ɔgressive disease was defined as development of new-lesions or 25% increase in the sum of the products of the two largest :pendicular diameters of all measurable tumors. Duration of ponse in patients who achieved complete or partial response s measured from the start of treatment to the date of disease ɔgression.

ıtistical methods

ɜults of this study were evaluated to determine whether the ɛetaxel plus irinotecan combination warranted further assess-nt in a phase III trial. Thus, this study was designed to conduct ɔ randomised phase II studies concurrently. We calculated the mber of patients required for each of the two studies based on Fleming's single-stage procedure (Fleming, 1982). In both dies, we set response rates of 40% as target activity level and % as the lowest level of interest with a power of 0.9 at a one-ed significance level of 0.05. As a result, a total of 100 qualified ients were to be enrolled, with 50 patients in each treatment n. The primary objective was to estimate the response rate to h regimens, particularly to irinotecan plus docetaxel.
Overall survival and progression-free survival were analysed by Kaplan–Meier method. The overall survival was measured m study entry to death. The progression-free survival was asured from study entry until the day of the first evidence of ease progression. If the disease had not progressed by the time this analysis, progression-free survival was considered censored the time of the analysis. All comparisons between patient ıracteristics, response rates, and toxicity incidences were formed by Pearson's χ^2 contingency table analysis.

SULTS

tient characteristics

ɔm October 1998 to August 1999, 108 patients were assigned to ɛive DC ($n=51$) or DI ($n=57$). Baseline patient characteristics ording to treatment arm are shown in Table 2. Patients were ll balanced between the two treatment arms in terms of gender, , performance status, disease stage, and histologic subtypes. ɛre were 23% stage IIIb patients and 74% had adenocarcinoma. patients were included in the survival evaluation, and all were essable for antitumor efficacy and toxicity.

eatment delivery

ients in both treatment arms received a median of two ıtment courses. Two or more courses were delivered to 72.5 71.9%, and four courses to 17.6 and 19.1% of patients in the DC and DI arms, respectively. Differences between arms in the number of chemotherapy courses administered were not statistically significant.

Response to treatment and survival

There were no complete responses. In the DC arm, 19 patients had partial responses for an overall response rate of 37% (Table 3). Among DI patients, 18 had partial responses for an overall response rate of 32%. The difference in response rate between arms was not significant ($P=0.55$). Progressive disease was noted in twice as many DI (25%) than DC (12%) patients. Early deaths within 3 months of treatment initiation occurred in 10% ($n=5$) of DC and 5% ($n=3$) of DI patients. The early deaths were treatment-related (three patients, all in the DC arm) or due to disease progression (five patients).

Overall and progression-free survival curves for the two treatment arms are shown in Figures 2 and 3. The median progression-free survival time was 20 weeks (95% confidence interval: 14–25 weeks) in the DC arm *vs* 18 weeks (95% confidence interval: 12–22 weeks) in the DI arm. Median survival times, 1-year survival rates, and 2-year survival rates were 50 weeks (95% confidence interval 34–78 weeks), 47 and 25%, respectively, in the DC arm, and 46 weeks (95% confidence interval: 37–54 weeks), 40 and 18%, respectively, in the DI arm. No significant differences were noted between groups in progression-free survival ($P=0.33$) or overall survival ($P=0.50$), although there were trends toward higher 1-year and 2-year survival rates in the DC.

ble 2 Baseline patient characteristics

		Docetaxel/ cisplatin	Docetaxel/ irinotecan	χ^2 text
of patients		51	57	
ıder	Male/female	37/14	38/19	$P=0.537$
(years)	Median	62	60	
	Range	39–74	42–77	
	0/1	15/36	15/42	$P=0.830$
ology	Adenocarcinoma	36	44	$P=0.520$
	Squamous cell carcinoma	13	9	
	Others	2	4	
ease stage	IIIb/IV	11/40	14/43	$P=0.820$
n metastasis	(+)/(−)	4/47	11/46	$P=0.086$

= performance status.

Table 3 Overall response to docetaxel/cisplatin (DC) or docetaxel/ irinotecan (DI) in patients with stages IIIb/IV non-small-cell lung cancer

Response	DC ($n=51$) No. pts	DI ($n=67$) No. pts
Complete response	0	0
Partial response	19	18
No change	23	25
Progressive disease	6	14
NE (TRD)	3	0
Response rate	37.3%*	31.6%*
95% Confidence intervals	24.1–51.9%	19.9–45.2%

pts = patients; NE = not evaluable; TRD = treatment-related death. *$P=0.55$.

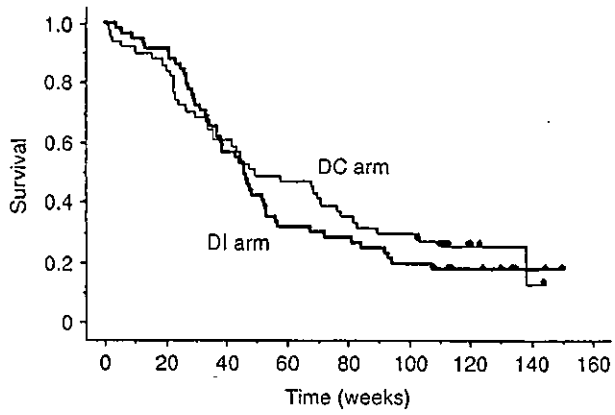

Figure 2 Overall survival according to treatment group, calculated by Kaplan–Meier method. Median survival times were 50 weeks for DC (docetaxel plus cisplatin) and 46 weeks for DI (docetaxel plus irinotecan). $P=0.50$ between treatment groups.

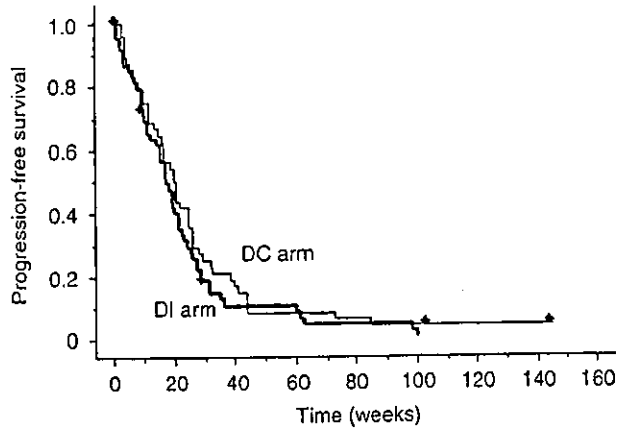

Figure 3 Progression-free survival according to treatment group, calculated by Kaplan–Meier method. Median progression-free survival times were 20 weeks for DC (docetaxel plus cisplatin) and 18 weeks for DI (docetaxel plus irinotecan). $P=0.33$ between treatment groups.

Table 4 Haematologic toxicity: maximum toxicity grade in any course

	Docetaxel/ cisplatin (% pts)			Docetaxel/ irinotecan (% pts)		
Toxicity/grade	**2**	**3**	**4**	**2**	**3**	**4**
Leucopenia*	31	43	4	26	40	16
Neutropenia*	10	31	43	4	23	61
Anaemia	47	10	2	46	7	0
Thrombocytopenia**	10	4	0	0	0	0
Febrile neutropenia		20			28	

pts = patients. *$P<0.01$ for grade 4; **$P<0.01$ for the sum of grades 2 and 3.

Second-line chemotherapy was administered to 61 patients (24 DC and 37 DI patients). A total of 22 patients in the DI group received cisplatin-based second-line chemotherapy and five had partial responses to this treatment (overall response rate, 23%). In particular, nine patients were subsequently treated with vinorelbine containing regimen and three patients had a partial response. Only two patients in the DC group received an irinotecan-containing regimen, one of whom had a partial response. Concerning as second-line chest irradiation, 8 patients in the DC group and 13 patients the DI group received.

Toxicity

Haematologic and nonhaematologic toxicities are listed in Tables 4 and 5. Grade 4 leucopenia and neutropenia occurred in a significantly higher percentage of DI than DC patients (leucopenia 16 *vs* 4%, $P<0.01$; neutropenia 61 *vs* 43%, $P<0.01$). On the other hand, there was a higher rate of grade $\geqslant 2$ thrombocytopenia in the DC than in the DI arm (14 *vs* 0%, $P<0.01$). Rates of anaemia (decrease in haemoglobin) and febrile neutropenia were similar in both groups.

Nonhaematologic toxicities including grade $\geqslant 2$ nausea (88 *vs* 51%, $P<0.01$), vomiting (39 *vs* 14%, $P<0.01$), and renal toxicity (increased serum creatinine; 12 *vs* 2%, $P<0.01$) were significantly more prevalent in the DC than in the DI arm, respectively. On the other hand, grade $\geqslant 2$ diarrhoea occurred significantly more often in DI than in DC patients (24 *vs* 42%, $P=0.01$). Other nonhaematologic toxicities, such as hepatic toxicity and peripheral neuropathy, were mild and occurred with similar frequency in both groups.

