients with stage I or II thymoma, primary resection is recommended. For patients with stage III thymoma, multimodality treatment, including chemotherapy, radiotherapy, and resection, is often applied for cure [2–4]. On the other hand, for patients with stage IVb thymoma who have distant or lymph node metastasis, or both, the main goal of treatment is not cure but disease control using chemotherapy [5, 6]. Between these stages, treatment for patients with stage IVa thymoma, defined as a tumor with pleural or pericardial dissemination, remains controversial because complete resection is generally considered difficult. In recent years, however, some surgeons have attempted complete surgical resection in multimodality therapy [2, 4, 7].

Two types of operation have been reported for pleural dissemination of invasive thymoma: resection of visible disseminated nodules as far as possible and extrapleural pneumonectomy (EPP), aiming at the resection of visible and invisible disseminated tumor cells. The former technique has been more frequently selected, but the latter has been rarely implemented. To elucidate the role of EPP for invasive thymoma with dissemination into the pleural cavity in multimodality therapy, we retrospectively analyzed our experiences.

Patients and Methods

Between February 1988 and April 2006, 49 consecutive patients with thymoma were treated at Tochigi Cancer Center. Tumor stages were 15 at stage I, 11 at stage II, 7 at stage III, 11 at stage IVa, and 5 at stage IVb. Pleural disseminations were present in 9 with stage IVa disease and 3 with stage IVb disease. The remaining 2 patients with stage IVa disease had pericardial dissemination. Of the 3 patients with stage IVb disease and pleural dissemination, 2 had lymph node metastasis in the anterior mediastinum or ipsilateral axilla, and 1 had contralateral multiple pulmonary metastases. We analyzed the 11 patients with pleural dissemination, excluding the pa-

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Table 1. Patient Characteristics, Treatments, and Outcomes

Pt	Age	Sex	Stage	Disease Status	Histology Type	Induction Chemo	Response	Operation
1	40	M	IVa	Primary	B2	No	...	T (ICR)
2	63	M	IVa	Primary	B2	CAMP ×4	PR	T (ICR)
3	33	M	IVa	Primary	B2	CAMP ×2; CHOP ×2	PR	T (ICR)
4	51	M	IVa	Primary	B3 + C	No	...	T + piecemeal (CR)
5	63	F	IVb	Primary	B2	PACE ×2	NC	EPP (CR)
6	49	M	IVa	Primary	B3	CAMP ×4	PR	None
7	29	M	IVa	Primary	B2	No	...	T + piecemeal (ICR)
8	62	F	IVa	Primary	B3	CAMP ×4	PR	T + piecemeal (ICR)
9	24	M	IVa	Recurrent	B2	CAMP ×2	PR	EPP (CR)
10	52	M	IVa	Primary	B2	CAMP ×4	PR	T + piecemeal (CR)
11	47	F	IVa	Primary	B3	CG ×3; CAMP ×2	NC	EPP (CR)

C = carcinoma; CAMP = cisplatin, doxorubicin, and methylprednisolone; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; CG = carboplatin and gemcitabine; CR = complete resection; EPP = extrapleural pneumonectomy; ICR = incomplete resection; NC = no change; PACE = cisplatin, doxorubicin, cyclophosphamide, and etoposide; PR = partial remission; T = thymectomy.

tient with pulmonary metastases. The Tochigi Cancer Center Institutional review board approved this retrospective analysis and waived the requirement of patient consent for the study. Staging was based on the Masaoka staging system [8]. According to the World Health Organization (WHO) classification [9], 7 tumors were categorized as type B2, 3 type B3, and 1 type B3+ thymic squamous cell carcinoma, respectively. In the last case, because the histology of the pleural disseminations was not thymic carcinoma but type B3 thymoma, we included this case in the study. The patients' characteristics are summarized in Table 1.

Therapeutic Strategy

Our basic therapeutic strategy for patients with thymoma and pleural disseminated was as follows:

1. Patients were treated with induction chemotherapy. The chemotherapy regimen consisted of cisplatin, doxorubicin, and methylprednisolone (CAMP) [5]. Surgical resection was attempted after four cycles of chemotherapy.
2. The usual surgical procedure was thymectomy with resection of the visible disseminated nodules in the pleural cavity. In some patients with unforeseen pleural disseminations found during the operation, adjuvant chemotherapy was administered after thymectomy.
3. Radiation therapy was applied if the tumor was incompletely resected or likely to remain. The radiation field in most patients included the area where the tumor was deemed likely to remain. We did not perform hemithoracic radiotherapy postoperatively.
4. Exceptionally, EPP was performed in selected patients whose cardiopulmonary function was sufficient to undergo pneumonectomy and in whom concomitant resection of the mediastinal mass in

Table 2. Treatments After Surgical Resection, Recurrences, and Outcomes

	Radiotherapy					Survival After Initial Treatment	
Pt.	Radiation Field	Dose (Gy)	Adjuvant Chemo	Recurrence	Treatment for Recurrence	Mon	Status
1	No		CAMP ×4	Pleura	EPP (CR)	249	NED
2	Med	50.7	No	Pleura	CDDP + VBL + BLM	13	TRD
3	Med + whole pleura	29.5–49.2	No	Pleura	CHOP	18	TRD
4	No		CDDP + VDS ×3	Pleura	CAMP + P + RT	202	AWD
5	Med + left upper TC + axilla	60	No	Lung	CAMP + L	161	NED
6	Med	60	No	Pleura	CAMP	154	AWD
7	No		CAMP ×4	Pleura	CAMP + P + RT	112	AWD
8	Lower TC (diaphragm)	50	No	Lung, pleura	CAMP[a]	93	AWD
9	No		CAMP ×2	Pleura, distant[b]	CAMP[a] + RT	80	DOD
10	Med + lower TC (diaphragm)	50.4	No	Pleura	RT	40	NED
11	No		No	None	No	35	NED

[a] Multiple regimen including CAMP. [b] Includes abdominal lymph nodes, bone, and liver metastases.

AWD = alive with disease; BLM = bleomycin; CAMP = cisplatin, doxorubicin, and methylprednisolone; CDDP = cisplatin; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; CR = complete resection; DOD = died of disease; EPP = extrapleural pneumonectomy; L = lung wedge resection; Med = mediastinum; NED = no evidence of disease; P = pleurectomy; RT = radiotherapy; TC = thoracic cavity; TRD = treatment-related death; VBL = vinblastine; VDS = vindesine.

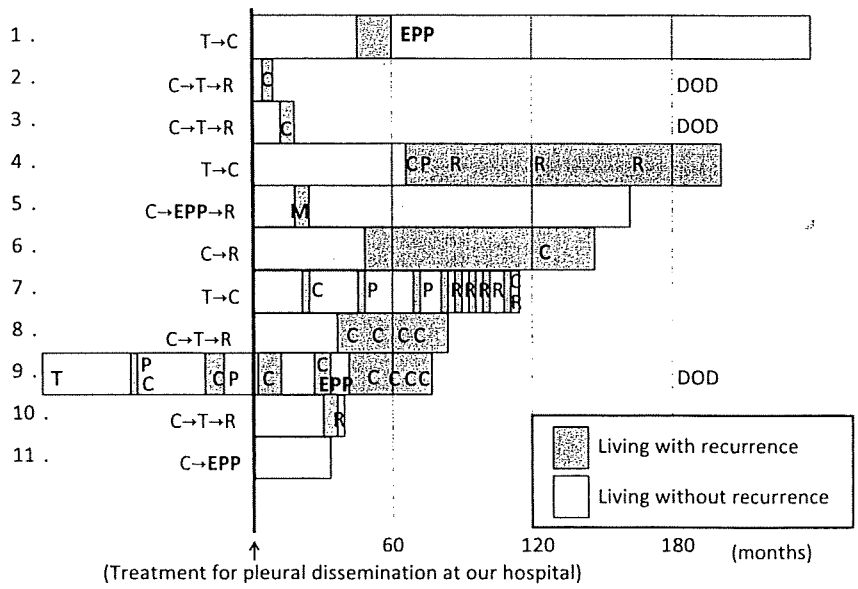

Fig 1. Clinical courses of all patients with pleural dissemination. (C = chemotherapy; DOD = died of disease; EPP = extrapleural pneumonectomy; M = pulmonary metastasectomy; P = pleurectomy; R = radiotherapy; T = thymectomy.)

conjunction with EPP was determined to be able to be safely completed. Additional disease conditions included recurrent pleural dissemination after standard surgery (patients 1 and 9) or the failure of induction chemotherapy (patients 5 and 11).

The patients were followed up every 1 to 3 months for 2 years after completion of the multimodality therapy and every 6 months thereafter. All patients were followed up until December 2008, and the median follow-up period for surviving patients was 112 months. We retrospectively reviewed the medical records of 11 patients with thymoma disseminating into the pleural cavity to clarify the outcome of our multimodality therapy, especially focusing on the role of EPP.

