Routine clinical and laboratory assessments and chest X-ray assessments were performed weekly or biweekly, where possible; CT examinations of the target lesion were performed every month, and magnetic resonance imaging of the whole brain and a bone scan were performed every 3 months. The objective responses of the patients were evaluated every month using the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines (Therasse *et al*, 2000). Tumour response was centrally evaluated by independent reviewers at an extramural conference and was performed for the intent-to-treat population. All adverse effects that occurred during gefitinib treatment were reported, and the severity of the effects was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Statistical analyses

The primary end point of this study was the response rate. A one-stage design using the binominal probability was used to determine the sample size. Assuming that a response rate of 50% would indicate potential usefulness, whereas a rate of 25% would be the lower limit of interest, and with $\alpha = 0.10$ (two side) and $\beta = 0.20$, the estimated accrual number was 23 patients. Estimating that the *EGFR*-positive rate would be about 20%, the screening number required to accrue 23 *EGFR*-positive patients was 115. After assuming an inevaluability rate of $<10\%$, the final required screening number was 125.

The secondary end points of this study were toxicity, OS, PFS, 1-year survival (1Y-S) and the disease control rate (DCR). Survival analyses were conducted on the intent-to-treat population using follow-up data available as of 30 April 2007. The survival curves were estimated using Kaplan–Meier plots.

RESULTS

Patient characteristics

Between March 2005 and January 2006, 118 patients were prospectively screened from 15 institutions; 117 of them underwent *EGFR* mutation analysis (tumour tissue was not available for one patient). The median time required for the *EGFR* mutation analysis was 12 days (range: 7–28 days). Among the 117 patients, *EGFR* mutations were detected in 32 patients (27%), 14 of whom had a deletion in or near E746-A750 (including one del E746-T751 ins A, two del L747-T751 and one del L747-T753 ins S) in exon 19. A further 17 had L858R, and one had a L861Q point mutation in exon 21 (Table 1).

Tissue samples from 17 patients (53%) were obtained by transbronchial biopsy. The *EGFR* detection rates for the surgical specimens and the bronchoscopic biopsy specimens were similar (30 *vs* 25%). The *EGFR* mutations were significantly more frequent in women ($P \leqslant 0.02$), in patients with adenocarcinoma ($P = 0.001$) and in people who had never smoked ($P < 0.001$) (Table 2). Finally, 28 patients (14 with deletions in exons 19 and 14 with point mutations in exon 21) were actually registered and received treatment with gefitinib, whereas four patients were dropped from the study as they became ineligible because of tumour progression during the time required for the mutation analysis.

Patient characteristics are listed in Table 3. In the initial screening, there were 56 female patients (48%), 97 patients (83%) with adenocarcinoma and 53 (45%) who had never smoked. The frequency of these characteristics was higher among the patients with *EGFR* mutations who were actually registered; namely, 18 patients (64%) were women, 27 (96%) had adenocarcinoma and 19 (68%) had never smoked. The median age of the 28 actually registered patients was 68 years; 24 patients (86%) had a good performance status (0–1), 22 (79%) had stage IV diseases and 17 (61%) were chemotherapy naive. Thoracic irradiation was contra-indicated in one patient with stage IIIA disease because of the large irradiation field that would have been required. All five patients with stage IIIB diseases had malignant effusions. Four patients had received adjuvant therapies; five had received platinum doublets or a combination of gemcitabine and vinorelbine as their first-line therapy. Two patients had received two regimens of platinum doublets followed by docetaxel or pemetrexed. One patient had received local radiation for pain control.

Table 1 Type of *EGFR* mutations ($n = 32$)

Characteristics	No. of patients	%
Exon 18	0	0
Exon 19	14	44
del E746-A750	10	32
del E746-T751 ins A	1	3
del L747-T751	2	6
del L747-T753 ins S	1	3
Exon 21	18	56
L858R	17	53
L861Q	1	3

EGFR = epidermal growth factor receptor.

Table 2 Relationship between patient characteristics and *EGFR* mutation status

	EGFR mutation positive ($n = 32$)		*EGFR* mutation negative ($n = 85$)		
Characteristics	No. of Patients	%	No. of Patients	%	*P*
Sex					
Male	11	34	50	59	
Female	21	66	35	41	<0.02
Histology					
Adenocarcinoma	31	97	66	78	
Nonadenocarcinoma	1	3	19	22	=0.001
Smoking status					
Never	21	66	31	36	
Current/former	11	34	54	64	<0.001

EGFR = epidermal growth factor receptor.

Response and survival

The objective tumour responses are listed in Table 4. The overall response rate and DCR were 75% (95% CI: 57.6–91.0%) and 96% (95% CI: 87.0–96.4%), respectively. Five out of ten male patients (50%), six out of nine smokers (67%) and five out of eight male smokers with adenocarcinoma (63%) achieved a PR. One female nonsmoker with squamous cell carcinoma also achieved a PR. Among the registered patients with *EGFR* mutations, the response rate was no different between current/former smokers and those who had never smoked (67 *vs* 79%) or between chemotherapy-naive and postchemotherapy patients (77 *vs* 73%). Female and patients with a mutational deletion in exon 19 tended to have a higher response rate than male (89 *vs* 50%) and patients with a missense mutation in exon 21 (86 *vs* 64%), respectively.

The median follow-up time was 18.6 months (range: 13.8–23.4 months). The median PFS time was 11.5 months (95% CI: 7.3 months to -) (Figure 1A). The median OS has not yet been reached, and the 1Y-S was 79% (95% CI: 63.4–93.8%) (Figure 1B).

Table 3 Patient characteristics of all registered patients ($n = 28$)

Characteristics	No. of patients (%)
Age	
Median	68
Range	49–89
Performance status	
0	11 (39)
1	13 (47)
2	4 (14)
Sex	
Male	10 (36)
Female	18 (64)
Histology	
Adenocarcinoma	27 (96)
Squamous cell carcinoma	1 (4)
Large cell carcinoma	0 (0)
Adenosquamous carcinoma	0 (0)
Other	0 (0)
Smoking status	
Never	19 (68)
Current/former	9 (32)
Stage	
IIIA[a]	1 (3)
IIIB	5 (18)
IV	22 (79)
Prior cancer therapy	
Chemotherapy	
No	17 (61)
One regimen (adjuvant)	4 (14)
One regimen (not adjuvant)	5 (18)
Two regimens	2 (7)
Recurrence after surgery	11 (39)
Radiation	1 (4)

[a]Unresectable, no indication for thoracic radiation because of a large radiation field.

Table 4 Response rate ($n = 28$)

Response	No. of patients	Response rate (%)	95% CI
Complete response	1	3.6	
Partial response	20	71.4	
Stable disease	6	21.4	
Progressive disease	0	0.0	
Not evaluable[a]	1	3.6	
Overall response	21	75.0	57.6–91.0
Disease control rate	27	96.4	87.0–96.4

CI = confidence interval. [a]One patient was not evaluable because of a poor evaluation of efficacy.

Safety and toxicity

Toxicity was evaluated in all eligible patients (Table 5). The most frequent adverse events were rash, dry skin, diarrhoea, stomatitis and elevated AST/ALT levels. Two patients experienced grade 3 rash and one patient experienced grade 3 keratitis; however, these patients all achieved a PR, and the adverse effects subsided after pausing gefitinib treatment for around 2 weeks. Four patients experienced grade 3 hepatotoxicity; three of these patients had to discontinue treatment for this reason.

One patient developed interstitial lung disease (ILD) (Ando *et al*, 2006). Ground-glass opacity was detected in the right upper lobe 19 days after the start of gefitinib administration, resulting in the cessation of treatment. However, the lesion enlarged into bilateral lung fields on day 25, and steroid therapy was initiated. Nonetheless, the patient died of respiratory failure on day 48. Two patients also experienced grade 1 ILD. They recovered without steroid administration.

Subsequent treatment after disease progression

Of the 14 patients who become refractory to gefitinib and exhibited disease progression, 10 received chemotherapy as their first treatment regimen after gefitinib (Table 6); 5 patients received platinum doublets and 1 patient received vinorelbine as a second-line treatment; and 3 received docetaxel and 1 received platinum doublet as a third-line treatment. In all, 4 out of the 10 patients (40%) had a PR. Of the nine patients who become refractory to the first treatment regimen after gefitinib, six received chemotherapy as their second regimen after gefitinib, including one who received gemcitabine, one who received docetaxel, and one who was re-treated with gefitinib as a third-line therapy; two other patients received docetaxel and one was re-treated with gefitinib as a fourth-line therapy. Two of the six patients (33%) had a PR. The two patients who received gefitinib re-treatment both had SD.

BAC features, *EGFR* amplification and T790M mutation in exon 20

A total of 110 tissue samples were available for pathological review, of which 90 were from adenocarcinoma; 33 of these specimens (37%) revealed proportional BAC components in the specimen. Among them, 15 were considered extensive and the remaining 18 were found to have minor BAC components. The 39 surgical specimens included 36 from adenocarcinomas. The *EGFR* mutations were detected in 12 out of the 36 adenocarcinoma specimens. None of the samples with a BAC component, micropapillary pattern or mucin production was associated with an *EGFR* mutation (Table 7).

Data on *EGFR* gene copy numbers were available in only 12 samples. We used the criteria for defining a high *EGFR* gene copy number (gene amplification or high polysomy, as determined using FISH) that were described in a previous report (Cappuzzo *et al*, 2005). A total of 7 out of the 12 samples had a high gene copy number (FISH positive), and 6 (3 with *EGFR* mutations) out of the 7 samples had proportional BAC components. In all, 5 out of the 12 samples were FISH negative, only 1 (with no *EGFR* mutation) of which had a BAC component. Two patients that were FISH negative, BAC negative and *EGFR* mutation positive had SD when treated with gefitinib.