Table 5 Nonhaematologic toxicity: maximum toxicity grade in any course

	Docetaxel/ cisplatin (% pts)			Docetaxel/ irinotecan (% pts)		
Toxicity/grade	**2**	**3**	**4**	**2**	**3**	**4**
Diarrhoea*	18	6	0	26	12	4
Nausea*	53	33	0	33	18	0
Vomiting**	33	2	4	14	0	0
Peripheral neuropathy	2	0	0	2	0	0
AST increase	8	2	2	7	0	2
ALT increase	14	4	0	9	2	2
ALP increase	8	2	0	4	0	0
Creatinine increase*	10	0	2	0	0	2

pts = patients; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase. *$P<0.01$ for the sum of grades 2, 3, and 4; **$P=0.01$ for the sum of grades 2, 3, and 4.

There were three treatment-related deaths in the DC arm, which were due to febrile neutropenia and sepsis (one of these patients also developed perforation of the oesophagus). No treatment-related deaths occurred in the DI arm. The difference in incidence of treatment-related deaths was not significant.

DISCUSSION

Results of this randomised phase II study showed that the doublet chemotherapy regimens DC and DI had comparable activity in patients with advanced NSCLC. A primary goal of this study was to determine whether the DI combination should be studied in the phase III setting. Although there were no differences between DI and DC – a third-generation cisplatin-containing regimen – in overall and progression-free survival, patients who received DI tended to have lower 1-year and 2-year survival rates. Furthermore, overall toxicity was not reduced in the DI arm compared with the DC arm. Leucopenia and neutropenia were the major toxicities in both groups. As expected, emesis and renal toxicity were more prevalent in patients receiving DC, and diarrhoea occurred more frequently with DI.

Cisplatin has played a prominent role in the treatment of NSCLC, despite a relatively unimpressive single-agent response rate and a relatively severe toxicity profile. In 1995, the Non-Small Cell Lung Cancer Collaborative Group published a pivotal meta-analysis of chemotherapy in lung cancer and demonstrated the advantage of cisplatin-based regimens over best supportive care (Non-Small Cell Lung Cancer Collaborative Group, 1995). In the 1990s, third-generation chemotherapeutic agents, including paclitaxel, docetaxel, vinorelbine, gemcitabine and irinotecan, were shown to have higher response rates often coupled with fewer adverse effects (no renal toxicity, no massive dehydration, less emesis, etc.) than cisplatin. For example, single-agent paclitaxel (Ranson *et al*, 2000), docetaxel (Roszkowski *et al*, 2000), or vinorelbine (The Elderly Lung Cancer Vinorelbine Italian Study Group, 1999) significantly improved survival compared with best supportive care in patients with advanced NSCLC. Studies of single-agent gemcitabine (Perng *et al*, 1997) or irinotecan (Negoro *et al*, 2003) demonstrated a survival benefit comparable to that of second-generation chemotherapy regimens (cisplatin plus vindesine, cisplatin plus etoposide). Based on the above results, we thought that combination chemotherapy consisting of third-generation agents might improve outcome for patients with advanced NSCLC.

Only one published study compared cisplatin-based and noncisplatin-based regimens that included third-generation

agents. Georgoulias *et al* (2001) conducted a randomised study of cisplatin plus docetaxel (CD) *vs* gemcitabine plus docetaxel (GD) in 441 advanced NSCLC patients. The noncisplatin regimen provided a comparable response rate (CD 32.4%, GD 30.2%) and median survival time (CD 10 months, GD 9.5 months) but with less toxicity. The authors stated that the non-cisplatin GD regimen would likely be more acceptable to patients based on convenience of administration. However, several randomized trials reported at recent international meetings showed slightly shorter survival times with noncisplatin compared with cisplatin-based combinations. Preliminary results of the EORTC-Lung Cancer Group phase III study of cisplatin plus paclitaxel *vs* cisplatin plus gemcitabine *vs* paclitaxel plus gemcitabine in 480 patients with advanced NSCLC revealed superior overall survival and progression-free survival with the cisplatin-based regimens (Van Meerbeeck *et al*, 2001). Moreover, in a recent Italian–Canadian intergroup study of 501 patients comparing gemcitabine plus vinorelbine with cisplatin plus vinorelbine or gemcitabine, the noncisplatin regimen provided only short-term and sporadic advantages in some quality-of-life components, but there were no significant differences in overall and progression-free survival (Gridelli *et al*, 2002).

The best known noncisplatin platinum-based chemotherapy regimen is the paclitaxel plus carboplatin doublet. A Southwest Oncology Group study compared vinorelbine plus cisplatin with paclitaxel plus carboplatin. No differences in the overall survival or quality of life were noted between the two treatment groups, but toxicity rates were significantly lower in patients who received paclitaxel plus carboplatin (Chen *et al*, 2002). Results of a recent ECOG randomised phase III trial evaluating four platinum-based chemotherapy regimens showed no significant differences in the overall survival, while the paclitaxel plus carboplatin combination was less toxic than cisplatin-based chemotherapy (Schiller *et al*, 2002). Based on these findings, the paclitaxel plus carboplatin regimen is considered a standard therapy for previously untreated patients with advanced NSCLC, with activity comparable to that of cisplatin-based regimens and better tolerability.

The utility of doublet regimens containing third-generation chemotherapeutic agents for advanced NSCLC thus needs to be evaluated against the paclitaxel plus carboplatin combination, and several such studies were reported or are ongoing. The Hellenic Cooperative Oncology Group is conducting a phase III randomised study of paclitaxel plus carboplatin *vs* paclitaxel plus gemcitabine, and final results indicate comparable activity, toxicity and total cost of the two regimens in patients with inoperable NSCLC (Kosmidis *et al*, 2002). The Taiwan group conducted a similar study and found that paclitaxel plus carboplatin and paclitaxel plus gemcitabine had similar efficacy in the treatment of NSCLC, but that paclitaxel plus carboplatin was more cost-effective (Chen *et al*, 2002).

As mentioned in the introductory paragraphs, we conducted a phase I study of docetaxel plus irinotecan (DI) in patients with advanced NSCLC, and had a promising response rate of 48% and median survival time of 48 weeks (Masuda *et al*, 2000). Although we recommended docetaxel 50 mg m^{-2} on day 1 plus irinotecan 50 mg m^{-2} on days 1, 8, and 15 in the phase I study, more than half of patients could not receive irinotecan on day 15 because of haematologic toxicities. Accordingly, the day-15 irinotecan dose was omitted and the day-2 docetaxel dose moved to day 8 and increased from 50 to 60 mg m^{-2} in this randomised phase II trial.

It has been reported that second-line chemotherapy compared with best supportive care may increase the overall survival in patients with advanced NSCLC, and more studies in this regard are needed. In a recent study in which patients received cisplatin-based chemotherapy followed by docetaxel or supportive care alone, the median survival was significantly longer in the docetaxel-treated patients (Shepherd *et al*, 2000). In our study, 52% of patients were treated with second-line chemotherapy. Of these, 19 (33%) DI patients received cisplatin-based second-line chemotherapy, five of whom (26%) responded. Thus, cisplatin-based chemotherapy is capable of exerting antitumour activity in patients who have relapsed after having received noncisplatin-containing regimens.

Only two patients in the DC group received an irinotecan-containing regimen, one of whom had a partial response. As there were only two patients, we cannot judge whether irinotecan-containing regimen is effective for the patients after having received cisplatin-containing regimen.

In conclusion, docetaxel plus irinotecan combinations may be reasonable treatment options for NSCLC patients who cannot tolerate cisplatin. However, as there was no significant difference in the overall survival and no reduction in overall toxicity, DI has not improved on results obtained with DC. Thus, we will not select docetaxel/irinotecan as the experimental regimen in the next phase III study of first-line treatment of advanced NSCLC.

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Effect of re-treatment with gefitinib ('Iressa', ZD1839) after acquisition of resistance

A 70-year-old man with adenocarcinoma of the lung developed pulmonary metastases 7 months after middle and lower lobectomy of the right lung in October 1998. He received four courses of first-line chemotherapy with docetaxel/irinotecan from June to September 1999. The best response was stable disease and, after 6 months of treatment, there was evidence of progressive disease with increase in size and number of pulmonary metastases. Therefore, we recommended enrollment in a phase I study of gefitinib ('Iressa') [1], an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor.

The patient began to take gefitinib 700 mg/day in March 2000. Remarkable tumor regression was immediately achieved in April 2000 (Figure 1). This response lasted for 18 months. However, pulmonary metastases again developed (considered to be progressive disease), and gefitinib was discontinued in October 2001. The patient received a combination of nedaplatin, a second-generation platinum complex with high antitumor activity against non-small-cell lung cancer [2], and gemcitabine in November 2001. Significant tumor regression was achieved, and a total of six courses from November to April 2002 were administered. Pulmonary metastases progressed again and pulmonary effusion developed in August 2002. Although progressed, he had few symptoms, and was considered to have a performance status of 0. We planned to use a chemotherapy regimen that had not previously been used for this patient, but instead commenced re-treatment with gefitinib at the patient's request on September 3, 2002 (gefitinib 250 mg/day had by this time been approved for use in Japan). One month later, a significant response had been achieved (Figure 1).