The patients were evaluated with computed tomography (CT) for response after induction chemotherapy and completion of the multimodality treatment. Complete remission (CR) was defined as the complete disappearance of all objective evidence of disease on CT for at least 4 weeks. Partial remission (PR) was defined as a decrease of at least 50% in the sum of the product of the perpendicular diameter of measurable lesions for at least 4 weeks. Disease progression was defined as an increase of at least 25% in tumor size or new lesions. All other circumstances were classified as no change (NC).

Statistical Analysis

Survival was measured from the first day of treatment at our hospital for thymoma accompanied by pleural dissemination until death from any cause or the last date of follow-up. Local recurrence-free survival was measured from the date of resection until local recurrence or death from any cause or the last date of follow-up. Survival and local recurrence-free survival curves were calculated using the Kaplan-Meier method, and differences in local recurrence-free survival were determined by the log-rank test. Statistical analysis was conducted using StatView 5.0 software (SAS Institute Inc, Cary, NC).

Results

The clinical courses of the 11 patients, none of whom had myasthenia gravis, are reported in Tables 1 and 2 and Figure 1. All but 1 patient had pleural dissemination at the first presentation. Patient 9 had pleural recurrence 4 years after thymectomy and came to our hospital after two resections for pleural dissemination.

Induction chemotherapy was performed in 8 patients: CAMP therapy was applied in 7 patients, and a regimen including cisplatin, doxorubicin, cyclophosphamide, and etoposide (PACE) was administered in patient 5. CHOP, consisting of cyclophosphamide, doxorubicin, vincristine, and prednisone, was used in patient 3 after acute renal failure caused by tumor lysis syndrome during CAMP therapy [10]. Patient 11 received carboplatin and gemcitabine before CAMP therapy. Among the patients with induction chemotherapy, 6 had a partial response and 2 showed no change, and the response rate was 75%. The response rate to CAMP therapy was 85%.

Surgical resection was performed in 10 patients in the initial multimodality therapy: 7 underwent thymectomy with or without resection of the visible pleural tumors, 2 underwent EPP as the initial operation, and 1 had EPP for recurrent pleural dissemination after two resections of pleural dissemination at a previous hospital. Adjuvant chemotherapy was administered to 5 patients.

Six patients received postsurgical radiotherapy. The radiation field was the mediastinal tumor bed in patients 2 and 6 because pleural dissemination had disappeared after induction chemotherapy. In addition to the mediastinum, the whole left pleural surface in patient 3, more than half of the left pleural surface and left axilla in patient 5, and the lower third of the right hemithorax in

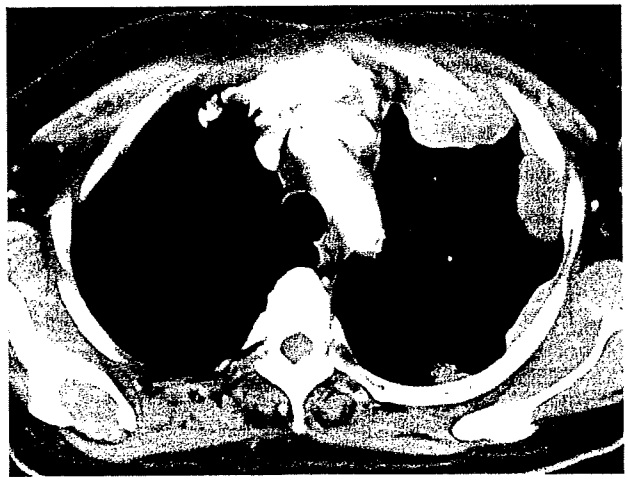

Fig 2. Chest computed tomography scan shows multiple pleural nodules in the left thorax in patient 5. This patient also had an ipsilateral axillary lymph node metastasis.

patient 10 were irradiated. Only the lower third of the left hemithorax was irradiated in patient 8.

Patient 6 was not treated surgically because CT after induction therapy documented the tumor had invaded the main pulmonary artery. The patient was treated with radiotherapy.

Recurrence developed in 10 patients, consisting of pleural recurrence in 7, pulmonary metastasis in 1, and metastases to multiple organs in 2. Treatment for recurrence was mostly chemotherapy and radiotherapy. Surgical resections were EPP in 1 patient, pleurectomy in 2, and pulmonary metastasectomy in 1. Among the 10 patients who had recurrence after initial treatment, patients 2 and 3 died during chemotherapy for recurrence. Patient 2 received chemotherapy consisting of cisplatin, vinblastine, and bleomycin for recurrent pericardial tumors, and died 13 months after the initiation of treatment due to bleomycin-induced pneumonitis. Patient 3 received CHOP therapy for pleural recurrence. Fulminant rhabdomyolysis occurred on the day 7 of the second course of chemotherapy, and the patient died of acute renal failure 18 months after the diagnosis of primary tumor [10].

Four EPP cases are detailed:

- Patient 1: A 40-year-old man underwent thymectomy with combined resection of the pericardium for invasive thymoma with pleural dissemination, which persisted because of numerous miliary nodules. The patient received four cycles of CAMP adjuvant chemotherapy. Pleural recurrence developed 5 years after the operation, and left EPP was performed. The patient's postoperative course was uneventful, and he was alive without recurrence at 188 months after EPP.
- Patient 5: A 63-year-old woman who had invasive thymoma with pleural dissemination and axillary lymph node metastasis received PACE therapy at a previous hospital. Because the chemotherapy had no effect, the patient was referred to our hospital (Fig 2) and underwent left EPP and excision of axillary lymph node metastasis. Her postoperative course was uneventful. Pulmonary metastasis developed 1 year after EPP. She received four courses of CAMP therapy and then underwent wedge resection of the right upper lobe of the lung. The patient was alive without recurrence at 157 months after EPP.
- Patient 9: A 24-year-old man with recurrent disseminated thymoma in the pleural cavity was referred to Tochigi Cancer Center. The patient had undergone thymectomy at age 15 years and recurrent pleural nodules were twice removed at ages 19 and 22 at another hospital. He received four courses of CAMP therapy, resulting in partial response, and underwent left EPP at 25 years old with postoperative chemotherapy; however, pleural recurrence and abdominal lymph node, bone, and liver metastases developed 2 years after EPP. The patient died of disease at age 31.
- Patient 11: A 47-year-old woman was diagnosed with a left lung cancer with pleural dissemination at another hospital. Carboplatin and gemcitabine therapy was performed, without response. The needle biopsy specimen was reexamined and the diagnosis was changed to invasive thymoma. Thereafter, the patient received CAMP therapy, but the tumor did not respond and she underwent left EPP. The patient's postoperative course was uneventful, and she was alive without recurrence 31 months after the operation.

The surgical approach for EPP was posterolateral thoracotomy in patients 1, 9, and 11 and median sternotomy combined with continuous anterior thoracotomy in patient 5.

The 8 patients without EPP in the initial multimodality therapy had pleural recurrence, and after retreatment with chemotherapy and radiotherapy, 1 patient was alive without disease, 4 were alive with pleural recurrence, and

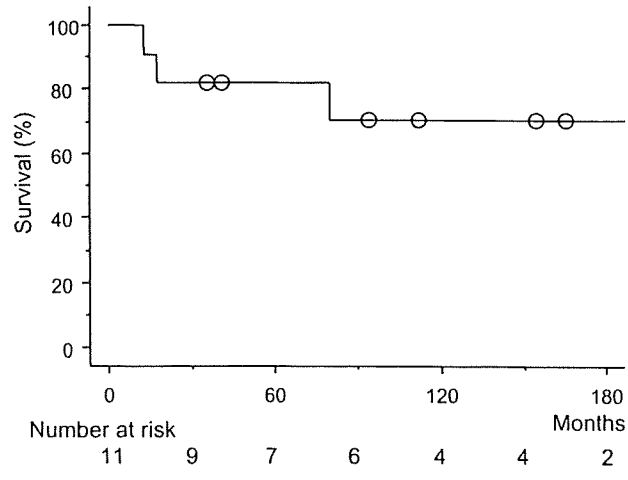

Fig 3. Survival curve of the 11 patients with disseminated thymoma and pleural dissemination. Overall survival was 81% at 5 years and 70% at 10 years.

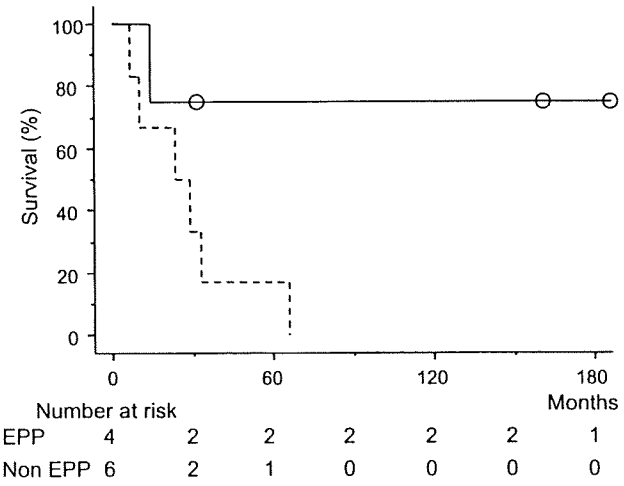

Fig 4. Local recurrence-free survival curves of 10 patients who underwent surgical resection according to the operative procedures. The 5- and 10-year local recurrence-free survival rates were both 75% for the EPP group, and 16% and 0%, respectively, for the non-EPP group ($p = 0.06$).