Another *EGFR* mutation, T790M in exon 20, has been reported to be associated with resistance to gefitinib (Kobayashi *et al*, 2005; Pao *et al*, 2005). We checked for this mutation in six patients who did not respond to gefitinib; however, the mutation could not be identified in any of the patients.

DISCUSSION

We performed a multicentre phase II study examining the use of gefitinib for advanced NSCLC in patients with *EGFR* mutations, prospectively recruiting patients at the time of genetic screening and avoiding a selection bias. All patients were registered in a central database. All tissues were delivered from the local participants to the central facility, where they were reviewed by a pathology specialist and the *EGFR* mutation status was evaluated. The median time for the *EGFR* mutation detection analysis was 12 days, which is probably an acceptable time lag before the start of treatment for advanced NSCLC. However, a shorter period would clearly be desirable for routine clinical practice. Indeed, 4 out of the 32 *EGFR*-positive patients were dropped from the study because of disease progression before their actual registration

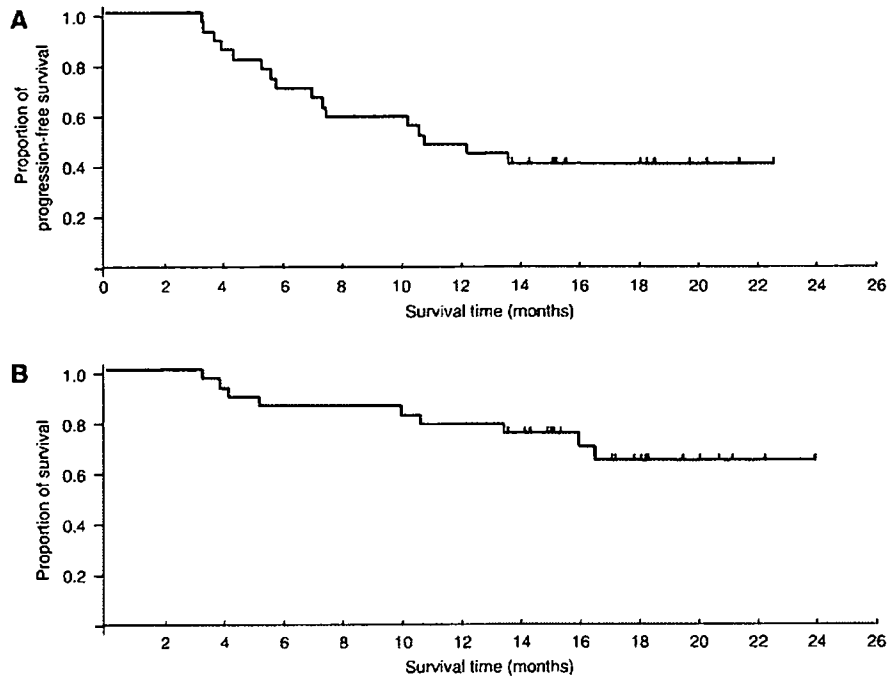

Figure 1 (**A**) Progression-free survival (PFS) and (**B**) overall survival (OS) of all eligible patients ($n = 28$). The median PFS was 11.5 months. The median OS has not yet been reached. The 1-year survival rate was 79%.

Table 5 Common adverse events ($n = 28$)

	No. of patients (%)			
Adverse events	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haematologic				
Anaemia	12 (43)	3 (11)	0 (0)	0 (0)
Leucopaenia	4 (14)	1 (4)	2 (7)	0 (0)
Neutropaenia	4 (14)	1 (4)	1 (4)	0 (0)
Thrombocytopaenia	3 (11)	0 (0)	0 (0)	0 (0)
Nonhaematologic				
Rash	10 (36)	11 (39)	2 (7)	0 (0)
Dry skin	9 (32)	10 (36)	0 (0)	0 (0)
Nail changes	5 (18)	2 (7)	0 (0)	0 (0)
Keratitis	0 (0)	0 (0)	1 (4)	0 (0)
Fever	0 (0)	1 (4)	0 (0)	0 (0)
Fatigue	3 (10)	3 (10)	3 (10)	0 (0)
Diarrhoea	7 (25)	1 (4)	0 (0)	0 (0)
Constipation	1 (4)	0 (0)	0 (0)	0 (0)
Stomatitis	8 (29)	1 (4)	0 (0)	0 (0)
Gastritis	1 (4)	0 (0)	0 (0)	0 (0)
Anorexia	2 (7)	1 (4)	0 (0)	0 (0)
Nausea	3 (11)	1 (4)	0 (0)	0 (0)
Vomiting	2 (7)	2 (7)	1 (4)	0 (0)
Dyspnoea	2 (7)	0 (0)	1 (4)	0 (0)
ILD	2 (7)	0 (0)	0 (0)	1 (4)[a]
Vertigo	1 (4)	1 (4)	0 (0)	0 (0)
Dysgeusia	0 (1)	1 (4)	0 (0)	0 (0)
Elevated AST/ALT	10 (36)	2 (7)	4 (14)	1 (4)[a]
Elevated creatinine	2 (7)	1 (4)	2 (7)	0 (0)

ALT = alanine transaminase; AST = aspartate transaminase; ILD = interstitial lung disease. [a]Same patient.

could occur. Yatabe *et al* (2006) has developed a rapid assay to detect *EGFR* mutations, and we have decided to use this assay in a phase III trial. The *EGFR* mutation rates in transbronchial biopsy samples were found to be the same as those in surgical specimens, suggesting that this assay can also accommodate stage IV NSCLC. We detected the two characteristic types of *EGFR* mutations (in exons 19 and 21) in 44 and 56% of the patients, respectively (Table 1); these percentages are identical to those in previous reports from Japan (Shigematsu *et al*, 2005; Asahina *et al*, 2006; Inoue *et al*, 2006; Yatabe *et al*, 2006; Yoshida *et al*, 2007). In summary, we confirmed the feasibility of using the *EGFR* detection assay in daily practice.

The overall response rate was 75%, which was comparable to those of other phase II studies of gefitinib in patients with *EGFR* mutations (Asahina *et al*, 2006; Inoue *et al*, 2006), despite our study permitting the entry of patients who had previously received up to two chemotherapy regimens. The DCR of 96% was relatively high, and the median PFS of 11.5 months and 1Y-S of 79% were also very promising. In a Korean study, Lee *et al* (2006) also reported a very promising response rate (56%) and 1Y-S (76%) for gefitinib in a prospective study of selected NSCLC patients with adenocarcinoma and never/light smokers, defined as having smoked no more than 100 cigarettes during one's lifetime. In the screening process for the present study, *EGFR* mutations were significantly more frequent in women, patients with adenocarcinoma and those who had never smoked. However, among the patients who were selected according to their *EGFR* mutation status, no differences in response were observed between never smokers and current/former smokers or between chemotherapy-naive and postchemotherapy patients. In a retrospective study, Han *et al* (2006) directly compared clinical predictors (smoking history, gender and histology) and the *EGFR* mutation status for their ability to predict response and survival. They showed that female never smokers with adenocarcinoma (three clinical predictors) had a 33% response rate, whereas patients with a positive *EGFR* mutation status had a 62% response rate. Furthermore, in a multivariate analysis, only a positive *EGFR* mutation status was associated with an improved OS, suggesting that the *EGFR* mutation status should be analysed whenever possible to optimise response predictions based on clinical

Table 6 Subsequent treatments after failure to respond to gefitinib ($n = 28$)

Gefitinib treatment	No. of Patients	1st regimen after gefitinib	No. of patients	2nd regimen after gefitinib	No. of patients
1st line	17	Plt doublet	5	Gem or Doce	2
				Gefitinib[a]	1
		VNR	1	—	-
2nd line[b]	4	Doce	2	Doce	1
		Plt doublet	1	Doce	1
2nd line	5	Doce	1	Gefitinib[a]	1
3rd line	2	—	—	—	—
Total	28		10		
Response			4/10		2/6

Doce = docetaxel; Gem = gemcitabine; Plt = platinum; VNR = vinorelbine. [a]Both patients had an SD response after gefitinib re-treatment. [b]First regimen as systemic chemotherapy after adjuvant treatment.

Table 7 Bronchial alveolar carcinoma (BAC) features and *EGFR* mutation status

	EGFR mutation		
	+	−	*P*-value
Surgically resected adenocarcinoma case	12	24	
BAC component			
Yes	8	17	1.0
No	4	7	
Micropapillary pattern			
Yes	4	12	0.48
No	8	12	
Mucin production			
Yes	1	5	1.0
No	11	19	

EGFR = epidermal growth factor receptor.

background factors. In the present study, *EGFR* mutations were detected in 16 out of 40 (40%) female never smokers with adenocarcinoma who underwent the screening process, and 14 out of these 16 patients (88%) achieved a response after undergoing gefitinib therapy. We could not compare the predictive powers of clinical predictors and the *EGFR* mutation status with regard to the clinical benefits of gefitinib in this study. Thus, the need for *EGFR* mutation testing among clinically favourable patients remains uncertain. Decisions regarding the first-line therapy of choice for patients with *EGFR* mutations or a clinically favourable profile (nonsmoker with adenocarcinoma) must also await the results of an ongoing randomised phase III study in an Asian population (IPASS: Iressa Pan-Asian Study) comparing platinum doublets with gefitinib.

