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Epidermal Growth Factor Receptor Gene Mutations and Increased Copy Numbers Predict Gefitinib Sensitivity in Patients With Recurrent Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
To evaluate epidermal growth factor receptor (*EGFR*) mutations and copy number as predictors of clinical outcome in patients with non–small-cell lung cancer (NSCLC) receiving gefitinib.

Patients and Methods
Sixty-six patients with NSCLC who experienced relapse after surgery and received gefitinib were included. Direct sequencing of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* was performed using DNA extracted from surgical specimens. Pyrosequencing and quantitative real-time polymerase chain reaction were performed to analyze the allelic pattern and copy number of *EGFR*.

Results
Thirty-nine patients (59%) had *EGFR* mutations; 20 patients had deletional mutations in exon 19, 17 patients had missense mutations (L858R) in exon 21, and two patients had missense mutations (G719S or G719C) in exon 18. No mutations were identified in *ERBB2*. Response rate (82% [32 of 39 patients] *v* 11% [three of 27 patients]; $P < .0001$), time to progression (TTP; median, 12.6 *v* 1.7 months; $P < .0001$), and overall survival (median, 20.4 *v* 6.9 months; $P = .0001$) were significantly better in patients with *EGFR* mutations than in patients with wild-type *EGFR*. Increased *EGFR* copy numbers (≥ 3/cell) were observed in 29 patients (44%) and were significantly associated with a higher response rate (72% [21 of 29 patients] *v* 38% [14 of 37 patients]; $P = .005$) and a longer TTP (median, 9.4 *v* 2.6 months; $P = .038$). High *EGFR* copy numbers (≥ 6/cell) were caused by selective amplification of mutant alleles.

Conclusion
EGFR mutations and increased copy numbers were significantly associated with better clinical outcome in gefitinib-treated NSCLC patients.

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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase of the *ErbB* family that has been implicated in cell proliferation and survival and is frequently overexpressed in many solid tumors, including non–small-cell lung cancer (NSCLC). Gefitinib (Iressa; AstraZeneca, Osaka, Japan) is an orally active, selective EGFR tyrosine kinase inhibitor that binds to the adenosine triphosphate–binding pocket of the EGFR kinase domain and blocks downstream signaling pathways. Two phase II studies, IRESSA Dose Evaluation in Advanced Lung Cancer 1 and 2 (IDEAL 1 and 2), have demonstrated that gefitinib monotherapy exerts an antitumor activity in patients with advanced NSCLC who had previously received platinum-based chemotherapy.[1,2] Gefitinib was approved in Japan for the treatment of inoperable or recurrent NSCLC in July 2002.

The IDEAL trials and retrospective studies have revealed that women, never smokers, patients with adenocarcinoma, and Japanese patients have higher response rates to gefitinib.[1-4] Among patients with adenocarcinoma, histologic subtypes have been studied; one study showed that responses were more frequent in patients with bronchioloalveolar carcinoma (BAC) features (38% *v* 14%; $P < .001$),[3] whereas another study showed that the response rate was higher in patients with a papillary-dominant subtype (76% *v* 21%; $P = .002$).[5]

Although no predictive molecular markers had been identified at the time of approval, somatic mutations in the kinase domain of *EGFR* have been subsequently linked to gefitinib sensitivity. According to three initial reports, 20 of 24 gefitinib-responsive tumors contained *EGFR* mutations, whereas 19 nonresponsive tumors did not contain any mutations.[6-8] The mutations were detected in exons 18 to 21 of *EGFR*, close to the region coding the adenosine triphosphate–binding pocket of the kinase domain, and most of them were observed in two hotspots: in-frame deletions including amino acids at codons 747 to 749 in exon 19 and an amino acid substitution at codon 858 (L858R) in exon 21. Analyses of surgically resected NSCLC tumors revealed that such mutations were more frequent among women, never smokers, patients with adenocarcinoma, and Japanese or East Asian patients,[7-13] consistent with the known clinical predictors of gefitinib sensitivity.

To evaluate the exact predictive value, we studied consecutive patients with recurrent NSCLC who received gefitinib therapy. To insure high-quality genetic analyses of the archived tissues, we used methanol-fixed, paraffin-embedded surgical specimens, which are known to preserve DNA better than formalin-fixed tissues,[14] and performed laser capture microdissection (LCM).

Recently, some other biomarkers of NSCLC have been studied. The *EGFR* and chromosome 7 copy numbers in NSCLC were assessed using fluorescence in situ hybridization (FISH), and more than 3.0 *EGFR* copies per cell (balanced polysomy or gene amplification) were detected in 39 (22%) of 183 patients.[15] A correlation between an increased *EGFR* copy number and gefitinib sensitivity was also proposed in another study.[16] In yet other studies, mutations in the kinase domain of *ERBB2 (HER2)*, a gene coding another receptor tyrosine kinase of the ErbB family, were detected in 16 (3.6%) of 445 patients with lung adenocarcinoma.[17,18] In the current study, we also analyzed the *EGFR* copy number and the presence of *ERBB2* mutations to assess their impact on clinical outcome.

The expression of EGFR and related proteins has been more widely studied using immunohistochemistry. Some studies suggested that high expression of phosphorylated Akt[19,20] or low expression of phosphorylated mitogen-activated protein kinase[20,21] was associated with better outcome in gefitinib-treated patients, but in general, methods, criteria, and results were inconsistent among studies. We thought that protein expression should be analyzed in another exploratory study, and in the current study, we focused on the genetic analyses.