2 died during chemotherapy for recurrence. In contrast, 3 of the 4 patients with EPP had no local failure and were alive without recurrence at 31, 157, and 188 months after their operations (Fig 1 and 4).

Figure 3 shows the overall survival curve of the 11 patients with thymoma with pleural dissemination. Overall survival rates were 81% at 5 years and 70% at 10 years. Figure 4 shows the local recurrence-free survival curves of 10 patients who underwent operations according to the procedure. Local recurrence-free survival was 75% at both 5 and 10 years for the EPP group, and 16% and 0%, respectively, for the non-EPP group ($p = 0.06$).

Comment

Although surgical resection is considered the mainstay of therapy for thymoma, the standard treatment for stage IVa thymoma with pleural dissemination has not been established. This is partly because the proportion of patients with this stage of thymoma is small, and complete resection is usually difficult to achieve. Masaoka and colleagues [8] created a classification in 1981 that is now widely used. They analyzed 96 patients with thymoma, but only 8 patients were classified with having stage IVa disease. The 5- and 10-year survival rates for this stage were reported to be 50% and 0%, respectively.

Although Wilkins and colleagues [11] recommended the excision of all pleural disseminated tumors, it is usually impossible to remove numerous pleural tumors, unless EPP is performed; therefore, radiation or chemotherapy, or both, are the treatments of choice for stage IVa thymoma. Ichinose and colleagues [12] treated 8 patients with thymoma and pleural dissemination mainly by radiotherapy. Only 2 of these patients underwent operations. The 5-year survival rate was 87.5%, and the authors suggested that radiotherapy should play a primary role in the treatment of this disease condition.

In 1993 Rea and colleagues [13] reported the results of induction chemotherapy for advanced thymoma patients, including stage IVa disease, to improve resectability. They used therapy consisting of cisplatin, doxorubicin, vincristine, and cyclophosphamide; the response rate was 100% and the complete remission rate was 43%.

To improve local control for these patients, multimodality therapy, which usually includes induction chemotherapy, surgical resection, adjuvant chemotherapy, and radiotherapy, has been developed [2, 5, 14]. Although different chemotherapy regimens were used and different proportions of stage IVa patients were included, survival rates were higher than previously documented outcomes. These results warrant the use of multimodality therapy for stage IVa thymoma.

The surgical procedure in multimodality therapy remains to be determined. Two surgical techniques are used for invasive thymoma that has disseminated into the pleural cavity: resection of visible disseminated nod-

Table 3. Published Cases of EPP for Thymoma With Pleural Dissemination in English Literature

Author		Disease			
Year	(Reference no.)	Age	Sex	Status[a]	Survival from EPP
1991	Nakahashi [17]	18	F	Primary	5 y, 10 m, NED
1994	Higashiyama [16]	56	F	Recurrence	3 y, 6 m, NED
1997	Shih [18]	32	F	Primary	2 y, 6 m, NED
2006	Wright [19]	25	M	Recurrence	16 y, 6 m, DOD
		47	F	Recurrence	15 y, 2 m, NED
		34	F	Primary	7 y, 2 m, DOD
		34	F	Primary	4 y, 6 m, DOD
		53	M	Primary	2 y, 5 m, NED
2008	Ishikawa (current study)	40	M	Recurrence	15 y, 8 m, NED
		63	F	Primary	13 y, 1 m, NED
		25	M	Recurrence	6 y, 8 m, DOD
		47	F	Primary	2 y, 7 m, NED

[a] Disease status at the presentation of pleural dissemination.

DOD = died of disease; EPP = extrapleural pneumonectomy; m = month; NED = no evidence of disease; y = year.

ules as far as possible, and EPP, aimed at resecting visible and invisible disseminated tumor cells. The former operation is used frequently, but EPP is rarely implemented. EPP for stage IVa thymoma was suggested as a curative operation by Bergh and colleagues [15] in 1978. Since then, more than 10 EPP procedures for thymoma have been documented as case reports (Table 3) [12, 16–18]. Although the follow-up periods were relatively short and publication bias may have affected the results, all patients were reported to be alive without recurrence at publication.

Two retrospective studies dealing with surgical treatment, including EPP for stage IVa thymoma, have recently been published. Wright [19] reported 5 patients, including a long-term survivor without recurrence after an operation for pleural recurrence, and advocated that EPP can be performed safely and improve survival in selected patients. Huang and colleagues [7] reported 18 patients with stage IVa thymoma treated with multimodality therapy. Among them, 4 patients underwent EPP to achieve complete resection. High-dose hemithoracic radiotherapy was also initiated after EPP. They reported 3 of the 4 patients were alive without recurrence.

In our hospital, thymectomy with resection of pleural nodules in multimodality therapy was the treatment strategy for disseminated thymoma; however, after we experienced a long-term disease-free survivor (patient 1) who underwent EPP for recurrent pleural dissemination, we applied EPP for selected patients who had recurrent pleural dissemination after multiple resections and in whom the tumor had failed to respond to induction chemotherapy. As a result, 3 of the 4 patients treated with EPP were alive without recurrence, and 2 survived for more than 10 years after EPP. Although multimodality therapy using CAMP therapy was able to prolong survival even after recurrence, EPP was associated with long-term disease-free survival. We considered that EPP was able to provide complete resection of invisible disseminated tumor cells and improve local control compared with other surgical procedures.

Although we did not encounter any operative deaths, EPP is thought to be a more invasive operation compared with lung-preserving operations, and patient selection is essential. A sufficient cardiopulmonary function is essential when applying EPP. In primary disseminated cases, complicated resection of the mediastinal mass, such as the combined resection of great vessels, in addition to EPP seems to be intolerable. In addition to these, two good indications for EPP are considered to be thymoma with extensive and confluent pleural dissemination that can be completely resected only by EPP, as in the Huang series, and chemoresistant disseminated thymoma, which is considered a less controllable disease if it recurs postoperatively. For these candidates, we consider that EPP may become a treatment of choice at the first attempt to resect these tumors. Needless to say, patients with myasthenia gravis are not good candidates for EPP. As experience of EPP for thymoma with pleural dissemination is limited, a prospective multicenter study is needed to elucidate the role of EPP in the treatment of thymoma.

In conclusion, our retrospective study revealed that multimodality therapy, including chemotherapy, surgical resection, and radiotherapy, prolonged survival of patients with thymoma and pleural dissemination. Furthermore, EPP as part of multimodality therapy for selected patients showed a possibility of improving local control, leading to cure. A prospective multicenter study is warranted to establish a treatment strategy that includes EPP for stage IVa thymoma.

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ORIGINAL ARTICLE

Schedule-dependent synergism and antagonism between pemetrexed and docetaxel in human lung cancer cell lines in vitro

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Abstract
Background Pemetrexed and docetaxel show clinical activities against a variety of solid tumors including lung cancers. To identify the optimal schedule for combination, cytotoxic interactions between pemetrexed and docetaxel were studied at various schedules using three human lung cancer cell lines A-549, Lu-99, and SBC-5 in vitro.
Methods Cells were incubated with pemetrexed and docetaxel simultaneously for 24 or 120 h. Cells were also incubated with pemetrexed for 24 h, followed by a 24 h exposure to docetaxel, and vice versa. Growth inhibition was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell cycle analysis. Cytotoxic interactions were evaluated by the isobologram method.
Results Simultaneous exposure to pemetrexed and docetaxel for 24 and 120 h produced antagonistic effects in all three cell lines. Pemetrexed (24 h) followed by docetaxel (24 h) produced additive effects in A-549 cells and synergistic effects in Lu-99 and SBC-5 cells. Docetaxel followed by pemetrexed produced additive effects in A-549 and Lu-99 cells and antagonistic effects in SBC-5 cells. The results of cell cycle analysis were fully consistent with those of isobologram analysis, and provide the molecular basis of the sequence-dependent difference in cytotoxic interactions between the two agents.
Conclusions Sequential administration of pemetrexed followed by docetaxel may provide the greatest anti-tumor effects for this combination in the treatment of lung cancer.