In contrast, 50% of the men, 67% of the smokers and 63% of the men who were smokers achieved a PR in this study. Furthermore, one female nonsmoker with squamous cell carcinoma also responded to gefitinib. The histological type of this tumour was reassigned by a pulmonary pathologist, and the tumour was finally confirmed to be a squamous cell carcinoma. Squamous cell carcinoma harbouring an *EGFR* mutation is rarely seen but has been previously reported (Asahina *et al*, 2006). In a Japanese phase II trial of gefitinib for unselected chemotherapy-naive patients (Niho *et al*, 2006), the response rates among smokers, men, and patients with nonadenocarcinoma were 19, 13 and 10%, respectively. Thus, NSCLC patients who are either smokers, men or have a nonadenocarcinoma histology are unlikely to receive gefitinib treatment as a first-line treatment instead of standard chemotherapies (platinum doublets), which yield a response rate of about 30% (Schiller *et al*, 2002). Therefore, *EGFR* mutation screening may have a higher impact on the selection of responders to gefitinib treatment among these kinds of Asian patient subset (for example, smokers with adenocarcinoma, and nonsmoking men or women with nonadenocarcinoma).

The benefit of chemotherapy in general among patients with *EGFR* mutations, compared with *EGFR* mutation-negative patients, remains uncertain. Previous studies (Bell *et al*, 2005) have suggested that patients with *EGFR* mutations tend to be more sensitive to chemotherapy than those with wild-type *EGFR*. In the present study, 40 and 33% of the patients responded to first- and second-line chemotherapy regimens after gefitinib, respectively. These relatively high response rates for refractory NSCLC suggest that patients with an *EGFR* mutation-positive status are generally sensitive to chemotherapy. Large-scale multivariate analyses, using pooled data from prospective phase II or III trials in which the *EGFR* mutation status was clearly confirmed, are needed to clarify this point.

The toxicities observed in the present study were mostly tolerable. Most of the common adverse events, like rash, diarrhoea or hepatotoxicity, were mild and subsided after gefitinib administration was paused for a short period. One male smoker with adenocarcinoma died of ILD. Thus, even among patients who are selected based on their *EGFR* mutation status, men or smokers may still be at risk for developing ILD; therefore, biomarkers to predict ILD are needed.

Patients with exon 19 mutations tended to have a higher response rate than those with a missense mutation in exon 21, consistent with the findings of previous reports (Jackman *et al*, 2006; Riely *et al*, 2006). The Spanish Lung Cancer Group also reported on a prospective phase II study of erlotinib in advanced NSCLC patients with *EGFR* mutations (Paz-Ares *et al*, 2006). The overall response rate was 82%. They also showed a difference in response rates between patients with mutations in exons 19 and 21 (95 and 67%, respectively). Exon 11 c-kit mutations are more closely correlated with a good prognosis in patients with gastrointestinal stromal tumour, who may benefit from lower doses of imatinib, whereas patients with exon 9 mutations may require higher doses (Debiec-Rychter *et al*, 2006). In the case of *EGFR*, functional differences between mutation types may also exist.

We found no discernible associations between the *EGFR* mutation frequency and the presence of a BAC component. Several reports, including that of Hirsch *et al* (2005) suggest that a higher *EGFR* copy number is correlated with BAC histological features. We also found an association between a high *EGFR* copy number and the presence of a BAC component, even though the number of specimens examined was relatively small. In a study on erlotinib, the presence of a BAC component was clearly associated with *EGFR* amplification. As the *EGFR* mutation rate is lower in western populations than in Asian populations, the *EGFR* gene copy number might be a more useful biomarker in western populations, especially with regard to the use of erlotinib.

In conclusion, gefitinib treatment for patients with advanced NSCLC harbouring an *EGFR* mutation demonstrated a promising activity in patients with a good performance status. Patient screening according to *EGFR* mutation status may be a useful tool in daily practice and will likely have a great impact on the selection of patients who are likely to benefit from gefitinib treatment.

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Multidisciplinary Treatment for Advanced Invasive Thymoma with Cisplatin, Doxorubicin, and Methylprednisolone

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Background and Objectives: Advanced invasive thymomas are not usually manageable by surgical resection and radiotherapy. We reviewed our experience with a multidisciplinary approach and evaluated chemotherapy in the treatment of invasive thymoma.

Patients and Methods: Seventeen consecutive patients with invasive thymoma were treated with multimodality therapy consisting of chemotherapy, surgery, and/or radiotherapy. Four patients had stage III disease with superior vena cava invasion, nine had stage IVa disease, and four had stage IVb disease. The chemotherapy regimen consisted of cisplatin, doxorubicin, and methylprednisolone (CAMP). Chemotherapy was administered in a neoadjuvant setting to the 14 patients and in an adjuvant setting to the remaining three patients. Surgical resection was intended in all patients. After those treatments, chemotherapy and/or radiation therapy were performed.

Results: All but one of the 14 patients with induction chemotherapy responded to the CAMP therapy, and the response rate was 92.9%. Seven of these patients underwent complete remission after surgical resection and chemoradiotherapy, and the others underwent partial remission. All three patients treated with surgical resection and then chemotherapy with or without radiotherapy also achieved complete remission. Tumor progression after multimodality therapy occurred in 10 patients. After retreatment, eight of these patients were alive at the time of analysis, with a median survival time after recurrence of 30 months. The 5- and 10-year overall survival rates for all patients were both 80.7%. The major side effect of CAMP therapy was acceptable neutropenia.

Conclusions: CAMP therapy was highly effective for invasive thymomas, and the multimodality therapy containing this chemotherapy brought about good disease control in the majority of patients. We believe that this multidisciplinary treatment with CAMP therapy, surgery, and radiotherapy is a justifiable initial treatment for patients with advanced invasive thymoma. Furthermore, appropriate treatments are essential for the long-term survival of patients with recurrences after multimodality therapy.

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In patients with thymoma, surgical resection with or without radiation therapy has been advocated as the treatment of choice for early-stage diseases.[1–3] Nevertheless, advanced-stage diseases such as tumors with great vessel invasion, pleural and/or pericardial dissemination, lymph node involvements, or distant metastases are difficult to manage by surgery and radiotherapy, and the treatment strategy for those diseases remains controversial.[4,5]

Chemotherapy has been shown to have significant antitumor activity against unresectable, recurrent, or metastatic thymomas.[6–9] Recently, multimodality therapy using chemotherapy has been examined in the treatment of advanced thymomas.[10–12] Investigators have demonstrated that combined-modality therapy can improve outcomes for advanced thymoma patients. Nevertheless, the chemotherapy regimens and treatment schedules in these studies were varied, and an optimal treatment strategy has not yet been determined. Furthermore, although it is well known that thymoma has a slow-growing nature and a late recurrent tendency, few reports contained longer follow-up data or results of retreatment of recurrences.[13–15]

To improve the outcome of patients with advanced invasive thymomas, we have conducted a study of multimodality therapy including chemotherapy. Here, we report the results with a longer follow-up.

PATIENTS AND METHODS

From February 1988 to September 2003, 38 patients with thymoma were referred to our hospital. Their clinical characteristics are shown in Table 1. Of these patients, 17 consecutive patients with advanced invasive thymoma, (four patients with stage III disease, nine with stage IVa disease, and four with stage IVb disease) including four patients with recurrent tumor, were enrolled in the study of multimodality therapy including chemotherapy, surgery, and/or radiotherapy. In all but three patients, pathologic diagnosis of thymoma was obtained by thoracotomy, transthoracic needle biopsy, or fiberoptic bronchoscopic biopsy before initiation of treatment. Among the patients without pretreatment histologic diagnosis, one patient had multiple recur-

TABLE 1. Profile of Patients with Thymoma

Sex	
Male	17
Female	21
Age (yr)	
Median (range)	57 (25–75)
World Health Organization tumor type	
A	2
AB	6
B1	3
B2	22
B3	5
Masaoka stage	
I	15
II	4
III	6
IVa	9
IVb	4

rent pleural tumors after surgical treatment and chemotherapy for thymoma, and the remaining two had anterior mediastinal mass suspected invasive thymoma on computed tomography (CT) that were located at unsuitable places for needle biopsy. Clinical staging was determined by the medical history and physical examination, chest radiography, and chest CT. Other imaging modalities such as magnetic resonance imaging, echocardiography, or venography were performed when indicated. The staging was based on the Masaoka staging system.[16] All patients gave written informed consent for the study.

The treatment strategy of the multimodality therapy was as follows: (a) If a tumor of stage III with invasion to the great vessels or stage IV disease was distinctly demonstrated on diagnostic imaging at the initial staging, induction chemotherapy was conducted. After three or four cycles of the chemotherapy, surgical resection was attempted when the residual tumor was found, and consolidation chemotherapy and/or radiotherapy were given. (b) When stage IV disease was found on operation despite a clinically earlier stage, surgery for debulking the tumor was attempted. After that, chemotherapy was administered as a postsurgical adjuvant treatment, and then radiation therapy was applied if indicated.

The chemotherapy regimen consisted of cisplatin (20 mg/m^2 per day, continuous infusion on days 1 through 4), doxorubicin (40 mg/m^2 intravenously on day 1), and methylprednisolone (1000 mg/day intravenously on days 1 through 4 and 500 mg/day intravenously on days 5 and 6) (CAMP). Treatment cycles were repeated every 21 to 28 days. Prophylactic granulocyte colony stimulating factor was not routinely used. Surgery was intended through a median sternotomy in all patients. Resection was defined as complete (R0) if all gross disease was removed and if all surgical margins were free of the tumor. An incomplete resection meant that the surgical margins were microscopically positive (R1) or that gross residual tumors (R2) were left at the end of the operation. Radiation therapy was administered to the mediastinal or residual tumor areas using opposite anterior and posterior parallel fields and doses of more than 50 Gy. When malignant pericardial effusion was noted during the operation, whole mediastinal irradiation was carried out.