This is an interesting case in which acquired resistance to gefitinib could be overcome. There are some possible explanations. First, resistance to gefitinib might naturally change over time, but there is no report of this so far. Secondly, because platinum-based cytotoxic chemotherapy was administered after the first treatment with gefitinib, the proportion of sensitive or resistant cells might have been modified. Thirdly, treatment with cytotoxic chemotherapy might produce genetic changes in EGFR or other unknown associated genes that regulate resistance to gefitinib. Saltz et al. reported that a combination of the EGFR inhibitor cetuximab (C225) and irinotecan produced a 22.5% partial response in patients with irinotecan-refractory colorectal cancer with high EGFR expression [3]. In contrast to that report, cytotoxic agents have the possibility of modifying resistance to cytostatic agents. Recently, two large phase III studies to compare concurrent use of conventional platinum-based chemotherapy (carboplatin/paclitaxel or cisplatin/gemcitabine) and gefitinib with conventional chemotherapy alone were reported [4, 5]. No differences in overall survival were found. These results suggested that gefitinib and chemotherapy may be targeting the same cells with the possibility of overlapping activity. If cytotoxic agents altered sensitivity to gefitinib by genetic modification, chemotherapy followed by gefitinib might be superior to concurrent use. Gefitinib is a very promising agent, but little knowledge is available concerning the types of cases for which gefitinib should be administered, or how gefitinib should be combined with conventional cytotoxic agents. Further investigations are needed to answer these questions.

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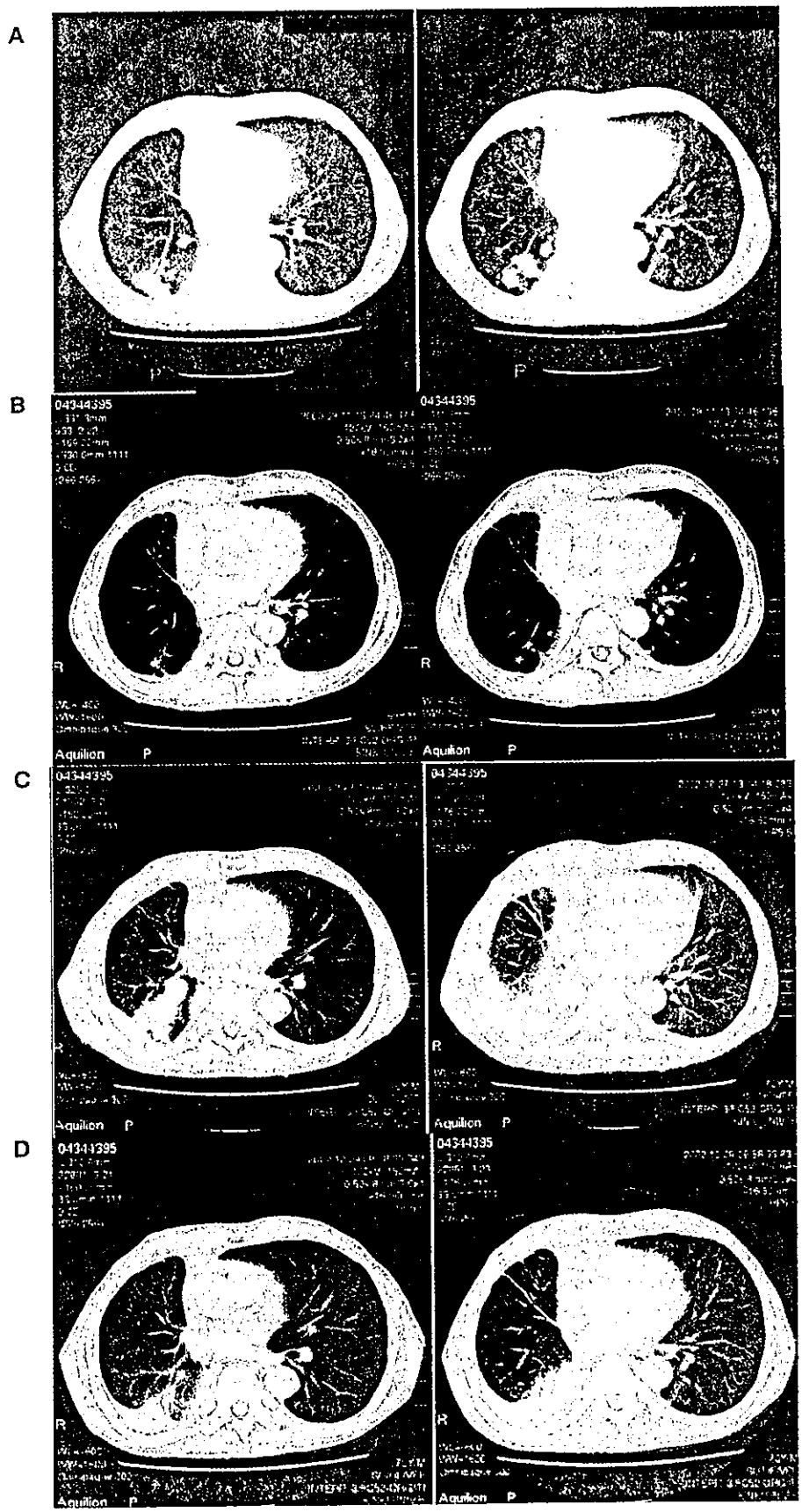

Figure 1. A 70-year-old man with adenocarcinoma of the lung. CT scan before treatment of gefitinib (A), after initiation of treatment (B), before re-treatment (C) and after initiation of re-treatment (D).

British Journal of Cancer (2004) 90, 2092–2096

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Combination phase I study of nedaplatin and gemcitabine for advanced non-small-cell lung cancer

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To establish the toxicities and maximum tolerated dose (MTD) of nedaplatin with gemcitabine, and to observe their antitumour activity, we conducted a combination phase I study in advanced non-small-cell lung cancer (NSCLC). Patients received nedaplatin (60–100 mg m^{-2} given intravenously over 90 min) on day 1, and gemcitabine (800–1000 mg m^{-2} given intravenously over 30 min) on days 1, 8, every 3 weeks. In total, 20 patients with locally advanced or metastatic NSCLC who received no prior chemotherapy or one previous chemotherapy regimen were enrolled. The most frequent toxicities were neutropenia and thrombocytopenia; nonhaematological toxicities were generally mild. Three out of six patients experienced dose-limiting toxicities (neutropenia, thrombocytopenia and delayed anaemia) at dose level 4, 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine, which was regarded as the MTD. There were three partial responses, for an overall response rate of 16.7%. The median survival time and 1-year survival rate were 9.1 months and 34.1%, respectively. This combination is well tolerated and active for advanced NSCLC. The recommended dose is 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine. This combination chemotherapy warrants a phase II study and further evaluation in prospective randomised trials with cisplatin- or carboplatin-based combinations as first-line chemotherapy for advanced NSCLC.

British Journal of Cancer (2004) **90,** 2092–2096. doi:10.1038/sj.bjc.6601817 www.bjcancer.com
Published online 20 April 2004

Keywords: combination phase I study; maximum tolerated dose; nedaplatin; gemcitabine; non-small-cell lung cancer

Based on the results of a meta-analysis (Non-Small Cell Lung Cancer Collaborative Group, 1995), cisplatin-based chemotherapy is considered the best available therapy for patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC). Although several new agents with novel mechanisms and significant activity against NSCLC have been introduced, such as taxanes, gemcitabine and vinorelbine, any of these agents used in combination with a platinum agent provide equivalent survival improvement (Kelly *et al*, 2001; Schiller *et al*, 2002; Fossella *et al*, 2003). The prognosis of advanced NSCLC patients who receive cisplatin-based chemotherapy is still poor, and the renal and gastrointestinal toxicities caused by cisplatin often limit its clinical use. Therefore, development of different treatment strategies is necessary.

Nedaplatin is a second-generation platinum derivative that has shown equivalent antitumour activity and lower toxicity – less nausea, and lower nephrotoxicity and neurotoxicity – than cisplatin (Kameyama *et al*, 1990; Ota *et al*, 1992). A phase I study demonstrated the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies of nedaplatin was 120 and 100 mg m^{-2}, respectively, and the dose-limiting toxicity (DLT) was thrombocytopenia (Ota *et al*, 1992). Two independent phase II studies of nedaplatin for NSCLC showed response rates of 14.7 and 20.5%, respectively, and 16.7 and 12.5% with the patients who had received chemotherapy previously (Fukuda *et al*, 1990; Furuse *et al*, 1992a). Based on these promising results, a randomised study of nedaplatin–vindesine *vs* cisplatin–vindesine was conducted for previously untreated NSCLC patients in Japan and indicated that nedaplatin-based chemotherapy yielded similar response rates and overall survival (Furuse *et al*, 1992b). Leucopenia, renal toxicities and gastrointestinal toxicities were more frequent in the cisplatin–vindesine arm, while thrombocytopenia was more frequent in the nedaplatin–vindesine arm.