Keywords Pemetrexed · Docetaxel · Isobologram · Lung cancer

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Introduction

Lung cancer is the leading cause of cancer mortality in industrialized countries, with non-small cell lung cancer (NSCLC) accounting for nearly 80% [1]. Although surgery may be curative in early-stage NSCLC, most patients present with inoperable advanced disease. These patients managed with best supportive care alone have a median survival time of only 5 months and a 1-year survival rate of approximately 10% [2]. First-line treatment for patients with advanced NSCLC includes platinum compounds combined with vinorelbine, gemcitabine, or taxanes [3]. This is associated with improved quality of life, but only moderate survival advantages when compared with best supportive

care alone. Therefore, there is an emergent need for effective second-line treatments for NSCLC patients who experience disease progression after first-line chemotherapy. Currently, erlotinib, docetaxel, and pemetrexed are approved as second-line drugs by the US Food and Drug Administration for patients whose tumors have progressed after platinum-based treatments [4, 5].

Small cell lung cancer (SCLC) accounts for approximately 12% of all lung cancers [6]. Compared with NCSLC, SCLC has a rapid doubling time, and earlier development of wide spread metastasis. SCLC is highly sensitive to initial radiotherapy and chemotherapy. The most commonly used regimens include etoposide, cisplatin, doxorubicin, or cyclophosphamide [7]. For limited-stage patients, chemotherapy associated with thoracic radiation was able to produce a cure rate of 10–20%. In extensive disease, the combinations of these agents yields responses of 50–70%, with 20–30% complete remissions, but most patients die from recurrent diseases. The identification of new agents is critical for further progress in the treatment of SCLC, and the evaluation of a variety of agents including docetaxel and pemetrexed has been underway [8–10].

Pemetrexed is a new antifolate that has significant activity against a broad spectrum of solid tumors including lung cancer [11, 12]. Pemetrexed inhibits multiple enzymes involved in folate metabolism including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase [13]. Pemetrexed arrests cells mainly in S phase and induces apoptosis against tumor cells [14]. Against lung cancers, pemetrexed is non-inferior to docetaxel, with lower hematologic toxicity, and febrile neutropenia and a similar rate of non-hematologic toxicities [12].

The taxanes, paclitaxel and docetaxel, have significant activity in lung cancer. Both inhibit microtubule dynamics and cause G2/M cell cycle arrest. However, there are several differences between them in the pharmacokinetics and pharmacologic actions [15, 16]. Docetaxel demonstrated greater affinity for the tubulin-binding site, wider cell cycle activity, longer intracellular retention time and higher intracellular concentration in tumor cells, more potent antitumor activity in in vitro and in vivo models, and more potent induction of bcl-2 phosphorylation and apoptosis. Paclitaxel has a non-linear pharmacokinetic behavior, while docetaxel demonstrated linear pharmacokinetics and less schedule dependence than paclitaxel.

The combination of pemetrexed and docetaxel may play a major role in the second-line treatment of lung cancers. The wide range of antitumor activity of these agents, their different cytotoxic mechanisms and different toxicity profiles, and the absence of cross-resistance provide the rationale for combining these agents. Since both pemetrexed and docetaxel are cell cycle-specific, disturbances of the cell cycle produced by one drug may influence the cytotoxic effects of the other. Furthermore the drug schedule may play a significant role in the outcome, and therefore, how the drugs are combined requires careful consideration.

We showed that the ordered treatment of pemetrexed followed by paclitaxel may be synergistic, whereas simultaneous administration was potentially antagonistic in a variety of solid tumor cell lines [17]. What is not clear is whether such schedule dependency will be as important for pemetrexed and docetaxel as for pemetrexed and paclitaxel in the treatment of lung cancers. The present study was aimed at characterizing the cytotoxic effects of various pemetrexed and docetaxel combinations and schedules on three human lung cancer cell lines using the isobologram method of Steel and Peckham [18]. Flow cytometry was performed to understand the molecular basis of the schedule-dependent synergism and antagonism of the pemetrexed and docetaxel combination.

Materials and methods

Cell lines

Three human lung cancer lines, A-549 (lung adenocarcinoma), Lu-99 (giant-cell lung cancer), and SBC-5 (small cell lung cancer) were used. A-549 cells were purchased from the American Type Culture Collection (Rockville, MD). Lu-99 and SBC-5 cells were obtained from Health Science Research Resources Bank (Tokyo). These cells were growing as a monolayer in 75-cm^2 plastic tissue culture flasks containing RPMI1640 medium (Sigma Chemical Co., St Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma) and antibiotics (penicillin G and streptomycin) in a humidified atmosphere of 95% air/5% CO_2 at 37°C. Under these conditions, the doubling times of these cells were 20–30 h.

Drugs

Pemetrexed and docetaxel were kindly provided by Eli Lilly and Company (Indianapolis, IN) and Sanofi-Aventis K.K. (Tokyo, Japan), respectively. Drugs were dissolved with RPMI1640 and stored at −80°C. Drugs were diluted with RPMI-1640 plus 10% FBS before use.

Cell growth inhibition using combined anti-cancer agents

Growing cells were collected by trypsinization, separated and resuspended to a final concentration of 5.0×10^3 cells/ml in fresh medium containing 10% FBS and antibiotics. Cell suspensions (100 μl) were dispensed into the individual wells of a 96-well tissue culture plate with a lid (Costar, Corning, NY). Each plate had one 8-well control column

containing medium alone and one 8-well control column containing cells but no drug. Eight plates were prepared for each drug combination.

Simultaneous and continuous exposure to pemetrexed and docetaxel

After a 20–24 h incubation for cell attachment, solutions of docetaxel and pemetrexed (50 μl) at different concentrations were added to individual wells in final volumes of 200 μl per wells. The plates were incubated under the same conditions for 120 h.

Simultaneous 24 h exposure to pemetrexed and docetaxel

After cell attachment, solutions of docetaxel and pemetrexed (50 μl) at different concentrations were added to individual wells in final volumes of 200 μl per wells. The plates were also incubated under the same conditions for 24 h. The cells were then washed twice with culture medium, and then fresh medium (200 μl) and antibiotics were added. The cells were cultured again for four additional days in drug-free medium.

Sequential exposure to pemetrexed (24 h) followed by docetaxel (24 h) or vice versa

After cell attachment, medium containing 10% FBS (50 μl) and solutions of docetaxel or pemetrexed (50 μl) at different concentrations were added to individual wells. The plates were then incubated under the same conditions for 24 h. The cells were washed twice and fresh medium was added, followed by the addition of solutions of docetaxel or pemetrexed (50 μl) at different concentrations. The plates were incubated again under the same conditions for 24 h. The cells were then washed twice, and the cells were cultured for three additional days in drug-free medium.

MTT assay

Viable cell growth was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [19]. For all 4 cell lines examined, we established a linear relation between the MTT assay value and the cell number within the range shown.

Isobologram

The dose–response interactions between pemetrexed and docetaxel were evaluated at the IC_{50} level by the isobologram method of Steel and Peckham (Fig. 1) [18]. The IC_{50} was defined as the concentration of drug that produced 50% cell growth inhibition; i.e. a 50% reduction of absorbance. The theoretical basis of the isobologram method and the procedure for making the isobologram has been described in detail [18, 20, 21]. Based on the dose–response curves of pemetrexed and docetaxel, three isoeffect curves were constructed (Fig. 1). If the agents act additively by independent mechanisms, combined data points would lie near the Mode I line (hetero-addition). If the agents act additively by similar mechanisms, the combined data points would lie near the Mode II lines (iso-addition) [14, 16, 17].

Since we cannot know in advance whether the combined effects of two agents will be hetero-additive, iso-additive, or an effective intermediate between these extremes, all possibilities should be considered. Thus, when the data points of the drug combination fell within the area surrounded by mode I and/or mode II lines (i.e. within the envelope of additivity), the combination was described as additive.

We used this envelope to evaluate not only the simultaneous exposure combinations of pemetrexed and docetaxel, but also to evaluate the sequential exposure combinations, since the second agent under our experimental conditions could modulate the cytotoxicity of the first agent.

A combination that gives data points to the left of the envelope of additivity (i.e. the combined effect is caused by lower doses of the two agents than is predicted) can confidently be described as supra-additive (synergism). A combination that gives data points to the right of the

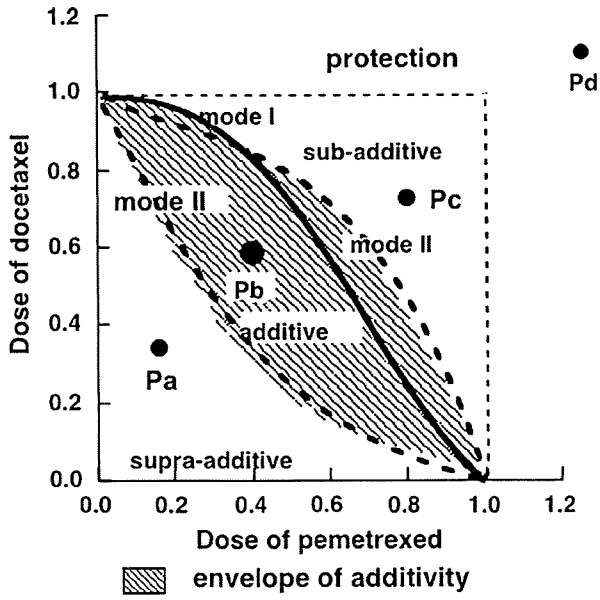

Fig. 1 Schematic representation of an isobologram (Steel and Peckham). The envelope of additivity, surrounded by mode I (*solid line*) and mode II (*dotted lines*) isobologram lines, was constructed from the dose–response curves of pemetrexed alone and docetaxel alone. The concentrations that produced 50% cell growth inhibition were expressed as 1.0 in the ordinate and the abscissa. Combined data points *Pa*, *Pb*, *Pc* and *Pd* show supra-additive, additive, sub-additive, and protective effects, respectively

envelope of additivity, but within the square or on the line of the square can be described as sub-additive (i.e. the combination is superior or equal to a single agent but is less than additive). A combination that gives data points outside the square can be described as protective (i.e. the combination is inferior in cytotoxic action to a single agent). A combination with both sub-additive and/or protective interactions can confidently be described as antagonistic.