The patients were evaluated with CT for response after induction chemotherapy and completion of the multimodality treatment. A complete remission (CR) was defined as the complete disappearance of all objective evidence of disease on CT for at least 4 weeks. A 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).

Survival was measured from the first day of treatment until death or the last date of the follow-up (March 31, 2004). The survival curves were calculated according to the Kaplan–Meier method, and comparisons among the curves were made by means of the log-rank test. The median follow-up time of all patients (n = 17) was 54 months (range, 2–193 mo), and median follow-up time of surviving patients (n = 14) was 62 months (range, 6–193 mo).

RESULTS

Of the 17 patients, eight were women and nine were men, ranging in age from 25 to 72 years (median, 51 yr) (Table 2). Pretreatment pathologic diagnoses were obtained in 14 patients, and the tumor histology of the remaining three patients (patients 15–17) was revealed after chemotherapy and surgical treatment. Histologic types of the thymoma were B2 tumor in 14 patients and B3 tumor in three patients, according to the World Health Organization classification.[17] All four patients who were diagnosed as having stage III disease were found to have a tumor with superior vena cava invasion on diagnostic imaging. Nine patients with stage IVa disease had pleural tumor dissemination and/or pericardial effusion, and four with stage IVb disease had pulmonary metastasis or lymph node involvement.

A summary of treatments and outcomes is listed in Table 3. CAMP therapy was administered in a neoadjuvant setting to 14 patients (Figures 1 and 2). One complete response and 13 partial responses were obtained, with an overall response rate of 92.9% (95% confidence interval [CI], 66.1–99.8%). After chemotherapy, nine patients underwent surgical resection of the residual tumor with curative intent. However, R0 resection was performed in only two patients, R1 resection in one patient, and R2 resection in six patients. Postsurgical radiotherapy was performed in eight patients. Among the remaining four patients, one complete responder for CAMP therapy had no additional treatment. Two partial responders received radiotherapy because of the unresectable tumor, and the other one refused further treatment.

Three patients (patients 1, 2, and 11) who were categorized at the initial staging as having stage I to III disease were found on operation to have stage IVa disease with pleural dissemination or malignant pericardial effusion. The patients underwent resection of the main tumor and extended

TABLE 2. Characteristics of Patients with Advanced Invasive Thymoma

Patient No.	Age (yr)	Sex	Histology	Disease Stage	Site of Disease
1	40	M	B2	IVa	Pleural dissemination
2	59	F	B2	IVa	Pericardial effusion, pericardium, aorta, lung
3	72	M	B2	IVa	Pericardial effusion, pericardium, SVC, lung
4	63	M	B2	IVb	Mediastinal lymph nodes, pleural effusion
5	38	F	B2	III	SVC
6	33	M	B2	IVa	Pleural dissemination, lung
7	65	F	B2	IVb (rec)	Pulmonary metastasis, pleural dissemination
8	66	F	B2	IVb (rec)	Pulmonary metastasis
9	62	F	B2	III	SVC
10	56	M	B3	IVa (rec)	Pleural dissemination
11	29	M	B2	IVa	Pleural dissemination, pericardium, lung
12	49	M	B3	IVa	Pleural dissemination, pericardium, pulmonary artery
13	51	F	B2	III	SVC, lung
14	62	F	B3	IVa	Pleural dissemination
15	25	M	B2	IVa (rec)	Pleural dissemination
16	29	M	B2	IVb	Pulmonary metastasis
17	62	F	B2	III	SVC

Rec, recurrent case; SVC, superior vena cava.

TABLE 3. Summary of Treatments

Patient No.	Previous Treatment	Cycles of CAMP Therapy	Response to CAMP Therapy	Subsequent Treatment	Total Response	Sites of Tumor Progression	Progression-Free Survival (mo)	Treatment for Recurrences	Overall Survival (mo)
1	S (R2)	4	NA		CR	Pleura	61	S (R0)	193+
2	S (R2)	4	NA	RT	CR		180		180+
3		4	PR	S (R1), CAMP × 2, RT	CR	Pleura, lung	45	RT	180+
4		4	PR	S (R2), RT	PR	Pericardium	11	CT[1]	13
5		4	PR	S (R0), RT	CR		169		169+
6		2+CT[2]	PR	S (R2), RT	PR	Pleura	17	CT[2]	18
7		2	PR		PR		2		2
8		3	CR		CR	Pulmonary metastasis	7	S (R0)	88+
9		2	NC	S (R2), RT	PR	Primary site	42	RT	72+
10		4	PR	RT	CR	Pleura	32	RT	67+
11	S (R2)	4	NA		CR	Pleura	24	CAMP × 2, S (R0)	56+
12		4	PR	RT	PR		54		54+
13		4	PR	S (R0)	CR		43		43+
14		4	PR	S (R2), RT	CR	Pleura	23	CAMP × 4	37+
15		4	PR		PR	Pleura	18	CAMP × 4, S (R0)	29+
16		4	PR	S (R2), RT	CR		9		9+
17		4	PR	S (R2), RT	PR		6		6+

CR, complete remission; CT[1], CDDP+VLB+BLM; CT[2], CPA+ADM+VCR+prednisone; NA, not assessable; NC, no change; PR, partial remission; R0, complete resection; R1, microscopically incomplete resection; R2, macroscopically incomplete resection; RT, radiation therapy; S, surgery.

thymectomy combined with a partial resection of the pericardium, parietal pleura, and/or lung. Even after the resection, patients 1 and 11 retained numerous miliary pleural tumors in the hemithorax, and patient 2, with malignant pericardial effusion, had a residual mass on the aortic arch. These patients received four cycles of CAMP therapy after surgery, and only patient 2 underwent subsequent whole mediastinal radiation therapy.

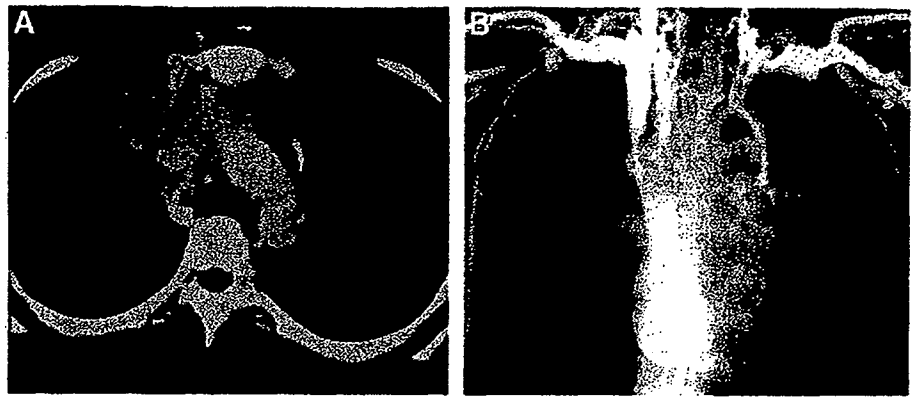

FIGURE 1. Patient 5 before chemotherapy. (*A*) CT scan showing a large anterior mediastinal tumor invading the superior vena cava. (*B*) Venous phlebogram illustrating an almost complete obstruction of the superior vena cava at the level of the junction of bilateral brachiocephalic veins.

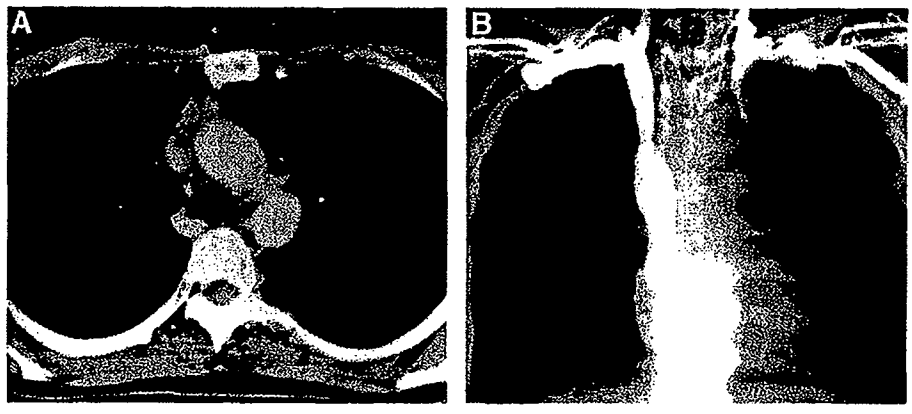

FIGURE 2. Patient 5 after four cycles of induction chemotherapy. (*A*) CT scan revealing considerable shrinkage of the tumor. (*B*) Venous phlebogram demonstrating the marked improvement of superior vena cava obstruction.

After completion of the multimodality therapy, 10 patients achieved CR and seven achieved PR; the overall remission rate was 100%. Tumor progression after treatment was observed in six (60%) of 10 CR patients and in four (57%) of seven PR patients, with a median progression-free survival of 24 months (range, 7–61 mo). The remaining six patients (four CR patients and two PR patients), 35% of the total population, had no tumor progression six to 180 months after the initiation of the multimodality therapy.