Gemcitabine, an analogue of deoxycytidine, is a pyrimidine antimetabolite, that shows a reproducible response rates of >20% with a median survival time of 9 months, offering a quality of life benefit in comparison with best supportive care (Abratt *et al*, 1994; Anderson *et al*, 1994; Gatzemeier *et al*, 1996; Anderson *et al*, 2000). The main toxicity of gemcitabine is mild-to-moderate myelosuppression. The combination of gemcitabine and cisplatin showed synergistic effects in preclinical studies because gemcitabine inhibited the repair of DNA damage caused by cisplatin (Bergman *et al*, 1996), and achieved high response rates along with improvements in median survival time in clinical setting (Sandler *et al*, 2000; Schiller *et al*, 2002; Alberola *et al*, 2003).

Recently, carboplatin has attracted attention ahead of nedaplatin because it has similar activity to cisplatin with fewer nonhaematological toxicities. The available data suggest that carboplatin–paclitaxel or carboplatin–gemcitabine should be considered among standard regimen for advanced NSCLC (Kelly *et al*, 2001; Grigorescu *et al*, 2002; Rudd *et al*, 2002; Schiller *et al*, 2002).

It seems that nedaplatin has activity and toxicity profiles similar to those of carboplatin, although no randomised trial has not been done to allow direct comparison (Fukuda *et al*, 1990; Furuse *et al*,

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Received 30 December 2003; revised 1 March 2004; accepted 2 March 2004; published online 20 April 2004

1992a; Ota *et al*, 1992). Moreover, Matsumoto *et al* (2001) demonstrated that the combination of nedaplatin and gemcitabine resulted in enhanced inhibition of tumour growth *in vivo* and the antitumour efficacy of the combination was superior to that of cisplatin–gemcitabine or carboplatin–gemcitabine. Based on the results of a preclinical study, we designed the present phase I study of the efficacy of the combination of nedaplatin and gemcitabine for advanced NSCLC. The purpose of this study was to establish the toxicities and MTD of this combination, to determine the RD for phase II studies, and to observe their antitumour activity.

PATIENTS AND METHODS

Patient eligibility

Patients with histologic or cytologic confirmation of locally advanced or metastatic NSCLC who received either no prior chemotherapy or one previous chemotherapy regimen were eligible. The eligibility criteria were as follows; (1) measurable lesions; (2) age $\leqslant$75 years; (3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1; (4) adequate organ function (a white blood count (WBC) $\geqslant 4000\,\mu l^{-1}$, a neutrophil count $\geqslant 2000\,\mu l^{-1}$, a platelet count $\geqslant 100\,000\,\mu l^{-1}$, a haemoglobin count $\geqslant 9.5\,g\,dl^{-1}$, serum total bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, serum transaminase $\leqslant 2\times$ upper normal limits, a serum creatinine $\leqslant$ upper normal limits, blood urea nitrogen (BUN) $\leqslant 25\,mg\,dl^{-1}$, $PaO_2 \geqslant 60$ mmHg or $SpO_2 \geqslant 90\%$]; and (5) normal electrocardiogram (ECG). At least 4 weeks must have passed after the completion of previous therapy and the patients had to have recovered from the toxic effects of previous therapy. The exclusion criteria consisted of pulmonary fibrosis or interstitial pneumonitis with symptoms or apparent abnormalities on chest X-ray, massive pleural effusion or ascites, acute inflammation, pregnancy, lactation, symptomatic brain metastases, active concurrent malignancies, severe drug allergies, severe heart disease, cerebrovascular disease, uncontrollable diabetes mellitus or hypertension, severe infection, active peptic ulcer, ileus, paralysis intestinal, diarrhoea and jaundice. This study was performed at Kinki University School of Medicine and was approved by the Institutional Review Board. Written informed consent was obtained from all patients. This study was conducted in accordance with Declaration of Helsinki.

Pretreatment and follow-up studies

Prior to entry, a complete history was taken and physical examination including age, height, weight, performance status, histological diagnosis, tumour stage, contents of previous treatment and presence of a complication was performed. The pretreatment laboratory investigations included a complete blood cell count, differential WBC count, platelet count, serum electrolytes, total protein, albumin, total bilirubin, transaminase, alkaline phosphatase, lactate dehydrogenase, BUN, creatinine, creatinine clearance and urinalysis. After the initiation of therapy, a complete blood cell count with a differential WBC count was performed at least twice a week. Blood chemistry profiles and chest X-ray films were obtained weekly. The lesion measurements were performed during at least every second course. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 and tumour responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (Therasse *et al*, 2000). Time to progression was measured from the date of registration to the date of first progression or death from any cause. Survival time was also measured from the date of registration to the date of death or latest follow-up, and was calculated using the Kaplan–Meier method (Kaplan and Meier, 1958).

Drug administration and dose escalation

The treatment schedule included nedaplatin, diluted with 500 ml of normal saline, given intravenously over 90 min on day 1, and gemcitabine with 100 ml of normal saline, given intravenously over 30 min after the completion of nedaplatin infusion on days 1 and 8, every 3 weeks. All patients were allowed to receive antiemetics with dexamethasone and granisetron, and post-therapy hydration with 1000 ml of normal saline. Granulocyte colony-stimulating factor (G-CSF) prophylaxis was not administered. Doses of gemcitabine on day 8 were given if the WBC count was $>2000\,\mu l^{-1}$ and/or the platelet count was $>750\,000\,\mu l^{-1}$, and/or allergic reaction, fever, elevation of transaminase and pneumonitis were less than grade 2, and/or the other nonhaematological toxicities were less than grade 3. The subsequent courses were withheld until the toxic levels returned to those specified in the eligibility criteria. The doses of both drugs were decreased by one dose level if DLTs occurred. In the case of the initial dose level, the doses of nedaplatin and gemcitabine were reduced by 20 and 200 mg m^{-2}, respectively.

Dose escalations were performed as listed in Table 1. Intrapatient dose escalation was not allowed. At least three patients were treated at each dose level, and three additional patients were entered at the same dose level if DLT was observed in one of the first three patients. The MTD was defined as the dose level at which more than two of three patients, or three of six patients experienced DLT. The definition of DLT was as follows: (1) grade 4 leukopenia, (2) grade 4 neutropenia for more than 4 days, (3) thrombocytopenia $<20\,000\,\mu l^{-1}$, (4) grade 3 febrile neutropenia, (5) grade 3 nonhaematologic toxicity except for nausea/vomiting, (6) delay of administration of gemcitabine on day 8 over a week for toxicities.

RESULTS

Between August 2001 and February 2003, 20 patients were enrolled in this study. The total and the median number of courses were 56 and 3 (range 1–6), respectively. The patients' characteristics are shown in Table 2. The majority of patients had a PS of 1. There

Table 1 Dose-escalation schema

Dose level	Nedaplatin dose (mg m^{-2})	Gemcitabine dose (mg m^{-2})	No. of patients (courses)
1	60	800	3 (8)
2	80	800	3 (10)
3	80	1000	8 (18)
4	100	1000	6 (20)

Table 2 Patients' characteristics

No. of patients		20
Age, years	Median	63.5
	Range	36–74
Sex	Male/female	17/3
Performance status	0/1	5/15
Histology	Adeno/squamous	13/7
Stage	IIIB/IV	4/16
Prior therapy	None	5
	Surgery	5
	Radiation	6
	Chemotherapy	14
	CDDP-based	3
	CBDCA-based	4
	Nonplatinum	4
	UFT	2
	Gefitinin	1

were five previously untreated patients (level 3, two patients; level 4, three patients) and 15 (75%) previously treated patients. Of the previously treated patients, five had received prior surgery, five had prior radiotherapy, and 14 had prior chemotherapy. Seven had received platinum-based chemotherapy (cisplatin, three patients; carboplatin, four patients), and four a nonplatinum regimen. Responses to previous chemotherapy included partial response in five patients, stable disease in seven, progressive disease in one, and not evaluable in one. The median interval from previous treatment was 16 weeks (range 4–92.5 weeks). Out of 20 patients, 18 were assessable for toxicity and response. Two patients at level 3 were excluded from the toxicity and response evaluation because they had refused this study after registration.