Data analysis

Findings were analyzed as described previously [22]. To determine whether the condition of synergism (or antagonism) truly existed, a Wilcoxon signed-rank test was performed to compare the observed data with the predicted minimum (or maximum) data for an additive effect. Probability values (P) ≤ 0.05 were considered significant. Combinations with $P > 0.05$ were regarded as having an additive/synergistic (or additive/antagonistic) effect. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, CA).

Flow cytometric analysis

SBC-5 cells were treated with 5.0 μM pemetrexed alone, or 1.5 nM docetaxel alone or their combination simultaneously for 24 h. The cells were also treated with pemetrexed for 24 h followed by docetaxel for 24 h or the reverse sequence. The cells were harvested at 72 h and the cell cycle profiles were analyzed by staining intracellular DNA with propidium iodide in preparation for flow cytometry with the FACScan · CellFIT system (Becton-Dickinson, San Jose, CA). The size of the sub-G1, G0/G1 and S+G2/M fractions was calculated as a percentage by analyzing DNA histograms with the ModFitLT 2.0 program (Verity Software, Topsham, ME) [23].

Results

Figure 2 shows the dose–response curves for pemetrexed in A-549, Lu-99, and SBC-5 cells. The dose–response curves were plotted on a semi-log scale as a percentage of the control. The IC_{50} values of pemetrexed against these cells were 1.5 ± 0.4, 0.42 ± 0.10, 1.3 ± 0.2 μM, respectively ($n = 5$). The IC_{50} values of docetaxel against these cells were 1.7 ± 0.2, 1.0 ± 0.1, and 0.82 ± 0.13 nM, respectively ($n = 5$).

The dose–response curves in Fig. 3 show the effect of simultaneous exposure (24 h) (panel a), sequential exposure to pemetrexed followed by docetaxel (panel b), and vice versa (panel c) on the growth of SBC-5 cells. The

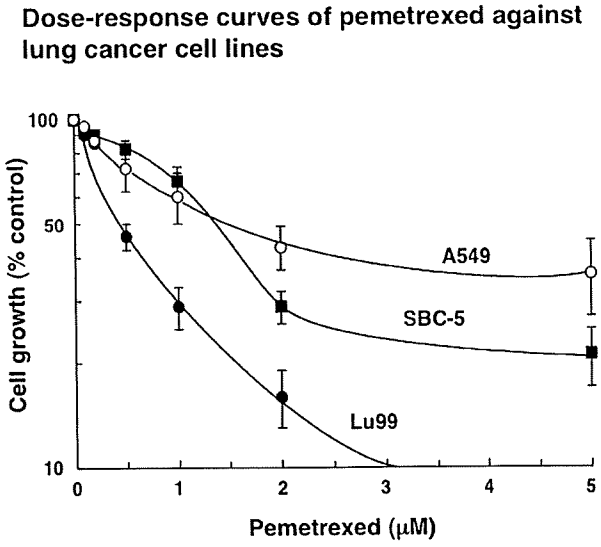

Fig. 2 The dose–response curves of 24 h exposure to pemetrexed against A-549, Lu-99, and SBC-5 cells. Cell growth inhibition was measured using the MTT assay after 5 days and was plotted as a percentage of the control (cells not exposed to drugs). Each point represents the mean ± SEM for at least three independent experiments

pemetrexed concentrations are shown on the abscissa. Dose–response curves in which the docetaxel concentrations are shown on the abscissa are based on the same data (figure not shown). Three isoeffect curves (mode I and mode II lines) were constructed based on the dose–response curves of pemetrexed alone and docetaxel alone. Isobolograms at the IC_{50} level were generated based on these dose–response curves for the combinations.

Simultaneous exposure to docetaxel and pemetrexed for 24 h

Figure 4a shows isobolograms of SBC-5 cells after simultaneous exposure to pemetrexed and docetaxel. The combined data points fell in the areas of subadditivity and protection. The mean values of the observed data (0.71) were larger than those of the predicted maximum values (0.60). The observed data and the predicted maximum data were compared by Wilcoxon signed-rank test. The difference was significant ($P < 0.05$), indicating antagonistic effects (Table 1). Quite similar effects were observed in A-549 and Lu-99 cells (Table 1, isobolograms not shown).

Sequential exposure to pemetrexed for 24 h followed by docetaxel for 24 h

Figure 4b shows isobolograms of SBC-5 cells exposed first to pemetrexed and then to docetaxel. The combined data points fell in the area of supraadditivity. The mean values of the observed data (0.46) were smaller than those

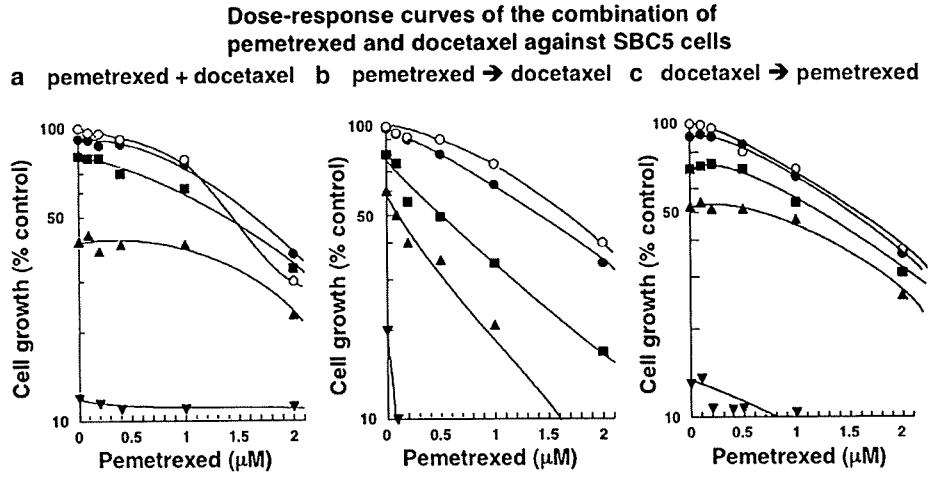

Fig. 3 Schedule dependence of the interaction between docetaxel and pemetrexed in SBC-5 cells. Cells were exposed to these two drugs simultaneously for 24 h (**a**), pemetrexed first for 24 h followed by docetaxel for 24 h (**b**), and vice versa (**c**). The cell number after 5 days was measured using the MTT assay and was plotted as a percentage of the control (cells not exposed to drugs). The concentrations of docetaxel are shown on the abscissa. The concentrations of pemetrexed were 0 (*open circle*), 0.2 (*filled circle*), 0.5 (*filled square*), 1.0 (*filled triangle*) and 2.0 (*filled inverted triangle*) μM, respectively. Data are mean values for three independent experiments; SE was < 25%

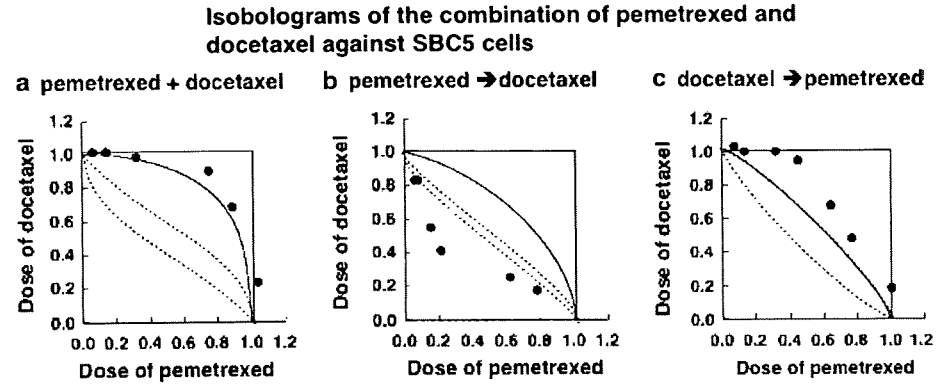

Fig. 4 Isobolograms of simultaneous exposure to docetaxel and pemetrexed for 24 h in SBC-5 cells (**a**). The combined data points fell in the areas of subadditivity and protection. Data are mean values for at least three independent experiments; SE was <25%. Isobolograms of sequential exposure to pemetrexed (24 h) followed by docetaxel (24 h) in SBC-5 cells (**b**). All data points of the combinations fell in the area of supraadditivity. Data are mean values for at least three independent experiments; SE was <20%. Isobolograms of sequential exposure to docetaxel (24 h) followed by pemetrexed (24 h) in SBC-5 cells (**c**). All data points of the combinations fell in the areas of subadditivity and protection. Data are mean values for at least three independent experiments; SE was <25%

of the predicted minimum values (0.60) (Table 1). The difference was significant ($P < 0.05$), indicating synergistic effects. Quite similar effects were observed in Lu-99 cells (Table 1, isobolograms not shown), while additive effects were observed in A-549 cells (Table 1, isobolograms not shown).