Treatment for recurrences was performed in all 10 patients. Complete surgical resection for the recurrences with or without preoperative CAMP therapy was accomplished in four patients. Patients 1 and 15 underwent an extrapleural pneumonectomy for pleural dissemination. Patient 8, who had recurrence after extrapleural pneumonectomy for the primary tumor, had a wedge lung resection for pulmonary metastasis, and patient 11 received a partial pleurectomy. For patients with unresectable recurrent tumors, radiotherapy was performed in three patients, and chemotherapy was performed in three patients whose tumors were unsuitable for radiotherapy. Two of the patients treated with chemotherapy died during the retreatment, one from recurrent tumor and the other from fulminant rhabdomyolysis.[18]

The 5- and 10-year overall survival rates of all patients were both 80.7% (95% CI, 60.9–100%) (Fig. 3). The survival curves according to stages of disease are shown in Figure 4. The 10-year survival rates of patients with stage III and stage IVa disease were 100 and 88.9% (95% CI, 68.4–100%), respectively. In stage IVb, the 5-year survival rate was 37.5% (95% CI, 0–93.6%), and only patient 8 survived for more than 5 years after CAMP therapy and resection for recurrence. In the 10 patients with recurrence, the median survival time and 5-year survival rate after retreatment were 30 months (range, 1–132 mo) and 30.0% (95% CI, 1.6–58.4%), respectively.

Toxicity of CAMP Therapy and the Multidisciplinary Treatment

The side effects of CAMP therapy are shown in Table 4. Seventy-one cycles were administered (median, four cycles;

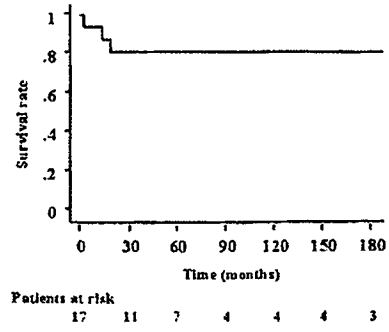

FIGURE 3. Overall survival of patients with advanced invasive thymoma who were treated with the multimodality therapy.

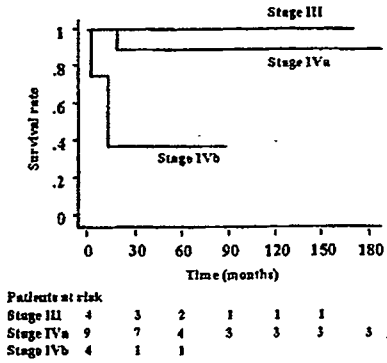

FIGURE 4. Survival according to the Masaoka staging system. In univariate analysis, there was a significant difference between stage IVa and stage IVb disease ($p = 0.036$), but there were no significant differences between stage III and stage IVa disease ($p = 0.564$) and stage III and IVb disease ($p = 0.123$).

TABLE 4. Toxic Effects of Cisplatin, Doxorubicin, and Methylprednisolone Therapy

NCI-CTC grade (%)	0	1	2	3	4	5
Leukocytes	14	12	39	27	8	
Neutrophils	10	9	21	34	26	
Hemoglobin	75	12	11	3		
Platelets	55	36	6	3		
Nausea/vomiting	31	26	36	5	1	
Infection	92	3		3	1	1

range, two to eight cycles), and the major adverse effects were leukopenia and neutropenia. Although 60% of cycles were associated with grade 3 or 4 neutropenia, almost all patients in the study received no granulocyte colony stimulating factors or no dose reduction of all three drugs. Treatment delays (median, 1 wk; range, 1–6 wk) were performed in eight patients because of neutropenia and patients' wishes. Chemotherapy-related death occurred in patient 7. She had multiple pulmonary metastases and pleural recurrences complicated with myasthenia gravis, pure red cell aplasia, and hypogammaglobulinemia. She died of pneumonia after the second cycle. Another peculiar complication of tumor lysis syndrome developed in patient 6, with a huge thymoma of predominantly lymphocytic type during the first cycle.[18]

After CAMP therapy and surgical treatment, mild cardiac dysfunction was observed in two patients (patients 2 and 3[19]) who received whole mediastinal irradiation because of malignant pericardial effusion. No other severe complications were encountered.

DISCUSSION

Complete surgical resection is considered essential in the treatment of thymomas, even for advanced diseases and recurrences.[1–3] Nevertheless, 20 to 40% of patients who undergo surgery for thymoma receive incomplete resection or biopsy alone.[1–3] Moreover, at the initial staging, some lesions are regarded as unresectable; these are usually advanced stage III or stage IV diseases, which are treated with chemotherapy and/or radiotherapy.[6–9]

We originated this aggressive multimodality therapy in February 1988 to improve the survival of patients with advanced or recurrent thymoma. In our study, eligible patients were limited to those with stage III lesions with great vessel invasion, stage IV lesions, or recurrences, because those tumors are not usually manageable by surgery and radiotherapy and are associated with unsatisfactory outcomes.[1–5] Our original chemotherapy regimen for invasive thymoma was designed from single-agent responsiveness for thymoma, which showed that cisplatin, doxorubicin, and corticosteroids had been the most active drugs.[20] Chemotherapy was not only administered in a neoadjuvant setting but also in a postsurgical adjuvant setting, because the initial stagings have not always been accurately estimated, even with CT and magnetic resonance imaging.

Neoadjuvant chemotherapy for invasive thymoma has been attempted in the treatment of locally advanced diseases because of the effectiveness of combination chemotherapy.[10–15] The chemotherapy regimens administered have been diverse, but almost all have included cisplatin and doxorubicin/epirubicin. The reported response rates have been documented to be 69 to 100%, and some patients receiving the treatment have had complete histologic remission. After induction chemotherapy for advanced tumors, the complete resection rates were around 70%. Of patients receiving the multimodality therapy using induction chemotherapy for locally advanced invasive thymoma, 5-year overall survival rates were reported to be between 55 and 95%,[13–15] because the study populations and treatment strategies were different.

In our 14 patients with neoadjuvant therapy, the response rate of CAMP therapy was 92.9%, which was better than or comparable with those of previous reports.[6–15] However, only two patients underwent complete resection, and seven underwent incomplete resection. The other tumors were interpreted as being unresectable after induction chemotherapy. Even after postsurgical radiotherapy, four patients without complete resection remained in PR, and two of them had a short survival. Our low complete resection rate is considered to be a result of the far advancement of the tumors: 13 of 17 patients had stage IV disease and/or recurrent tumors. Furthermore, CT was still incapable of predicting the possibility of performing a radical excision of the tumors after induction chemotherapy.

Patients undergoing incomplete resection or biopsy have been reported to show a significantly shorter survival than those with complete resection.[1–3] Blumberg et al.[2] reported that survival rates in patients with partial resection had been documented at 70 and 28% for 5 and 10 years, and 38 and 24% for biopsy, respectively. All three of our patients who had stage IV disease and were treated with surgery and then adjuvant chemotherapy with or without radiotherapy had distinct residual tumors after the operation. After the adjuvant therapy, two patients had pleural recurrences, but only after disease-free intervals of more than 5 and 2 years, respectively. In the remaining patient, postoperative CAMP therapy

and irradiation have managed the residual disease for more than 10 years. From our available data of those patients with the adjuvant therapy, we think that aggressive postsurgical treatment including chemotherapy is useful to cure or control residual lesions in patients with incomplete resection of the primary tumors, effectively maintaining their quality of life for a longer period.

In the multimodality therapy, some complications were noted. With chemotherapy, fatal infection and tumor lysis syndrome were observed in peculiar patients with parathymic syndrome of hypogammaglobulinemia and extensive lymphocytic thymoma associated with peripheral blood T-cell lymphocytosis,[18] respectively. No mortality was encountered in surgical treatment. After radiation therapy, mild cardiac dysfunction was observed in two patients who had whole mediastinal irradiation for malignant pericardial effusion.[19] This complication is probably caused by doxorubicin and radiation affecting the heart muscle synergistically. On the whole, we think that this multimodality therapy is tolerable as long as attention is paid to any peculiar conditions.

For the recurrent tumors in six patients exhibiting CR, we aggressively performed retreatment. Extrapleural pneumonectomy or partial pleurectomy was carried out in three patients with pleural recurrences, pulmonary metastasectomy was carried out in one patient who was in a postpneumonectomy state, and repetitive radiotherapy was carried out in two patients with mediastinal or diaphragmatic local recurrences. All six patients are still in good general condition 37 to 193 months after the initial treatment. From our experience, we consider that aggressive retreatment for recurrences even after the multimodality therapy is very important for controlling disease and maintaining good quality of life, as previous reports have also advocated.[21,22]

The treatment of advanced thymoma is still controversial. However, investigators have recently advocated the necessity of multimodal approaches to therapy that introduce the enhancement of tumor resectability, cure rate, and/or long-term disease control.[10–15] In studies of such multidisciplinary treatment, Shin et al.[12] and Kim et al.[15] have reported excellent results in the survival of patients with stage III or IV thymoma. Their study protocol was considered a precise long-term treatment, which consisted of induction chemotherapy (cisplatin, doxorubicin, cyclophosphamide, and prednisone), surgical resection, postoperative radiotherapy, and consolidation chemotherapy. From our study, we also recognize the importance of postsurgical adjuvant therapy for patients with advanced disease and/or incomplete resection as well as the importance of retreatment for recurrences after the multimodality therapy. Future studies on the treatment of advanced invasive thymoma should follow a meticulous scheme of a primary multidisciplinary approach to therapy and retreatment of recurrences.

In conclusion, CAMP therapy was highly effective for invasive thymomas. Although this study was limited by its small number of patients and its nonrandomized clinical trial design, we believe that the multimodality therapy containing this chemotherapy is justifiable for the initial treatment of patients with advanced thymoma such as stage III disease with major vessel invasion, stage IV disease, and recurrence. Further studies are warranted to determine the optimal treatment strategy.