Toxicities

The haematological and nonhaematological toxicities observed during the first course are shown in Tables 3 and 4, respectively. The most frequent toxicities observed in the first cycle were neutropenia and thrombocytopenia (Table 3). One-third of the patients had grade 3 thrombocytopenia, and one patient received a platelet transfusion during the first course. Three patients had grade 4 neutropenia for no longer than 4 days. The nadir for neutropenia and thrombocytopenia occurred on day 15 (median, range 5–18), and on day 15 (median, range 8–18), respectively. Nonhaematological toxicities generally were mild because none of the patients had experienced more than grade 3 in the first course (Table 4). The major toxicities following all courses are listed in Table 5. Grade 3 thrombocytopenia occurred in 16 out of 56 courses, and three patients received platelet transfusion (one patient at level 1, one at level 3 and one at level 4). However, no patient had haemorrhagic complications. The most frequent nonhaematological toxicities were elevation of transaminase activity, nausea and appetite loss, but all were mild. One previously untreated patient at level 3 experienced grade 3 pneumonitis after the fifth course, probably induced by this treatment, and the patient's condition improved after the administration of steroid. There was no treatment-related death. One of the 18 patients at level 4 underwent dose reduction after the first course due to neutropenia, and two patients at level 3 did not receive gemcitabine on day 8 because they had neutropenia, thrombocytopenia and high transaminase activity. Delays in the commencement of subsequent courses occurred in 11 courses, and the median length of the delay before starting the subsequent course was 21 days (21–35 days).

MTD and DLTs

At levels 1 and 2, none of the patients had developed a DLT. Haematological and nonhaematological toxicities were generally mild at these levels, although one patient had grade 3 thrombocytopenia at level 1. At level 3, two of six assessable patients had developed DLTs. Both could not receive their scheduled dose of gemcitabine on day 8 because they had neutropenia, thrombocytopenia and high transaminase activity. At level 4, three of six patients had developed DLTs. One patient received G-CSF for neutropenia, not lasting more than 4 days, which was considered as the DLT. Another patient required a platelet infusion because of thrombocytopenia $<20000\,\mu l^{-1}$. The third patient could not receive the second course due to the delayed anaemia, also considered as DLT. Therefore, dose level 4, 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine was regarded as the MTD. The recommended dose level for further phase II study was determined to be 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine (dose level 3 in this study).

Response and survival

There were three partial responses, for an overall response rate of 16.7%. As for squamous cell carcinoma, only one out of seven

Table 3 Haematological toxicity following first course of nedaplatin and gemcitabine

		WBC grade					ANC grade					plt grade					Hb grade				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	0	2	1	0	0	0	1	2	0	0	0	1	1	1	0	0	2	1	0	0
2	3	1	0	2	0	0	1	0	1	1	0	0	3	0	0	0	0	1	2	0	0
3	6	1	1	2	1	0	2	0	0	3	1	1	2	1	2	0	3	3	0	0	0
4	6	1	0	3	2	0	0	0	3	1	2	0	2	1	3	0	0	3	3	0	0

Table 4 Nonhaematological toxicity following first course of nedaplatin and gemcitabine

		Nausea grade					Vomiting grade					Fatigue grade					Transaminase grade				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	3	0	0	0	0	3	0	0	0	0	2	1	0	0	0	3	0	0	0	0
2	3	1	1	1	0	0	3	0	0	0	0	1	2	0	0	0	1	2	0	0	0
3	6	2	3	1	0	0	5	1	0	0	0	4	2	0	0	0	3	1	2	0	0
4	6	2	2	2	0	0	6	0	0	0	0	6	0	0	0	0	1	5	0	0	0

		Infection grade					Fever grade					Appetite loss grade					Constipation grade				
Dose level	No. of patients	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	3	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0
2	3	2	0	1	0	0	2	1	0	0	0	1	2	0	0	0	3	0	0	0	0
3	6	6	0	0	0	0	6	0	0	0	0	2	4	0	0	0	4	2	0	0	0
4	6	4	0	2	0	0	6	0	0	0	0	2	4	0	0	0	4	2	0	0	0

Table 5 Toxicities following all courses of nedaplatin and gemcitabine (56)

	Grade			
	1	2	3	4
WBC	13	26	10	0
ANC	15	15	13	3
Hb	24	27	1	0
Plt	22	14	16	0
Nausea	17	4	0	0
Vomiting	6	0	0	0
Appetite loss	21	0	0	0
Fatigue	15	0	0	0
Constipation	6	7	0	0
Transaminase	27	5	0	0
Neuropathy	5	0	0	0
Pneumonitis	0	0	1	0
Fever	1	0	0	0
Infection	0	3	1	0

patients had a partial response. The median progression-free survival time was 5.1 months. The median survival time and 1-year survival rate were 9.1 months and 34.1%, respectively. Out of 15 patients who had received prior treatment, two (13.3%) achieved a partial response, and there was no clear relationship between responses to previous treatment and responses to this regimen. For previously treated patients, the median survival time and 1-year survival rate were 9.2 months and 40.3%, respectively. Among five previously untreated patients, one (20%) achieved a partial response and the median survival time and 1-year survival rate were 12.0 months and 50.0%, respectively.

DISCUSSION

Many recent randomised clinical trials have shown that the combinations of cisplatin with one of the new agents, such as gemcitabine, taxanes or vinorelbine, is the standard therapy for patients with locally advanced or metastatic NSCLC (Non-Small Cell Lung Cancer Collaborative Group, 1995; Kelly *et al*, 2001; Schiller *et al*, 2002; Fossella *et al*, 2003). As it is known that cisplatin strongly promotes nephrotoxicity, neurotoxicity and gastrointestinal toxicity, second-generation platinum-containing compounds including carboplatin have attracted attention. Based on several randomised trials that have shown that the combination of carboplatin with paclitaxel produces similar response rates and overall survival with a more favourable toxicity profile than the combination of cisplatin with new agents (Kelly *et al*, 2001; Scagliotti *et al*, 2002; Schiller *et al*, 2002), combined therapy of carboplatin and paclitaxel is considered to be a standard therapy. More recently, the combination of carboplatin with gemcitabine has become attractive as a therapy for advanced NSCLC. Some randomised studies have indicated that carboplatin-gemcitabine regimen offers equivalent median survival compared with cisplatin-gemcitabine or mitomycin-vinblastine-cisplatin /mitomycin-ifosfamide-cisplatin (Danson *et al*, 2003; Zatloukal *et al*, 2003), and results in significant improvements in overall survival over those for gemcitabine alone or the older cisplatin-containing regimens (Grigorescu *et al*, 2002; Rudd *et al*, 2002; Sederholm, 2002). However, neutropenia and thrombocytopenia were more common in carboplatin-gemcitabine regimens than others; thrombocytopenia was particularly common.

Like carboplatin, nedaplatin is also a second-generation platinum derivative that appears to have a similar mechanism and toxicity profile to carboplatin, although direct comparison has not been performed. Moreover, *in vivo* study suggested that nedaplatin-gemcitabine resulted in more enhanced inhibition of tumour growth than cisplatin-gemcitabine or carboplatin-gemcitabine. These results prompted us to investigate nedaplatin-based combinations and to conduct this phase I study.

With respect to toxicities, the most frequent toxicities were haematological toxicities, especially neutropenia and thrombocytopenia. Eight of 18 patients (44.4%) developed more than grade 3 neutropenia after the first courses, and after 16 out of 56 (28.6%) courses overall. On the other hand, six out of 16 patients (37.5%) developed grade 3 thrombocytopenia after the first courses, and after 16 out of 56 courses (37.5%) overall. However, patients required platelet transfusions during only three courses. In addition, one previously untreated patient developed drug-related pneumonitis, which improved with the administration of steroid, at level 3 after the fifth course.

Overall, the toxicities of the combination of nedaplatin with gemcitabine were generally mild and this combination chemotherapy is both well tolerated and active against advanced NSCLC.

The overall response rate of 16.7%, the median survival time of 9.1 months, and 1-year survival rate of 34.1% in this study were quite acceptable because most patients had been given prior chemotherapy. As evaluation of antitumour activity was not a primary objective, and our patient population was small and heterogeneous, we are unable to draw definitive conclusions about the activity of this regimen. Currently, it is still controversial whether novel platinum compounds such as carboplatin and nedaplatin could replace cisplatin for the treatment of advanced NSCLC. However, when not only antitumour activity but also palliation are the main goals of treatment, these new platinum compounds might play a useful role because of their favourable toxicity profile. Therefore, nedaplatin-gemcitabine warrants a phase II study, and further evaluation in prospective randomised trials with cisplatin- or carboplatin-based combinations as a first-line chemotherapy for advanced NSCLC in order to investigate whether nedaplatin could replace cisplatin or carboplatin.

In conclusion, the combination of nedaplatin with gemcitabine is well tolerated and active for advanced NSCLC. The MTD and recommended dose level are 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine and 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine, respectively.

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Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer

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Received 5 February 2004; received in revised form 5 April 2004; accepted 15 April 2004

KEYWORDS
Gefitinib;
Non-small-cell lung cancer;
Prognostic factor;
Smoking index;
Female;
Performance status (PS);
Retrospective analysis

Summary

Background: The purpose of the study was to identify the potential predictive features associated with the response and survival benefit of gefitinib administration. We have retrospectively reviewed data of all patients who received a single regimen of gefitinib in our institution from August 1998 until July 2003.

Methods: Overall 101 patients with non-small-cell lung cancer (NSCLC) who have received a single use of gefitinib were analyzed. Potential factors associated with the response of gefitinib included smoking index, gender, histology, performance status (PS), number of pre-treatments, age and stage. Univariate analysis was performed for these strata by Fisher's exact test and multivariate analysis was then performed using the logistic regression model.