Sequential exposure to docetaxel for 24 h followed by pemetrexed for 24 h

Figure 4c shows isobolograms of SBC-5 cells exposed first to docetaxel, followed by pemetrexed. The combined data points mainly fell in the area of subadditivity. The mean values of the observed data were larger than those of the predicted maximum values ($P < 0.02$) (Table 1), indicating antagonistic effects. For A-549 and Lu-99 cells, most combined data points fell within the envelope of additivity and the mean values of the observed data were between those of the predicted minimum and maximum values (Table 1, isobolograms not shown), indicating an additive effect of this schedule.

Simultaneous exposure to pemetrexed and docetaxel for 5 days

For all three cell lines, combined data points fell in the areas of subadditivity and protection, indicating antagonistic effects (Table 1, isobolograms not shown).

Table 1 Mean values of observed data, predicted minimum, and predicted maximum of pemetrexed and docetaxel in combination at IC_{50} level

Schedule	Cell line	n[a]	Observed data	Predicted min.[b]	Predicted max.[c]	Effects
Pemetrexed + docetaxel (24 h)	A-549	8	0.72	0.31	0.55	Antagonism ($P < 0.02$)
	Lu-99	6	>1.0	0.41	0.62	Antagonism ($P < 0.05$)
	SBC-5	6	0.71	0.33	0.60	Antagonism ($P < 0.05$)
Pemetrexed (24 h) → docetaxel (24 h)	A-549	7	0.63	0.31	0.92	Additive
	Lu-99	7	0.29	0.50	0.67	Synergism ($P < 0.02$)
	SBC-5	7	0.46	0.60	0.82	Synergism ($P < 0.02$)
Docetaxel (24 h) → pemetrexed (24 h)	A-549	8	0.64	0.32	0.86	Additive
	Lu-99	8	0.63	0.32	0.85	Additive
	SBC-5	7	0.87	0.36	0.70	Antagonism ($P < 0.02$)
Pemetrexed + docetaxel (5 day)	A-549	6	0.79	0.51	0.68	Antagonism ($P < 0.05$)
	Lu-99	6	0.96	0.45	0.62	Antagonism ($P < 0.05$)
	SBC-5	4	0.73	0.20	0.57	Antagonism ($P < 0.05$)

[a] Number of data points

[b] Predicted minimum value for an additive effect

[c] Predicted maximum value for an additive effect

Cell cycle analysis

The isobologram analysis revealed that pemetrexed and docetaxel had a synergistic effect on two of the three lung cancer cell lines when sequentially administered with pemetrexed first and followed by docetaxel. In contrast, either simultaneous exposure or sequential addition in the reversed order (docetaxel to pemetrexed) resulted in antagonistic or additive effects. We confirmed these results by calculating the size of sub-G1 fractions, which correspond to apoptotic populations, on flow cytometry. As shown in Fig. 5, apoptosis-inducing effects of the two drugs were strongest when cells were exposed to pemetrexed first and followed by docetaxel. In contrast, the cytotoxic effects of docetaxel were significantly suppressed when pemetrexed was added simultaneously or afterward. These data are fully consistent with the results of isobologram analysis.

Cell cycle analysis also provided a clue to understand the mechanisms underlying this observation. Pemetrexed alone induced cell cycle arrest in late G1 to early S phase in SBC-5 cells (see Fig. 6 for representative results, and Table 2 for quantification and statistical analysis of three independent experiments). Docetaxel alone caused the loss of mitotic fractions along with massive apoptosis at a relatively low concentration (1.5 nM). When SBC-5 cells were exposed to both agents simultaneously, the cell cycle pattern was between the patterns of single-agent exposure, and the size of sub-G1 fractions was substantially

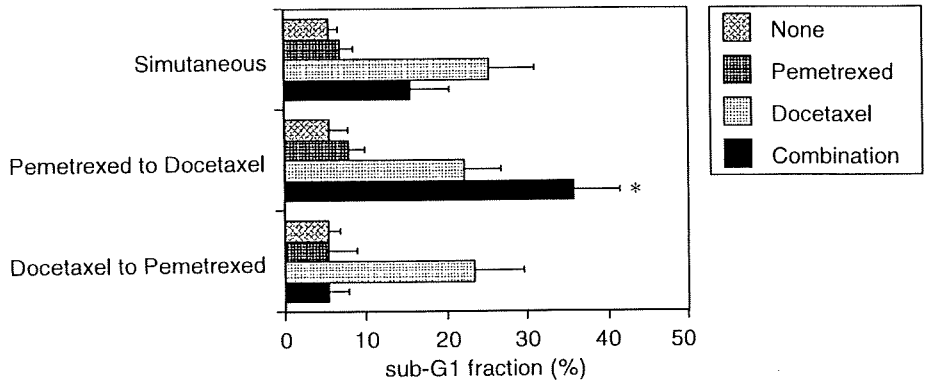

Fig. 5 SBC-5 cells were cultured in the absence (None) or presence of either 5.0 μM pemetrexed (Pemetrexed) or 1.5 nM docetaxel (Docetaxel) alone for 24 h; or in the presence of both drugs for 24 h (Simultaneous); or treated with pemetrexed for 24 h, followed by docetaxel for 24 h (Pemetrexed to Docetaxel); or treated with docetaxel for 24 h, followed by pemetrexed for 24 h (Docetaxel to Pemetrexed). After 72 h, DNA histograms were obtained to calculate the size of sub-G1 fractions as described in "Materials and methods". Data shown are the means ± SD of three independent experiments. The statistical difference was determined by one-way ANOVA with Bonferroni multiple comparison test. An *asterisk* denotes $P < 0.01$

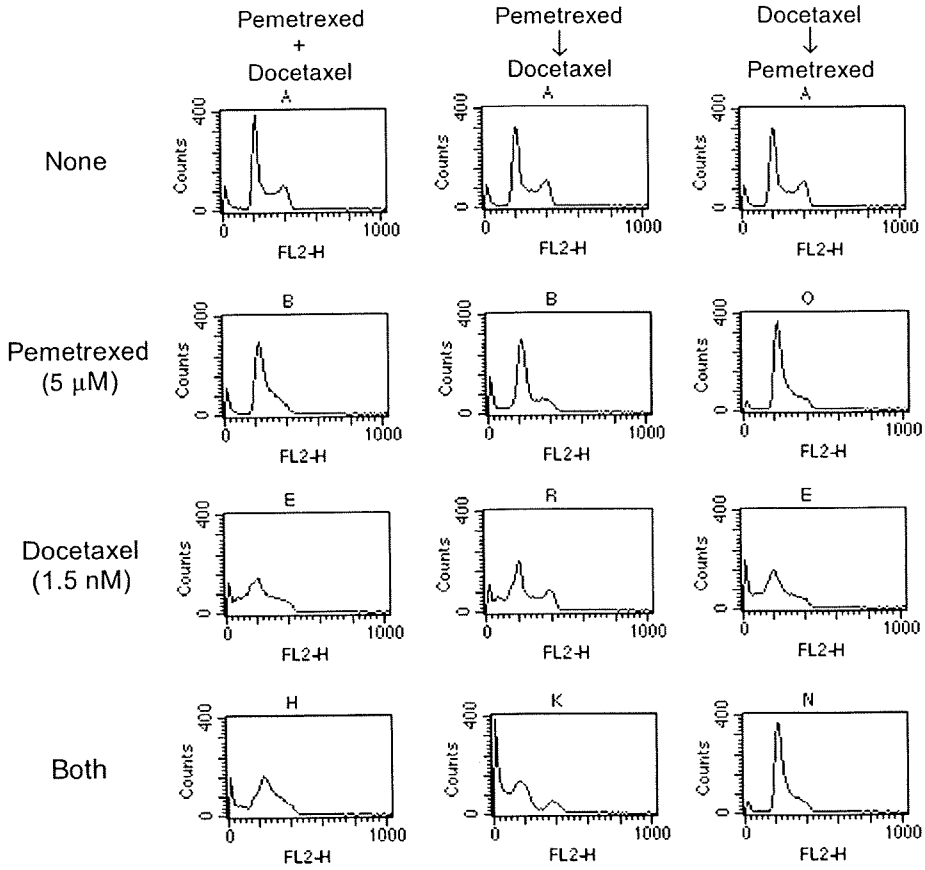

Fig. 6 Cell cycle analysis of SBC-5 cells treated with docetaxel and pemetrexed. *Left column* SBC-5 cells were treated with no drug, 5.0 μM pemetrexed, 1.5 nM docetaxel, or both drug simultaneously for 24 h. *Middle column* SBC-5 cells were treated with 5.0 μM pemetrexed for 24 h, followed by 1.5 nM docetaxel for 24 h. *Right column* SBC-5 cells were treated with 1.5 nM docetaxel for 24 h, followed by 5.0 μM pemetrexed for 24 h. Cells were harvested at 72 h and DNA histogram was obtained as described in "Materials and methods"