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ORIGINAL ARTICLE

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Oxaliplatin/fluorouracil/leucovorin (FOLFOX4 and modified FOLFOX6) in patients with refractory or advanced colorectal cancer: post-approval Japanese population experience

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Abstract
Background. The oxaliplatin/fluorouracil/leucovorin (FOLFOX regimen) is an effective and generally well-tolerated regimen in Western clinical studies of advanced colorectal cancer. In Japan, oxaliplatin was approved in April 2005.
Methods. To evaluate the objective tumor responses and feasibility (toxicities) of FOLFOX regimens (FOLFOX4 and modified FOLFOX6, mFOLFOX6) in a predominantly Japanese population with refractory or advanced colorectal cancer in Japan, 51 consecutive patients with histologically confirmed metastatic colon or rectum cancer who were treated between April 2005 and March 2006 were enrolled in a retrospective study. FOLFOX4 was used for treatment in 39% (first-line, 45%) of these patients, and mFOLFOX6 was used for treatment in 61% (first-line, 61%). Tumor responses were assessed radiologically, and toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 regarding toxicities other than peripheral sensory neuropathy.
Results. The objective response rates (in those who underwent first- or second-line therapy) were 50.0% and 8.7%, respectively. The tumor control rate (partial response [PR] + stable disease [SD]) was 80.4%. There were no toxicity-related deaths. Neutropenia grade 3 was experienced in 20% of patients, and often caused delay in the subsequent treatment course. Mild to moderate cumulative peripheral sensory neuropathy affected 78% of patients. The incidence of hypersensitivity reactions to oxaliplatin in our study was lower than that in reported in Western countries.
Conclusion. Both FOLFOX regimens have good efficacy in refractory or advanced colorectal cancer in a Japanese population, with an acceptable overall toxicity profile.

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Key words Oxaliplatin · FOLFOX · Colorectal cancer · Japanese population

Introduction

In 2000, it has reported that colorectal cancer (CRC) was diagnosed in more than 90000 patients per year in Japan, resulting in 36000 deaths per year.

Colorectal cancer accounts for 10% to 15% of all cancers and is the third leading cause of cancer-related death in Western countries. Approximately one-half of all patients develop metastatic disease. The prognosis for these patients is poor, although palliative chemotherapy has been shown to be able to prolong survival and improve the quality of life over best supportive care. For many years, the treatment of metastatic colorectal cancer was restricted to 5-fluorouracil (5FU) and the biomodulation of this agent.[1] Oxaliplatin and irinotecan, combined with continuous infusion of 5FU, significantly improved response rate, progression-free survival (PFS), and overall survival.[2–4] FOLFOX4 (oxaliplatin and leucovorin [LV] 5FU2) is more active than LV5FU2 alone, and has also shown superiority over IFL (irinotecan, FU bolus, leucovorin). Oxaliplatin (L-OHP), a new third-generation 1,2-DACH-platinum derivative, has a mechanism of action similar to that of other platinum derivatives.[5–9] However, its spectrum of antitumor activity in tumor models differs from those of cisplatin and carboplatin. In addition, it has also been observed to demonstrate activity against cisplatin-resistant colon carcinoma cell lines.[10] In addition, experimental data have shown synergistic activity of the oxaliplatin/FU combination. The clinical toxicity of oxaliplatin is also distinct from that of other platinum drugs: it has no renal toxicity and minimal hematotoxicity; it causes both a reversible acute, cold-related dysesthesia and a dose-limiting cumulative peripheral sensory neuropathy that usually regresses rapidly after treatment withdrawal. The recent availability of five active chemotherapeutic agents has doubled the median overall survival for metastatic CRC from 10 to 20 months.

Three combinations have shown excellent first-line efficacy in phase III trials – IFL with bevacizumab, FOLFOX, and FOLFIRI – however, neither of these combinations is clearly superior. Our clinical practice in Japan has been guided in a major way by extrapolation from the results of clinical trials conducted mainly in Western countries. To evaluate the value of FOLFOX regimens in the treatment of refractory or advanced CRC, a retrospective analysis study was designed to assess the feasibility (toxicities) and efficacy of combining oxaliplatin with the LV5FU2 schedule in a Japanese population. We herein report our experience with two FOLFOX regimens (FOLFOX4 and modified [m] FOLFOX6) in patients with advanced CRC, specifically, the toxicities and objective tumor response rates obtained.

Patients and methods

A retrospective analysis study was conducted at Kinki University Hospital in 51 consecutive patients with histologically confirmed metastatic colon or rectum cancer who were treated between April 2005 and March 2006. The primary objectives were to assess the feasibility (toxicities) and efficacy of two FOLFOX regimens (FOLFOX4 and mFOLFOX6) in a Japanese population. FOLFOX4 is a regimen comprising oxaliplatin 85 mg/m^2 as a 2-h infusion (day 1); LV 100/m^2 per day as a 2-h infusion (days 1 and 2); followed by a 5FU bolus 400 mg/m^2 per day and 5FU 600 mg/m^2 per day as a 22-h infusion (days 1 and 2). mFOLFOX6 is a regimen also comprising oxaliplatin 85 mg/m^2 as a 2-h infusion (day 1), LV 200 mg/m^{2}per day as a 2-h infusion (day 1), followed by a 5FU bolus 400 mg/m^2 (day 1) and 5FU 2400 mg/m^2 per day as a 46-h infusion (days 1 to 2). These therapies were administered on day 1 and repeated on day 2 of a 14-day treatment cycle. Routine antiemetic prophylaxis with a serotonin (5-HT3) antagonist (granisetron) and dexamethasone was given (Fig. 1). The use of implantable ports and infusion pumps allowed chemotherapy to be administered on an outpatient basis in some cases. Treatment was continued until either disease progression, the occurrence of unacceptable toxicity, or the patient refused further treatment. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 regarding toxicities other than peripheral sensory neuropathy and by following the oxaliplatin-specific scale (DEB-NTC). The definitions in the oxaliplatin-specific scale, which was developed as a specific scoring scale for oxaliplatin-inducing peripheral sensory neuropathy, are as follows: grade 1, transient dysesthesia and/or paresthesia lasting for less than 7 days; grade 2, transient dysesthesia and/or paresthesia lasting for 7 days or longer; and grade 3, dysesthesia and/or paresthesia with pain or function impairment that interferes with activities of daily living (such as difficulty with fastening buttons and writing). The response of measurable target lesions to treatment was objectively evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria after each four cycles of treatment. Complete response (CR) was defined as the disappearance of all disease. Partial response (PR) was defined as at least a 30% reduction in the sum of the longest diameters of all measured lesions by at least 4 weeks. Progressive disease (PD) was defined as an increase in lesions by 20%

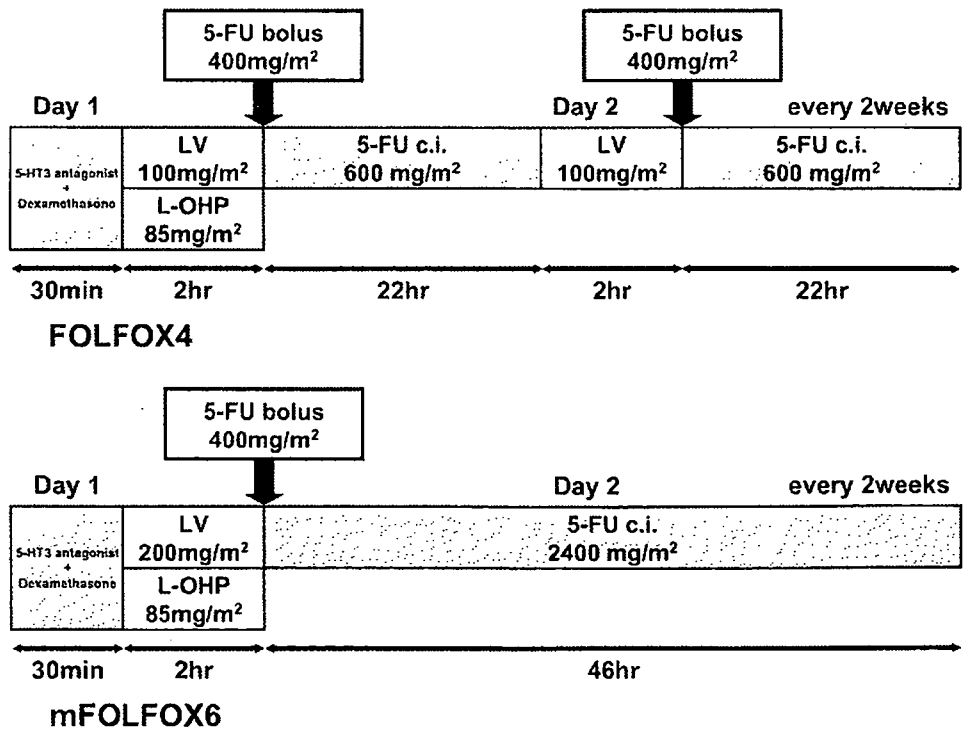

Fig. 1. Treatment schema for FOLFOX4 and mFOLFOX6 regimens. *LV*, leucovorin; *5-FU*, 5-fluorouracil; *L-OHP*, oxaliplatin; *c.i.*, continuous infusion

or greater, or the appearance of new lesions. Responses not falling into any of these categories were classified as stable disease (SD).