Results: The overall response rate was 19.8%. Univariate analysis revealed that significant predictive factors were associated with the response for 'adenocarcinoma', 'female', 'good PS' (0–1) and 'non-smoker' categories. Multivariate analysis limited the predictive factors associated with the response for 'female' ($P = 0.0032$), 'good PS' ($P < 0.02$) and 'non-smoker' ($P = 0.0417$). In survival analyses, 'female' ($P < 0.005$), 'good PS' ($P < 0.0001$), and a low level of the smoking index ($P < 0.05$) indicated significantly prolonged survival. Response and survival data in elderly patients were equivalent to those in younger patients. Adverse events (AEs) were generally mild and were almost always skin reactions and diarrhea. Interstitial lung disease (ILD) occurred in 4% of the group under observation.

Conclusions: Gefitinib provided clinical benefit for the following factors 'female', 'good PS' and 'non-smoker'. A low smoking index is reported as a novel predictive prognostic factor following a single regimen of gefitinib.

Abbreviations: NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; IDEAL-1, Iressa dose evaluated advanced lung cancer-1; PS, performance status; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; INTACT-1, Iressa NSCLC trial assessing combination treatment-1; INTACT-2, Iressa NSCLC trial assessing combination treatment-2

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0169-5002/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2004.04.032

1. Introduction

Patients with advanced non-small-cell lung cancer (NSCLC) have a poor prognosis with 1–5% 5-year survival rates [1]. A recent meta-analysis demonstrated that platinum-based combination chemotherapy is currently considered to be the most effective treatment for advanced NSCLC, and these have improved the median survival time (MST) by 2 months and caused a 10% increase in 1-year survival rates [2]. As platinum-based chemotherapy improves survival and quality of life in advanced NSCLC patients, most patients will receive second line chemotherapy. With recurrence or progression, docetaxel has been approved as a second line chemotherapy treatment due to demonstrated survival benefit compared with best supportive care (BSC) or vinorelbine/ifosfamide [3,4]. Currently, there is no proven effective chemotherapy for patients previously treated with platinum-based and docetaxel therapies.

The epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy because many types of cancer cells express or overexpress EGFR (including NSCLC, renal cell carcinoma and breast cancer) [5,6]. EGFR overexpression has been reported as a poor prognostic factor in many types of human solid tumors including NSCLC in several studies [7–9]. Currently, monoclonal antibodies that bind to the extracellular domain of EGFR and intracellular tyrosine kinase inhibitors have been developed [10,11]. Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways implicated in the proliferation, angiogenesis, invasion, metastasis and survival of cancer cells [12,13]. Several phase I trials demonstrated safety and tolerability of gefitinib in pretreated patients with solid tumors, in which trials an 11% response rate was seen in 100 patients with heavily pretreated advanced NSCLC [14]. On the other hand, in Japan, a phase I trial demonstrated five responders out of a total of 31 patients who all had adenocarcinoma of the lung [12]. To confirm anti-tumour activity and the safety profile of gefitinib, an international phase II study (IDEAL-1) and United States trial (IDEAL-2) were conducted as a second or third line treatment in patients with advanced NSCLC [15,16]. Patients enrolled in these studies were randomized into two different doses, 250 and 500 mg/day. These trials demonstrated that toxicity was mild and showed an encouraging response rate with an RR of 18.4 and 11.8% of patients in the 250 mg arm, respectively, and an improvement in disease related symptoms and quality of life were observed. The IDEAL-1 study has also confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' using multivariate analysis. Two large randomized phase III studies [17,18], which are standard chemotherapy (cisplatin/gemcitabine or carboplatin/paclitaxel) with or without gefitinib, failed to demonstrate a survival benefit for advanced NSCLC patients as a first line chemotherapy. Although the results of the phase III studies were negative, gefitinib is still considered a promising molecular targeted agent as a new generation treatment in patients with advanced NSCLC. Information on the clinical prognostic factors following a single regimen of gefitinib should be helpful in finding which patients are likely to receive benefit, and in the development of a future treatment. Although the previous phase II trial (IDEAL) showed that several predictive factors were associated with the response to gefitinib, the population was essentially biased towards the young, with good performance status (PS) and conserved, good organ functions.

In this study, to find factors associated with an objective response and survival benefit of gefitinib, we retrospectively analysed patients who received a single regimen of gefitinib at our institute.

2. Methods

All patients with stage IIIB or IV NSCLC, who received a single regimen of gefitinib from August 1998 until July 2003 at the Kinki University School of Medicine, Osaka, were retrospectively reviewed. We evaluated patients who participated in clinical trials (phase I trial, phase II trial; IDEAL-1), or phase II trial for investigating surrogate gene therapy, and in 53 patients who were administered the drug after marketing (including elderly or poor performance status patients). Patients who received gefitinib as part of a compassionate use program were excluded. All patients were checked for age, gender, histology, Eastern Cooperative Oncology Group (ECOG), PS, stage, pre-treatment regimen, number of prior regimen, and smoking status before treatment of gefitinib. Smoking status was evaluated by the Brinkmann index; number of cigarettes per day multiplied by number of years. We analyzed the response, overall survival rate and the adverse effects of gefitinib, and investigated predictive factors associated with response and prognosis. The response was assessed using physical examination, biochemical profile, chest X-ray, chest computed tomography (CT), head CT or magnetic resonance imaging (MRI) scan, abdominal echo-graphic or abdominal CT scan, bone scinti-graph, bronchoscope, and was evaluated according to the response eval-

uation criteria in solid tumor (RECIST) [19]. The severity of all the adverse events (AEs) that related to gefitinib administration was assessed by the NCPCTC (version 2.0) grading system. The predictive factors associated with the response that were analyzed in this study were age, gender, PS, histology, stage, number of prior regimen and smoking status. Variables were tested for any possible relationship with the response to gefitinib, at first by univariate analysis, and subsequently by the application of a multivariate model. Response rates were compared between strata using Fisher's exact test. Logistic regression models were used to explore observed differences and identify baseline factors that may independently predict for response rates. The survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Patient profiles

From August 1998 until July 2003 at our institute, a total of 105 patients, who were already cytologically or histologically diagnosed as NSCLC, were treated by a single regimen of gefitinib. Patients received gefitinib until disease progression or intolerable toxicity. Of these, 101 patients were evaluated as suitable for analysis; four patients were excluded from analysis because they received gefitinib as part of a compassionate use program. As shown in Table 1, the 101 patients included: 2 patients who received gefitinib at a once daily dose of 50 mg; single patients who each received 100, 225, 400, 525 and 700 mg, respectively; 89 patients who received 250 mg; and 5 patients who received 500 mg. In the phase I trial, we used an intermittent administration schedule with 14 days continuous dosing followed by 14 days off.

Table 1 Patient characteristics

	Number of patient ($N = 101$)
Phase I	7
50 mg	2
100 mg	1
225 mg	1
400 mg	1
525 mg	1
700 mg	1
Phase II (IDEAL-1)	11
250 mg	6
500 mg	5
Phase II (gene expression) (250 mg)	30
Post marketing (250 mg)	53

Table 2 Patient characteristics ($N = 101$)

	Number of patients
Age (year)	
Median (range)	62 (31–84)
<69	74
≥70	27
Gender	
Male	64
Female	37
Performance status	
0	15
1	62
2	17
3	7
Tumor histology	
Adenocarcinoma	81
Squamous	18
Large-cell	2
Stage	
III	18
IV	83
Previous treatment	
No treatment	5
Failed 1 previous chemotherapy regimens	53
Failed 2 previous chemotherapy regimens	34
Failed 3 previous chemotherapy regimens	9
Smoking (smoker:never-smoker)	55:46
Index[a] 0:1–999:1000	46:32:23

[a] Index: number of cigarettes per day multiplied by number of years.

Patient characteristics are shown in Table 2. The median age was 62 years (ranging from 31–84) and 74 patients (73.3%) were less than 69 years old. 63.4% of the patients were male, 76.2% had performance status (ECOG) 0–1, 80.2% had adenocarcinoma of which 83.2% had stage IV disease. Fifty-three patients had received one prior regimen, 43 had more than two prior regimens and only five had previously been untreated. 54.5% of them were smokers, and the non-smokers were almost all female. This study included patients

Table 3 Overall objective response

	Number	%
Number of patients evaluated	101	
Complete response (CR)	1	1.0
Partial response (PR)	19	18.8
Stable disease (SD)	52	51.5
Progressive disease (PD)	25	24.8
Not evaluable	4	4.0
Response rate % (95% CI)	19.8 (12.0–27.6)	
Disease control rate[a] % (95% CI)	71.3 (62.5–80.1)	

[a] CR + PR + S.D.

who had failed several previous chemotherapy regimens, and patients with an ECOG PS score of 3.