reduced. When SBC-5 cells were treated with docetaxel first and followed by pemetrexed, the cell cycle profile was almost identical to that of single exposure to pemetrexed, suggesting that the cell cycle effect of pemetrexed is dominant over that of docetaxel. As a result, the apoptosis-inducing effect of docetaxel was almost completely cancelled in the presence of pemetrexed. In contrast, when SBC-5 cells were treated with pemetrexed first and followed by docetaxel, the proportion of cells in sub-G1 phase was larger than that of cells treated with either pemetrexed or docetaxel alone. This was accompanied by a decrease in S-phase cells. Overall, the results of cell cycle analysis are fully consistent with those of isobologram analysis, and provide the molecular basis of the sequence-dependent differences in cytotoxic interactions between the two agents.

Discussion

In this study, we investigated the effects of pemetrexed in combination with docetaxel on lung cancer cell lines to determine the optimal schedule for this combination. Analysis of the drug–drug interaction effects was carried out using the isobologram method of Steel and Peckham [18], which provides a fundamental basis for assessing whether cytotoxicity induced by combinations of anticancer agents is greater, equal to, or smaller than would have been expected for the individual agents.

We demonstrated that a cytotoxic interaction between pemetrexed and docetaxel is schedule-dependent. Simultaneous exposure to pemetrexed and docetaxel for 24 h and 5 days showed antagonistic effects in all cell lines studied. Sequential exposure to pemetrexed for 24 h followed by docetaxel for 24 h showed synergistic effects in Lu-99 and SBC-5 cells, while it showed additive effects in A-549 cells. Sequential exposure to docetaxel followed by pemetrexed showed additive effects in A-549 and Lu-99 cells, but antagonistic effects in SBC-5 cells. We also used SW620 colon cancer cells for the study, and the combined effects for these schedules were quite the same as those of SBC-5 cells (data not shown).

These findings suggest that the sequential administration of pemetrexed followed by docetaxel may be more cytotoxic to cancer cells and optimal for this combination, while the simultaneous administration of pemetrexed and docetaxel may be less cytotoxic and suboptimal. It should be noted that the sequential administration of pemetrexed

Table 2 Effects of pemetrexed and docetaxel on cell cycle distribution of SBC-5 cells

Schedule	Pemetrexed + Docetaxel (%)	Pemetrexed ↓ Docetaxel (%)	Docetaxel ↓ Pemetrexed (%)
None			
Sub-G1	5.4	4.7	4.7
G1	48.4	51.3	51.3
S	24.9	22.3	22.3
G2/M	21.3	21.7	21.7
Pemetrexed (5 μM)			
Sub-G1	5.5	9.9	2.2
G1	62.8	61.6	68.2
S	28.4	18.1	20.0
G2/M	3.3	10.4	9.6
Docetaxel (1.5 nM)			
Sub-G1	25.2	17.6	21.3
G1	42.8	4.7	50.7
S	27.1	20.0	18.3
G2/M	4.9	17.7	9.7
Both			
Sub-G1	14.6	36.0	2.3
G1	52.1	40.1	66.4
S	22.7	12.2	26.0
G2/M	3.6	11.7	5.3

The proportion of cells in each phase of the cell cycle was calculated with the ModFitLT 2.0 program

followed by docetaxel might be more toxic for normal cells. Since, however, toxicity profiles of both agents are different, increasing overlapping toxicity would likely be mild.

Previously, we evaluated the cytotoxic effects of pemetrexed in combination with paclitaxel in vitro using A-549 cells, breast cancer MCF7, ovarian cancer PA1, and colon cancer WiDr cells in vitro [17]. The results were similar to those of the present study. Although slight differences are present, this would be due to the very strict definitions of synergism and antagonism in the isobologram method (Steel and Peckham). Our previous and present findings suggest that the simultaneous administration of pemetrexed and taxanes is less cytotoxic than the sequential administration of pemetrexed followed by taxanes, and latter schedule should be assessed in clinical trials for the treatment of lung cancer and other solid tumors.

In general, it is difficult to clarify the mechanisms underlying the cytotoxic effects of drug combinations. In this study, however, cell cycle analysis provided a clue to the molecular basis of schedule-dependent synergism and antagonism. The exposure of SBC-5 cells to pemetrexed led to synchronization of most cells that were in late G1 phase to the early S phase of the cell cycle, during which cells are relatively insensitive to docetaxel. This may explain the antagonistic effects of the simultaneous addition of the two agents. In the case of sequential exposure to docetaxel followed by pemetrexed, the cell cycle pattern was almost identical to that of cells treated with pemetrexed alone. This suggests that the cell cycle effect of docetaxel is transient and overcome by the addition of pemetrexed, which results in the abrogation of its cytotoxicity.

In contrast, the sequential exposure to pemetrexed followed by docetaxel produced a striking increase in apoptotic cells along with a decrease in cells in S phase. The effect of docetaxel on S phase cells no longer in pemetrexed-induced cell cycle arrest may cause the synergistic cytotoxicity. The decrease in S phase is compatible with this notion. However, the mechanisms underlying the cytotoxic effects of pemetrexed and docetaxel are still not well understood. The possibility that the drug interactions are due to some unknown mechanism related to complex perturbations of biochemical processes cannot be excluded.

In conclusion, our data show that the antitumor activity of pemetrexed and docetaxel is schedule-dependent. Sequential exposure to pemetrexed followed by docetaxel tended to produce synergistic effects, and would therefore be a suitable schedule, whereas simultaneous exposure to the two agents had antagonistic effects, and may be suboptimal. However, the question of how far these results can be applied in the treatment of patients remains unanswered. Further clinical studies are necessary to clarify whether the therapy sequence alters the antitumor effect and the toxicity of this combination. Our findings provide preclinical rationale for a novel, mechanism-based, therapeutic strategy to be tested in lung cancer patients.

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Conflict of interest statement None.

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ORIGINAL ARTICLE

A phase II trial of weekly chemotherapy with paclitaxel plus gemcitabine as a first-line treatment in advanced non-small-cell lung cancer

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Abstract
Purpose The efficacy and toxicity of combined paclitaxel (PTX) and gemcitabine (GEM) was evaluated as a protocol for first-line chemotherapy in 40 patients with advanced non-small-cell lung cancer (NSCLC).
Methods Paclitaxel, 100 mg/m^2, was administered intravenously (IV) as a 1-h infusion, followed by GEM, 1,000 mg/m^2, IV over 30 min on days 1 and 8 of a 21-day cycle. The median age of patients was 66 years with a range of 33–75 years. Nearly all patients (39/40) had an ECOG performance status of 0 or 1. Thirteen patients (32%) had initial stage IIIB disease and 27 patients (68%) had stage IV disease. Histological subtypes were adenocarcinoma (73%) and squamous cell carcinoma (25%).
Results Twenty-two patients (55%) achieved a partial response and none achieved a complete response, giving an overall response rate of 55% (95% confidence interval: 38.2–71.8%). Disease stability was achieved in 14 patients (35%), and 4 patients (10%) had progressive disease. The median survival time was 11.9 months (95% CI: 10.3–14 months), with a 1-year survival rate of 47.5%. Grade 3 or 4 hematological toxicities observed included neutropenia in 37.5%, anemia in 2.5%, and thrombocytopenia in 5.0% of these patients. Non-hematologic toxicities were mild, with the exception of grade 3 and 4 pneumonitis. There were no deaths due to toxicity.
Conclusion Weekly chemotherapy with PTX plus GEM is effective and is acceptable for the first line treatment of advanced NSCLC.

Keywords Non-small-cell lung cancer · First-line chemotherapy · Weekly chemotherapy · Gemcitabine · Paclitaxel

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Introduction

Lung cancer ranks among the most commonly occurring malignancies and currently is the leading cause of cancer-related deaths worldwide [21]. In Japan lung cancer is responsible for approximately 55,000 cancer-related deaths per year [5]. Even though the clinical usefulness of first-line chemotherapy has been established for the cases of advanced non-small-cell lung cancer (NSCLC), the prognosis is still extremely poor.