Results

Patient characteristics

A total of 51 patients with a median age of 61 years (range, 34–78 years) with refractory or advanced CRC were retrospectively analyzed. The patients' characteristics are listed in Table 1. Patients were treated with the FOLFOX4 (39%) or mFOLFOX6 regimens (61%). Twenty-eight patients (55%; FOLFOX4, 9; mFOLFOX6, 19) were treated in a first-line setting, and 23 patients (45%; FOLFOX4, 11; mFOLFOX6, 12) were treated in a second-line setting. Since April 2005, 4 months prior to beginning this study, we have used the FOLFOX4 regimen for inpatients, and from that time we selected the mFOLFOX6 regimen for all patients in the outpatient setting. The total number of chemotherapy cycles administered was 384, with a median of 8 cycles per patient (range, 1–12 cycles). The median dose intensity (actual/planned dose) was 93.4% for oxaliplatin and 100% for 5FU in the FOLFOX4 group and 91.2% for oxaliplatin and 94.8% for 5FU in the mFOLFOX6 group. The median dose intensity of oxaliplatin was 37 mg/m^2 per week (range, 31–42 mg/m^2 per week) in the FOLFOX4 group and 34 mg/m^2 per week (range, 23–42 mg/m^2 per week) in the mFOLFOX6 group.

Hematological toxicity

Several pertinent hematological toxicities are listed in Table 2, shown with numbers of patients who experienced them. The onset of neutropenia typically occurred between 10 and 14 days after treatment. Grade 3 and 4 neutropenia was observed in 20% of patients, with neutropenic fever being uncommon. Neutropenia often caused delay in the start of a subsequent treatment course. In all, 88 (23%) of 384 cycles were delayed due to toxicity, most commonly hematological: 64 (17%) for neutropenia and 24 (6%) for neurotoxicity. Using our administration schedule, no thrombocytopenia of over grade 3 was observed to develop. In addition, only one patient developed grade 3 anemia with transfusion.

Nonhematological toxicity

The most common nonhematological adverse effects of the FOLFOX regimens were peripheral neuropathy and lethargy (fatigue). These effects are listed in Table 3. Two patients experienced grade 2 hypersensitivity reactions (rash/hives, erythema, and one patient also experienced vomiting) during the administration of oxaliplatin. The symptoms rapidly resolved, in a few minutes, on symptomatic treatment (termination of infusion, use of steroids, and antagonists of type 1 and 2 histamine receptors). In using successful strategies over the next treatment courses (slowing the infusion rate, increasing the doses of steroids, and dose reduction of oxaliplatin), both patients were able to tolerate rechallenge of oxaliplatin, and one patient achieved a partial response. Peripheral neurotoxicity, characterized by paresthesia in a symmetric, glove-and-stocking distribution, occurred in 40 (78%) patients and there was no grade over 3.

Whenever the number of treatment cycles increases, neuropathy, within grade 2 level, tends to increase. The incidence of neurotoxicity along with the number of treatment cycles is listed in Table 4. Cold-related dysesthesia

Table 1. Characteristics of the study patients

Characteristics	$n = 51$
Sex, male/female	28/23
Age, years, median (range)	61 (34–78)
Performance status (ECOG), 0/1/2	19/25/7
Primary tumor, colon/rectum/rectosigmoid	28/20/3
Adjuvant therapy, +/–	18/33
Previous irinotecan therapy, +/–	15/36
Site of metastases, lung/liver/LN/peritoneum	22/21/18/7
FOLFOX4/mFOLFOX6	20/31
First-line/second-line	28/23
Dose reduction: +/–	7/44

ECOG, Eastern Cooperative Oncology Group; LN, distant lymph nodes

Table 2. Hematological toxicity (CTCAE V3.0)

	$n = 51$					
	Grade 1	Grade 2	Grade 3	Grade 4	All grades	Grade ≧3
Leucocytopenia	17	16	1	0	67%	2%
Neutropenia	18	15	9	1	84%	20%
Anemia	19	8	1	0	55%	2%
Thrombocytopenia	23	5	0	0	55%	0%

Table 3. Nonhematological toxicity (CTCAE V3.0)[a]

	$n = 51$					
	Grade 1	Grade 2	Grade 3	Grade 4	All grades	Grade ≧3
Anorexia	16	2	2	0	39%	4%
Nausea	13	2	2	0	33%	4%
Vomiting	5	2	2	0	18%	4%
Mucositis	10	1	2	0	25%	4%
Febrile neutropenia	–	–	0	0	0%	–
Hand-foot syndrome	2	0	0	0	4%	–
Pigmentation	4	0	–	–	7%	–
Allergy	0	2	–	–	4%	–
Lethargy	13	4	0	0	33%	–
AST/ALT elevation	22	2	0	0	47%	–
Diarrhea	8	2	2	0	23%	4%
Sensory neuropathy	31	9	0	0	78%	–

[a]Other than sensory neuropathy

Table 4. Incidence of neurotoxicity in FOLFOX regimens

	1–4 Cycles ($n = 9$)			5–8 Cycles ($n = 28$)			9–12 Cycles ($n = 14$)			All cycles ($n = 51$)		
Grade	1	2	3	1	2	3	1	2	3	1	2	3
Sensory neuropathy	5	0	0	19	2	0	7	7	0	31	9	0
	56%	0%	0%	68%	7%	0%	50%	50%	0%	60%	18%	0%

Grade	The oxaliplatin-specific scale (DEB-NTC)			
	0	1	2	3
Dysesthesia and/or paresthesia	No abnormality	Transient dysesthesia and/or paresthesia lasting less than 7 days	Transient dysesthesia and/or paresthesia lasting 7 days or more	Dysesthesia and/or paresthesia with pain or function impairment that interferes with activities of daily living

was reported in 31 patients (61%). Paresthesia lasting 7 days or longer (grade 2) occurred in 9 patients (18%). Peripheral neuropathy appeared in two forms. In the first form, an acute, transient, cold-exacerbated dysesthesia or paresthesia occurred shortly after the administration of oxaliplatin; it affected the hands, feet, perioral area, and throat; and typically lasted for several days after drug administration. In the second form, a delayed-onset, cumulative, dose-related peripheral neuropathy was characterized by paresthesias affecting the hands and feet that did not remit between cycles of treatment. Investigators also reported pharyngolaryngeal dysesthesia in only one patient; however, no patients had a laryngospasm-like syndrome.

Overall, 7 of the 51 patients (14%) required dose modification during treatment; dose reduction was required for oxaliplatin alone in 4 patients, for 5FU alone in 2 patients and for both agents in 1 patient. The majority of dose reductions were by one level (reduction to 65 mg/m^2 oxaliplatin and/or 75% of the starting dose of 5FU). No patients required a second-level dose reduction. The adverse events most commonly leading to dose reduction were neurotoxicity (1 patient in FOLFOX4 and 3 patients in mFOLFOX6) and diarrhea (2 patients in mFOLFOX6). In addition, 2 patients in the mFOLFOX6 setting underwent a dose reduction of oxaliplatin due to allergic reaction. The most common reason for treatment discontinuation was PD.

Antitumor activity

All 51 patients were able to be evaluated for response. Objective responses are listed in Table 5 and Table 6. There was no complete response. The overall objective response rates (in those who underwent first-line or second-line therapy) were 50.0% and 8.7%, respectively (Table 6). Stable disease was achieved in 49% of patients. The tumor control rate (PR + SD) was 80.4%.

Discussion

The recent advent of several new agents for the treatment of metastatic CRC has markedly enhanced the therapeutic armamentarium for this disease. Oxaliplatin in combination with infusional 5FU in the FOLFOX regimens has been shown to be effective in achieving improved response rate, time to progression, and survival time compared with 5FU/LV. In addition, recent large clinical phase III studies (N9741, EFC4584, GERCOR) showed that combination chemotherapy regimens, including irinotecan and oxaliplatin, markedly improved response rates and prolonged median survival over those seen with 5FU/LV),[11–13] and these combination chemotherapy regimens have supplanted 5FU/LV as a standard systemic approach for metastatic CRC. The median survival time (MST) has been gradually pro-

Table 5. Objective responses – (1)

FOLFOX4 (n = 20)				
CR	PR	SD	PD	NE
0	5 (25%) First-line, 3; second-line, 2	12 (60%)	3 (15%)	0
mFOLFOX6 (n = 31)				
0	11 (35.5%) First-line, 11; second-line, 0	13 (41.9%)	7 (22.6%)	0

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable

Table 6. Objective responses – (2)

First-line (n = 28)				
CR	PR	SD	PD	NE
0	14 (50%) FOLFOX4, 3; mFOLFOX6, 11	11 (39.3%)	3 (10.7%)	0
Second-line (n = 23)				
0	2 (8.7%) FOLFOX4, 2	14 (60.9%)	7 (30.4%)	0

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable

longed through the use of 5FU/LV with irinotecan and oxaliplatin. Currently, with the addition of molecular targeted agents, an MST of over 20 months has been reported.

Since April 2005, and the approval of oxaliplatin in Japan, clinical practice in this country has been conducted in a major way by extrapolation from the results of clinical trials conducted mainly in large Western phase III studies. The results of the present retrospective study demonstrate the efficacy and feasibility of FOLFOX regimens (FOLFOX4 and mFOLFOX6) as treatment for patients with advanced CRC in the Japanese population, as has been shown in Western populations. In this retrospective analysis study in a Japanese population, neutropenia grade 3/4 occurred in 20% of the patients who were assigned to receive oxaliplatin, but it was nonfebrile, whereas grade 3/4 vomiting and mucositis affected only 4% of the patients, while diarrhea affected 4%.

Lethargy has been described as the most frequent adverse event of the mFOLFOX6 regimen in a recent report by Braun et al.[14] In our study, 17 (33%) patients experienced lethargy similar to general fatigue symptoms.