3.2. Response to treatment

Table 3 shows an objective response observed in this study. Twenty responders were evaluated and the overall response rate was 19.8%. One patient achieved a complete response, 19 patients exhibited a partial response and 52 patients had stable disease, resulting in a disease control rate (objective responses plus stable disease) of 71.3%. When evaluated using patient characteristics, we determined the response rate detailed in Fig. 1. All patients that responded had adenocarcinoma of the lung as the histological subtype. In addition, for the factors 'female' and 'never-smoker', there were higher response rates than in 'male' and 'smoker' respectively, while RR was similar for age, stage and pre-treatment. The response rate of 'female' and 'never-smoker' were 37.8 and 32.6%, respectively. Using the Fisher's exact test, the predictive factors which were associated with a response were 'female' (37.8% versus 9.4%; $P = 0.0006$), 'adenocarcinoma' (24.7% versus 0%; $P = 0.0104$), 'good PS' (0–1) (26.0% versus 0%; $P = 0.0028$), and never-smoker (32.6% versus 9.1%; $P = 0.0025$). There were no significant differences for age, stage and pre-treatment (Table 4). A multivariate analysis was performed against the four significant predictive factors in univariate analysis (Table 5). Because the incidence of the female factor is very strongly correlated to the never-smoker factor, the statistical assay was rather unstable if the two factors were analyzed simultaneously. We then investigated two patterns of multivariate analysis. One analysis excluded smoking and the other excluded gender. If smoking status was extracted, then female and good performance status were statistically significant. If gender was extracted, then non-smoking and good performance were statistically significant. The odds of a response were over three times higher for patients with adenocarcinoma than for patients with other histologies, however, this is not considered to be statistically significant because the group in this study was of a small size and included a high percentage of adenocarcinoma.

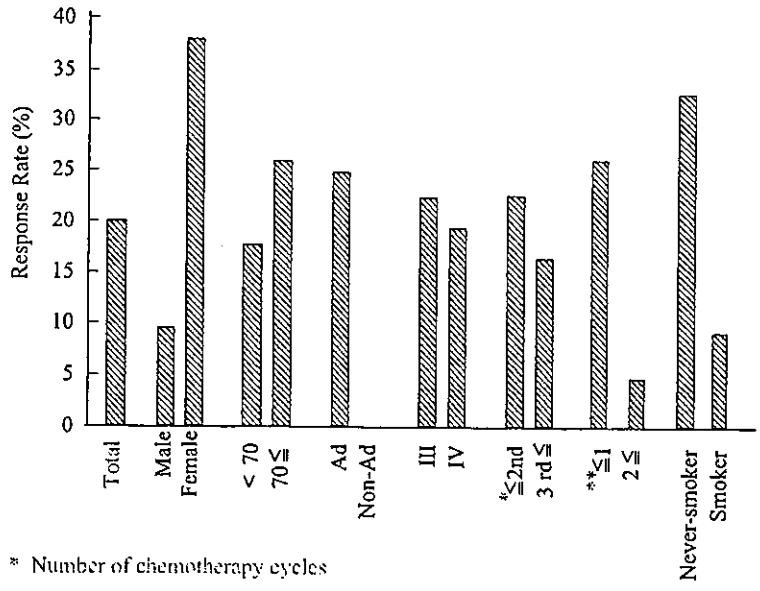

Fig. 1 Tumor response rate of the subgroups.

Table 4 Predictive factors associated with an objective response by univariate analysis

Parameter	*N*	Responder	RR (%)	*P*-value
Smoking index				
Non-smoker	55	15	32.6	
Smoker	46	5	9.1	0.0025
Gender				
Female	37	14	37.8	
Male	64	6	9.4	0.0006
Histology				
Adenocarcinoma	81	20	24.7	
Others	20	0	0.0	0.0104
PS				
0–1	77	20	26.0	
≥2	24	0	0.0	0.0028
Pre-treatment				
≤2 regimens	58	13	22.4	
≥3 regimens	43	7	16.3	N.S.
Age (years)				
≤70	74	13	17.6	
≥71	27	7	25.9	N.S.
Stage				
IIIB	18	4	22.2	
IV	83	16	19.3	N.S.

Abbreviations: N.S., not significant.

3.3. Toxicity

Drug-related AEs of all patients are shown in (Table 6). A total of 101 patients were evaluated for toxicity. The most frequent drug-related AEs were a rash, dry skin and diarrhea. Most of these AEs were mild (Grade 1 or Grade 2) and were controllable. Of all the drug-related AEs evaluated, Grade 3 or Grade 4 AEs were seen in less than 5%, and Grade 4 drug-related AEs were only pneumonitis. Grade 3 or 4 AEs required a treatment interruption, but recovered after discontinuation of gefitinib, except with pneumonitis. Four patients developed greater than Grade 3 pneumonitis requiring hospitalization. All patients had a fever and severe hypoxemia on admission. As soon as possible, all patients were administered steroid therapy. While two patients recovered with the steroid therapy, two patients died within 40 days after the administration of gefitinib. Hematological toxicities were not observed.

3.4. Survival

The median survival time of the patients who were 'good PS' (0 or 1) and 'poor PS' (2 or 3) was 353 and 97 days, respectively, and this difference was significant ($P = 0.0001$, log-rank test) (Fig. 2A). The MST of females was significantly longer than that of males (596 days versus 178 days, $P = 0.004$) (Fig. 2B). Furthermore, a low smoking index (<900) significantly prolonged survival (MST: 301 days versus 149 days, $P = 0.031$) (Fig. 2C). Age did not influence the survival benefit of the patients treated with gefitinib (Fig. 2D).

4. Discussion

Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways, and is one of the promising molecular targeted drugs used in the treatment of advanced NSCLC [16,17,20]. Although the large scale of the phase II study (IDEAL-1) [15] has already confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' by multivariate analysis, the population was essentially biased towards young people with good performance status who had conserved, good organ functions. To clarify the predictive prognostic fac-

Table 5 Predictive factors associated with an objective response by multivariate analysis

Parameter	Odds ratio	95% CI	*P*-value
Extraction of smoking			
Gender (female vs. male)	0.163	0.040–0.585	0.0032
Performance status (1 vs. 2)	0.061	0.000–0.415	0.0018
Histology (Adeno[a] vs. others)	3.326	0.435–infinity	N.S.
Extraction of gender			
Non-smoking (non vs. ≥1)	0.297	0.063–0.959	0.0417
Performance status (1 vs. 2)	0.096	0.000–0.628	0.0101
Histology (Adeno vs. others)	4.385	0.588–infinity	N.S.

Abbreviations: N.S., not significant; CI, confidence interval.
[a] Adenocarcinoma.

Table 6 Patients with drug-related adverse events (NCI-CTC)

Adverse event	Number of patients ($N = 101$)				
	Grade 1	Grade 2	Grade 3	Grade 4/5	Total
Rash	33 (32.6%)	21 (20.8%)	3 (3.0%)	0	57 (56.4%)
Dry skin	24 (23.7%)	3 (3.0%)	0	0	27 (26.7%)
Pruritis	9 (9.0%)	7 (7.0%)	0	0	16 (16.0%)
Diarrhea	19 (18.8%)	4 (4.0%)	0	0	23 (22.8%)
Nausea	6 (6.0%)	1 (1.0%)	0	0	7 (7.0%)
Vomiting	3 (3.0%)	0	0	0	3 (3.0%)
Anorexia	7 (7.0%)	0	0	0	7 (7.0%)
ALT increased	5 (5.0%)	2 (2.0%)	5 (5.0%)	0	12(13.0%)
AST increased	8 (8.0%)	2 (2.0%)	3 (3.0%)	0	13 (13.0%)
Pneumonitis	0	0	2 (2.0%)	2[a] (2.0%)	4 (4.0%)

[a] Treatment-related death (Grade 5).

tors in a practical setting, we retrospectively analysed the patients who received a single regimen of gefitinib at our institute. Multivariate analysis demonstrated that the predictive factors which were associated with a response were 'female', 'good PS' and 'never-smoker'. In survival analyses, the factors 'female', 'good PS', and a low smoking index also significantly prolonged survival.

The mechanism by which these factors produced better prognosis has not been clarified.