A number of new agents have become available recently for the treatment of unresectable and metastatic NSCLC in Japan, including the taxanes, gemcitabine (GEM), and vinorelbine. In randomized phase III trials, these agents in combination with a platinum compound have been associated with improved survival of patients having advanced NSCLC [8, 17, 23, 24]. However, a platinum compound is associated with a greater toxicity than other drugs used to treat NSCLC. In addition to nausea and vomiting, it causes neuropathy, profound fatigue, and renal toxicity. Some

patients are unable to tolerate the drug toxicity and terminate treatment early. Based on these observations, non-platinum regimens have been proposed as an alternative to the platinum-based combinations for treatment of advanced NSCLC [13].

Paclitaxel (PTX) and GEM are new anti-cancer agents having significant single-agent activity against advanced NSCLC. A recent clinical phase II study of 122 patients with previously untreated, unresectable stage III or IV NSCLC receiving a 3-h infusion of PTX at a dose of 210 mg/m^2 showed a good response rate of 35% [25]. Although PTX is usually given once every 3 weeks, Chan et al. [10] demonstrated that weekly administration of PTX at a dose of 80–90 mg/m^2 provides similar tolerability and a possible increase in efficacy.

Gemcitabine, a novel deoxycytidine analog, had a response rate of 20% with a single weekly administration in previously untreated advanced NSCLC [4]. As a first-line treatment, single-agent GEM has been shown to have anti-tumor activity equal to that of cisplatin/etoposide, resulting in less toxicity and a slightly better quality of life [27].

These agents have different mechanisms of action, and their toxicities are partially non-overlapping. Although the usual administration of PTX is once every 3 weeks, a weekly administration can increase efficacy with good tolerability [1, 2]. We demonstrated that weekly administration with PTX and GEM is a tolerable and active regimen for patients with advanced NSCLC previously treated with platinum-containing chemotherapy regimens [20]. Based on these findings, we designed a phase II trial to examine the efficacy and tolerance of the non-platinum-based combination of PTX and GEM administered weekly for patients with untreated advanced NSCLC.

Patients and methods

Patient selection

All patients with histologically or cytologically confirmed advanced NSCLC were eligible for this phase II trial. The subjects of this study were patients with clinical stage IV NSCLC or stage III with unresectable disease or for whom radiotherapy with curative intent is not possible. Patients with unresectable disease or radiotherapy with curative intent is not possible include those with pleural effusion and dissemination, those with intrapulmonary metastasis within the ipsilateral lobe, those with an irradiation field exceeding one-half of one lung, those with metastasis to the contralateral hilar lymph nodes, and those with reduced lung function. Other eligibility criteria included: age older than 20 years and younger than 76 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; measurable lesions; life expectancy $\geq$12 weeks; adequate bone marrow reserve with a WBC count $\geq$4,000 per mm^3; platelet count $\geq 10 \times 10^4$ per mm^3; and hemoglobin level $\geq$9.0 g/dL; liver function with a AST and ALT $\leq$2.5 $\times$ upper normal limit, unless as a result of liver metastases; and adequate renal function with a serum creatinine level $\leq$1.5 mg/dL. No prior radiotherapy treatment was allowed if the irradiated area was not the site of measurable lesion and the therapy was completed at least 2 weeks before enrollment into the study.

Patients were excluded for the following indications: $\geq$76 years of age (vinorelbine as single agent treatment), severe cardiovascular or cerebrovascular disease, uncontrolled diabetes or hypertension, active infection, pulmonary fibrosis, massive pleural effusion or ascites, active peptic ulcer, and severe neurological disorders. Patients were also excluded in case of previous malignancy and any evidence or history of hypersensitivity or other contraindications for the drugs used in this trial. Written informed consent was obtained from all patients.

Treatment

Paclitaxel, 100 mg/m^2, was administered IV during a 1-h infusion, followed by GEM, 1,000 mg/m^2, IV over 30 min on days 1 and 8 of 21-day cycle. Premedication for PTX consisted of dexamethasone 20 mg, diphenhydramine 50 mg, and ranitidine 50 mg IV for 30 min before PTX infusion. After the premedication for PTX was completed, a serotonin receptor antagonist was given as a 30-min infusion for prophylactic antiemetic therapy. Treatment was repeated every 3 weeks until maximum response plus two cycles or unacceptable toxicity. In stable disease, patients received a maximum of six cycles. At the investigator's discretion, patients were treated with up to eight cycles of the drug combination.

Dose modifications were planned according to hematologic and severe non-hematologic toxic effects. Once the doses were reduced, they were not increased. Patients who experienced grade 4 neutropenia, grade 4 thrombocytopenia, reversible grade 2 neurotoxicity, or liver dysfunction received reduced doses of both PTX, 75 mg/m^2, and GEM, 800 mg/m^2, for the next cycle. The next course of chemotherapy was started after 3 weeks when the leukocyte count was 3,000 per mm^3 or greater, the neutrophil count was 1,500 per mm^3 or greater, the platelet count was 75,000 per mm^3 or greater, serum creatinine was less than 1.5 mg/dL, GOT and GPT were less than twice the upper limit of the normal range, and the neurotoxicity was grade 1 or less. If hematologic recovery was not achieved by day 35 of treatment, the patient was withdrawn from the study.

Evaluation of responses and toxicity

Responses and toxicity were evaluated on the basis of tumor images obtained by computerized tomography (CT), laboratory results, subjective/objective symptoms, signs before, during, and after administration of the study drugs and during the period from completion of treatment to the final analysis. Measurable disease parameters were determined every 4 weeks by various means such as CT. Evaluation was performed in compliance with the response evaluation criteria in solid tumors (RECIST) guidelines for anti-tumor activity. Adverse events were assessed using the Common Terminology Criteria for Adverse Events version 3.0 (CTCAEv3.0). Patients were withdrawn from the study if evidence of tumor progression was observed. The institutional ethical review committee gave approval to the study.

Statistical analysis

The primary end point of the study was the response rate. Simon's two-stage design was used to determine sample size and decision criteria. It was assumed that a response rate of 40% in eligible patients would indicate potential usefulness, while a rate of 20% would be the lower limit of interest; $\alpha = 0.05$ and $\beta = 0.10$. Using these design parameters, the first stage of the study was to enroll 24 patients, and the regimen was rejected if fewer than five patients had an objective response. If six or more patients responded, the accrual was continued until 45 patients were enrolled (45 patients were required because of anticipated percentage of dropout cases). Combination therapy was considered effective if ≥ 14 of the 45 patients showed a response in the final analysis. Secondary end points were toxicity and overall survival. Response and survival rates were both calculated on an intent-to-treat basis. Overall survival and time to progression were measured from the start of this treatment until time of death or the date of the last follow-up clinical assessment. Survival curves were constructed using the Kaplan–Meier method (Fig. 1).

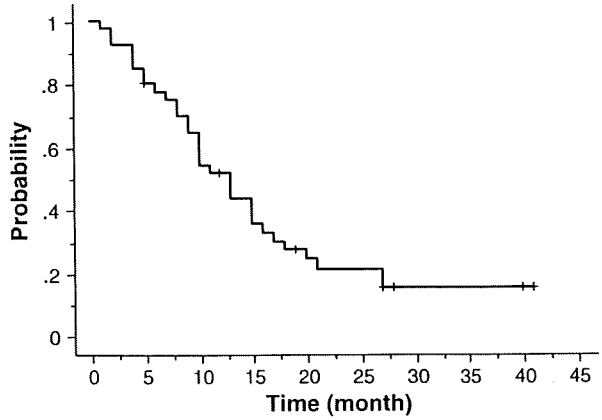

Fig. 1 Kaplan–Meier estimated overall survival curves. Median survival time, 11.9 months; 1-year survival rate, 47.5%

Results

Patient characteristics

A total of 40 patients were enrolled in the study between September 2001 and July 2004. The majority of patients were treated as outpatients. The clinical characteristics of the patients are listed in Table 1. The median age was 66 years with a range of 33–75 years. Nearly two-thirds of the patients were men. Twenty-four patients had an PS of 0, and 15 had PS of 1. Histological subtypes were 73% (29/40) adenocarcinoma and 25% (10/40) squamous cell carcinoma.

Table 1 Patient characteristics

Eligible patients	40
Gender	
Male	26
Female	14
Age (years)	
Median	66
Range	33–75
Performance status	
0	24
1	15
2	1
Histology	
Adenocarcinoma	29
Squamous cell	10
Large cell	1
Stage	
III	13
IV	27
Number of metastatic sites	
Median	2
Range	0–3
Location of metastases	
Bone	12
Lung nodules	10
Liver	9
Lymph nodes	8
Adrenals	6
Brain	3
Subcutaneous	1