The cumulative dose-limiting toxicity of oxaliplatin is peripheral sensory neuropathy, which reportedly occurs in about 70%–80% of patients: it typically resolves a few months after discontinuation of treatment, and may be exacerbated by cold stimulation. In our series, paresthesia lasting 7 days or longer was observed in 18% of patients and led to an oxaliplatin dose reduction for four patients after they had received a minimum of seven cycles (or at least 4 months) of chemotherapy.

The mechanism of this neurotoxicity has been elucidated to be as follows: the increased neuronal excitability is due to the action of oxaliplatin on voltage-gated sodium channels through the chelation of calcium by the oxaliplatin metabolite. The prevention of this neurotoxicity is a major goal, taking in to account the wide indications of this drug. Various different approaches have been either previously studied or are now being evaluated, based on pathogenic or practical concepts: (1) modification of the administration schedule; (2) substances acting upon sodium channels, such as calcium-magnesium, carbamazepine, gabapentine, venlafaxine; (3) detoxifying agents and antioxidants, such as glutathione, amifostine, alphalipoic acid, tocopherol; (4) substances used in other kinds of neuropathy, such as glutamine and alphalipoic acid; (5) neurotrophic factors, such as nerve growth factor (NGF), LIF; and (6) oxaliplatin analogs, with a DACH platin, without oxalate. Calcium-magnesium infusion appears to be an efficient and safe approach.

In this study, after September 2005, 32 patients (63%) were administered calcium-magnesium infusion for the prevention of the oxaliplatin-related neurotoxicity. Further studies are necessary for a better understanding and prevention of this potentially severe neurotoxicity.

In terms of antitumor activity, although the response rate (RR) in our population was slightly lower in comparison to that in previous Western clinical studies,[11–13] both of the oxaliplatin-based regimens demonstrated a promising objective RR in the first-line setting (50.0%) and in the tumor control rate (80.4%).

In a GERCOR study, the median survival was 21.5 months in 109 patients allocated to FOLFIRI then FOLFOX6 versus 20.6 months in 111 patients allocated to FOLFOX6 then FOLFIRI (P = 0.99). In first-line therapy, FOLFIRI achieved a 56% RR and 8.5-month median PFS, versus FOLFOX6, which achieved a 54% RR and 8.0-month median PFS (P = 0.26). Second-line FOLFIRI achieved a 4% RR and 2.5-month median PFS, versus

FOLFOX6, which achieved a 15% RR and 4.2-month PFS.[13] Although our study could not evaluate enough data for PFS and MST due to the short observation period after the approval of oxaliplatin in Japan, both the FOLFOX regimens we used seem to be beneficial as first-line and second-line therapy for refractory or advanced CRC in a Japanese population, with an overall response rate which is comparable to Western figures regarding first-line and second-line therapy. FOLFOX6 is the most useful of the FOLFOX regimens because it is simple and can be administered on an outpatient basis. When we use oxaliplatin in FOLFOX regimens, because 85 mg/m^2 is the approved dose for usage in Japan, the treatment is adapted for this dose, even in the mFOLFOX6 regimen.

In conclusion, the FOLFOX regimens we used were found to demonstrate good efficacy as chemotherapy regimens in our population, with an acceptable overall toxicity profile. However, attention must be paid to the occurrence of peripheral sensory neuropathy, which may influence a patient's quality of life, while also limiting the continuation of such treatment.

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perspectives Journal of proteome research

Personalized Medicine and Proteomics: Lessons from Non-Small Cell Lung Cancer

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Personalized medicine allows the selection of treatments best suited to an individual patient and disease phenotype. To implement personalized medicine, effective tests predictive of response to treatment or susceptibility to adverse events are needed, and to develop a personalized medicine test, both high quality samples and reliable data are required. We review key features of state-of-the-art proteomic profiling and introduce further analytic developments to build a proteomic toolkit for use in personalized medicine approaches. The combination of novel analytical approaches in proteomic data generation, alignment and comparison permit translation of identified biomarkers into practical assays. We further propose an expanded statistical analysis to understand the sources of variability between individuals in terms of both protein expression and clinical variables and utilize this understanding in a predictive test.

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Introduction

A personalized medicine approach uses appropriate biomarkers to select treatments best suited for an individual patient and disease phenotype. A multiple biomarker approach (e.g., proteomics) has the advantage over conventional single biomarkers of combining many different pieces of information. Here, we review the key features of state-of-the-art proteomic profiling and introduce recent analytic developments to build a proteomic toolkit for use in personalized medicine, and we describe how these may be applied in a viable method for exploiting predictive proteomic fingerprints in the clinic. The potential of our proteomics toolkit hopefully brings us one step closer to a practical personalized medicine.

Cancer therapy is moving toward individually selected treatments, chosen not only according to tumor cell type but also based on the patient's predicted responsiveness to different classes of therapy or susceptibility to therapeutic adverse events. This emerging personalized medicine approach draws on both genotype and phenotype information, including protein expression. To implement personalized medicine, we need to develop effective biomarker tests predictive of response to treatment or susceptibility to adverse events. The benefits of personalized medicine are exemplified by considering interstitial lung disease (ILD) among non-small cell lung cancer (NSCLC) patients, which is associated with various kinds of chemotherapy treatment. A personalized medicine approach, using a simple blood test to predict those NSCLC patients at risk of developing ILD, would clearly be of great value.

We review current thinking and present some novel developments in a number of areas that have to be integrated to develop and then practically apply such tests in a clinical setting:

- The large scale collection of reliable and high quality phenotypic and clinical data and blood samples.
- Protein analysis in blood.
- Data acquisition, handling, combining and analysis.
- Interpretation and utilization of results in a clinical setting.

Clinical Background

A Motivating Example: Gefitinib (IRESSA) Treatment of NSCLC. The concepts of proteomics-based personalized medicine discussed in this article are very generally applicable. A motivating example that we will refer to in order to illustrate the potential benefits of personalized medicine is ongoing work in attempting to develop a simple blood test to address the potential occurrence of ILD in seriously ill NSCLC patients, the target group for the NSCLC treatment gefitinib.

Gefitinib is a "small molecule" inhibitor of the enzyme tyrosine kinase of the epidermal growth factor receptor (EGFR) family, such as erbB1. It is an approved therapy for advanced NSCLC in many countries and offers important clinical benefits (tumor shrinkage and improvement in disease-related symptoms) for "end-stage" patients. The large phase III ISEL (IRESSA Survival Evaluation in Lung Cancer) trial demonstrated some improvement in survival with gefitinib which failed to reach statistical significance compared with placebo in the overall population and in patients with adenocarcinoma.[1] However, in preplanned subgroup analyses, a significant increase in survival was shown with gefitinib in patients of Asian ethnicity and in patients who had never smoked.[1]

Analysis of the biomarker data from a subset of patients in the ISEL study suggested that patients with pretreated advanced NSCLC who have tumors with a high EGFR gene copy number (detected by fluorescent in situ hybridization [FISH]) have a higher likelihood of increased survival when treated with gefitinib compared with placebo.[2] Increased HER2 gene copy number has also been seen in tumors from patients who are responsive to gefitinib.[3] Somatic-activating mutations of EGFR in tumor tissue have also been associated with increased gefitinib responsiveness in patients with NSCLC.[4-7] Such mutations are more commonly found in tumor samples from patients of Asian origin and non-smokers.[8]

Following the ISEL subgroup analyses, and the biomarker evidence, it has become important to clarify which patients are more suitable for treatment with gefitinib. Analyses for both somatic-activating mutations and gene copy number require tumor tissue, which is not always available from the time of diagnosis; therefore, a blood test may represent a more versatile option and be of great value to clinicians.

With respect to tolerability, the search for a blood test that might include both genetic and proteomic biomarkers to define patients at risk of adverse effects from a drug, for example interstitial lung disease with gefitinib, is a focus of research.

Interstitial Lung Disease as a Complication in NSCLC Patients. ILD is a disease that afflicts the parenchyma or alveolar region of the lungs.[9] The alveolar septa (the walls of the alveoli) become thickened with fibrotic tissue. Associated with drug use, it can present precipitously with acute diffuse alveolar damage (DAD). The lungs show so-called "ground glass" shadowing on chest radiology, and patients complain of severe breathlessness. There are no effective treatments but patients can be supported by oxygen supplementation, corticosteroid therapy, or assisted ventilation. The process of alveolar damage is however fatal in some patients. ILD is a comorbidity in patients with NSCLC.[10-16] Both diseases are associated with cigarette smoking,[17-20] and ILD is also considered to be associated with various kinds of lung cancer chemotherapy.[21-26]

In the ISEL study of gefitinib in NSCLC mentioned above, ILD-type events occurred in 1% of both placebo and gefitinib-treated patients.[1] Most ILD-type events occurred in patients of Asian origin, where placebo and treated patients had similar prevalences of respectively 4% and 3%. The rate observed in the gefitinib-treated arm was in line with earlier safety data from Japan and a large gefitinib post-marketing surveillance study in Japan (3322 patients), where the reported rate of ILD-type events was 5.8%.[27]

A simple blood test to predict the potential occurrence of ILD in seriously ill NSCLC patients before initiating treatments would clearly be of great value. This article describes the personalized medicine approach, which could be used to provide such a test. Consequently, the proteomics objectives of the preliminary phase of the study we describe were to verify the protein expression alterations in blood plasma from case patients (who developed ILD) and control patients (without ILD) treated by gefitinib, using a liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) proteomics platform.

Data and Sample Collection

To develop a personalized medicine test, it is essential to have access to an adequately sized collection of high quality tissue samples on which to perform proteomics analysis, with corresponding reliable diagnostic and clinical data.