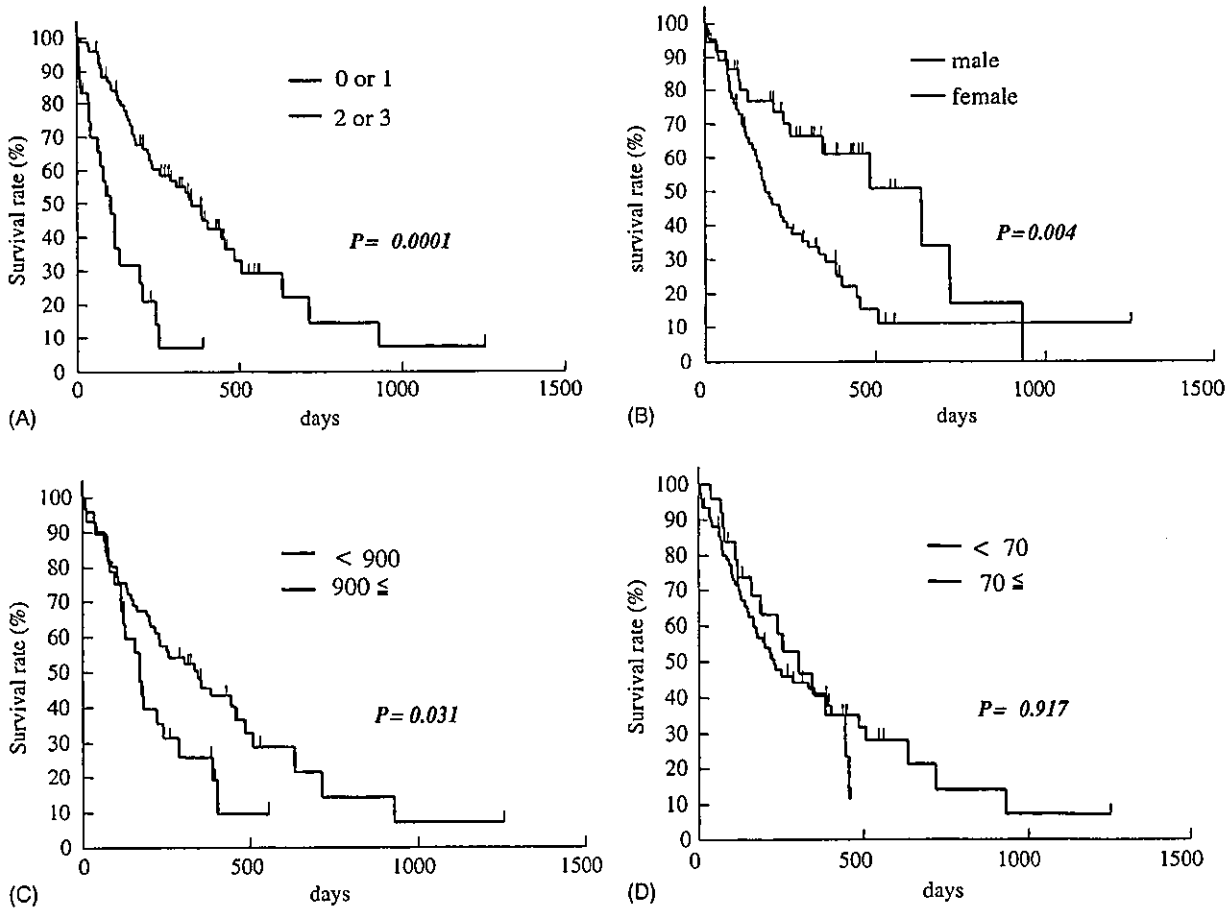

Fig. 2 A comparison of survival of: (A) PS 0, 1 vs. PS 2, 3; (B) gender: male vs. female; (C) smoking index: <900 vs. ≥900; and (D) age: <70 vs. ≥70.

Estrogen and progesterone may up-regulate EGFR in normal tissues [21], and activation of steroid hormones might impact on EGFR function in NSCLC [22]. Another explanation may be that the steroid hormone receptor might interact with EGFR and influence the response of an EGFR inhibitor.

Multivariate analysis in IDEAL-1 showed that PS was not a significant prognostic factor, however, the population of the study was restricted with regards to good PS. Although gefitinib was considered as an effector of symptom improvement in the phase II trial, the indication for patients with poor PS is controversial. Several authors described the case reports about the efficacy of gefitinib in NSCLC patients with poor PS [23,24] or with brain metastases [25]. Although 'good PS' were significant prognostic factor in this trial, gefitinib still might be a candidate drug for patients with poor PS, because of restriction of the use of other anti-cancer drug by their toxicities.

Elderly patients exhibited an equivalent response to young patients in this study. Recent data suggested, gefitinib is safe and well tolerated in elderly pretreated NSCLC patients [26]. A phase II study of gefitinib for elderly patients in NSCLC is needed.

A low smoking index was revealed as a predictive prognostic factor following a single regimen of gefitinib. Erlotinib is also administered orally and is a highly selective EGFR tyrosine kinase inhibitor [27] with a quinazolinamine-based structure similar to that of gefitinib. In the phase II study of erlotinib in NSCLC or bronchial alveolar carcinoma [28], a non-smoking history was also a prognostic factor. Chronic exposure to nicotine increases the expression level and phosphorylation status of EGFR and impairs its function [29]. Moreover, smoking produces overexpression of Her2/neu that binds to EGFR as a hetero-dimer in the tissue of normal bronchus. Expression of EGFR or Her2/neu or both in tissue samples by immunohistochemistry has not correlated in the response of gefitinib [30], however the different type of dimers formed between EGFR families might influence the response to gefitinib.

Four patients (4% of the patients) developed interstitial lung disease (ILD). Continuous smoking disrupted surfactant protein A or D [31,32], and the serum levels of the proteins were increased [33]. As 'smoking history' and 'male' are significant risk factors of ILD and also in treatment with gefitinib [34], a serum level of the surfactant protein A or D might be a predictive marker of ILD. Patients who are female and non-smokers are most likely to receive a high benefit and low risk with gefitinib treatment.

Although more basic biological research is needed to find the mechanism of action, we have found several predictive prognostic factors associated with the practical use of gefitinib. This is necessary clinical information which is important in order to set eligibility criteria for future clinical trials with gefitinib.

Acknowledgements

We would like to express our gratitude for advice received from Dr. Toshiji Nogami, Dr. Yusaku Akashi, Dr. Masaki Miyazaki and Dr. Kimio Yonesaka.

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Published online 29 January 2004

Nucleic Acids Research, 2004, Vol. 32, No. 2 611–622
DOI: 10.1093/nar/gkh223

Characterization of the 5′-untranslated region of YB-1 mRNA and autoregulation of translation by YB-1 protein

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Received August 16, 2003; Revised November 6, 2003; Accepted December 17, 2003

ABSTRACT

The eukaryotic Y-box binding protein YB-1 is involved in various biological processes, including DNA repair, cell proliferation and the regulation of transcription and translation. YB-1 protein is abundant and expressed ubiquitously in human cells, functioning in cell proliferation and transformation. Its concentration is thought to be highly regulated at both the levels of transcription and translation. Therefore, we investigated whether or not the 5′-UTR of YB-1 mRNA affects the translation of YB-1 protein, thus influencing expression levels. Luciferase mRNA ligated to the YB-1 mRNA 5′-UTR was used as a reporter construct. Ligation of the full-length YB-1 5′-UTR (331 bases) enhanced translation as assessed by *in vitro* and *in vivo* translation assays. Deletion constructs of the YB-1 5′-UTR also resulted in a higher efficiency of translation, especially in the region mapped to +197 to +331 from the major transcription start site. RNA gel shift assays revealed that the affinity of YB-1 for various 5′-UTR probe sequences was higher for the full-length 5′-UTR than for deleted 5′-UTR sequences. An *in vitro* translation assay was used to demonstrate that recombinant YB-1 protein inhibited translation of the full-length 5′-UTR of YB-1 mRNA. Thus, our findings provide evidence for the autoregulation of YB-1 mRNA translation via the 5′-UTR.

INTRODUCTION

Y-box proteins function as transcriptional and translational regulators of gene expression. They are found among prokaryotes and eukaryotes and are characterized by the evolutionary conservation of a cold shock domain (CSD). Recently, it was reported that a major protein component of messenger ribonucleoprotein (mRNP) particles in somatic cells is a member of the Y-box binding transcription factor family. This protein acts either as a repressor or an activator of protein synthesis (1–4). It has been hypothesized that YB-1 might play a role in promoting cell proliferation through the transcriptional regulation of various genes, including epidermal growth factor receptor, thymidine kinase, DNA topoisomerase II and DNA polymerase (5,6). The multiple biological roles of YB-1 include the modification of chromatin, the translational masking of mRNA, participation in a redox signaling pathway, RNA chaperoning and regulation of the stress response (7).

It has also been demonstrated that eukaryotic Y-box proteins regulate gene expression at the level of translation by binding directly to RNA (8,9). The rabbit Y-box protein, p50, is found in cytoplasmic mRNP particles in somatic cells and regulates translation by interacting with mRNA (2). The murine MSY1 protein and chicken Y-box protein both regulate transcription and translation (7,10–12). Furthermore, the Y-box family proteins, *Xenopus* mRNP3/mRNP4 and mouse MSY2, have also been found to be mRNA-masking proteins in germinal cells (13–15). Chen *et al.* (16) have reported that YB-1 is involved in the mRNA stability of the cdk4 gene; this stability is achieved by the binding of YB-1 to a specific sequence of the mRNA. YB-1 also stabilizes cap-dependent mRNA, since depletion of YB-1 results in accelerated mRNA decay (17).

Previously, we identified several proteins as partners of YB-1, including YB-1 itself, iron regulatory protein 2 (IRP2) and the ribosomal proteins S3A, L18A, L5, L23A and S5. We also provided evidence that YB-1 is involved in the translational regulation of an iron-related protein (18). Y-box binding proteins thus appear to perform critical functions in both

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