PATIENTS AND METHODS

Patients

After searching the pharmaceutical records of the National Cancer Center Hospital, 279 patients with NSCLC who had begun receiving gefitinib monotherapy (250 mg/d) between July 2002 and May 2004 were identified. Seventy-three of these patients had undergone surgical resection of primary NSCLC at the hospital and subsequently relapsed. Recurrences were not necessarily confirmed pathologically but were diagnosed clinically. Seven patients were ineligible for inclusion in this study because methanol-fixed tissues were not available (n = 5) or their informed consent to the genetic analysis was not obtained (n = 2); consequently, 66 patients were included.

Genetic Analyses of EGFR and ERBB2

On a protocol approved by the institutional review board of the National Cancer Center, we performed mutational analyses of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* and analyzed the *EGFR* copy number. Methanol-fixed, paraffin-embedded surgical specimens of primary NSCLC were collected retrospectively, and DNA was extracted from bulk tumor tissue, laser capture microdissected tumor tissue, and normal lung tissue from each patient. LCM was performed using a PixCell II LCM system (Arcturus Engineering Inc, Mountain View, CA) according to a previously described method.[22] If appropriate, tumor cells were captured separately from two areas with different histologic subtypes, such as an area with a BAC subtype and another area with stromal invasion. Nested polymerase chain reaction (PCR) was performed to amplify exons 18 through 24 of *EGFR* using previously described primers,[6] and standard PCR was used to amplify exons 18 through 24 of *ERBB2*. Direct sequencing of the PCR products was performed using ABI PRISM 3700 and 3100 DNA Sequencers (Applied Biosystems, Foster City, CA). All sequencing reactions were performed in both forward and reverse directions, and single nucleotide substitutions, insertions, and deletions were detected using an application program named NAMIHEI.[23] Pyrosequencing was performed to verify the sequencing data of the hotspots of *EGFR* and to assess the proportion of mutant alleles in the laser-captured tumor cells using a Pyrosequencing PSQ 96MA (Pyrosequencing, Uppsala, Sweden).[24] On the basis of the proportion of mutant alleles, *EGFR* mutations were divided into two patterns: balanced heterozygous (BH) pattern (< 60%) and mutant-allele-dominant (MD) pattern (≥ 60%). The cutoff level of 60% was decided because if more than 60%, the superiority of the mutant over the wild-type sequences was obvious on the direct sequencing chromatograms. Quantitative, real-time, TaqMan duplex PCR was performed to analyze the *EGFR* copy number using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The *EGFR* primers were 5′-GGAGGACCGTCGCTTGGT-3′ and 5′-AACACCGCAGCATGTCAAGA-3′; the probe (5′-CACCG-CGACCTGGCAGCCA-3′) was labeled with the reporter dye 6-carboxyfluorescein (FAM). RNaseP was coamplified in the same reaction mixture as the endogenous reference gene using TaqMan RNaseP Control Reagents (6-carboxyrhodamine [VIC] dye; Applied

Biosystems). The average *EGFR* copy number per cell was calculated from the differences in the threshold amplification cycles between *EGFR* and RNaseP. Peripheral-blood samples obtained from healthy volunteers were analyzed as normal controls. Decreased, normal, moderately increased, and highly increased *EGFR* copy numbers were defined as less than 1.5, 1.5 to 3.0, 3.0 to 6.0, and ≥ 6.0 copies per cell, respectively.

Pathologic Evaluation

We reviewed the histologic features of the 66 patients using hematoxylin and eosin–stained slides of tumor samples. Two board-certified pathologists (K.T. and Y.M.) who were unaware of the patients' outcome and mutational status examined all the specimens independently; in case of discrepancy, final diagnoses were established by consensus. Adenocarcinoma was categorized in two ways. The first categorization was based on the WHO's classification of lung tumors,[25] which includes four major subtypes of adenocarcinoma: papillary, acinar, BAC, and solid; the dominant subtype in the total tumor mass of each case was documented. The second categorization was based on a report from the Memorial Sloan-Kettering Cancer Center,[26] in which adenocarcinomas were classified into adenocarcinoma without BAC features (Ad), adenocarcinoma with BAC features (AwBF), BAC with focal invasion (BwFI), and pure BAC (PBAC). If two or more tumors were present in one patient, the diagnosis of the most invasive tumor in each case was documented.

Radiologic Evaluation

In patients who had measurable lesions, imaging studies were performed at baseline, approximately 4 weeks after the initiation of gefitinib treatment, and periodically thereafter throughout the treatment. One board-certified radiologist (U.T.) who was unaware of the patients' mutational status reviewed the baseline, first follow-up, and confirmatory imaging studies and classified the tumor responses into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) using standard bidimensional measurements.[27] Responders were defined as patients with CR or PR. In this study, SD was subdivided into minor response (MR) and no response. MR was defined as a ≥ 25% decrease in the sum of the products of the perpendicular diameters of all measurable lesions at any point during gefitinib treatment. Time to progression (TTP) was defined as the time from the start of gefitinib administration to confirmed disease progression or death.

Statistical Analysis

The associations among mutational status, *EGFR* copy number, patient characteristics, and tumor response to gefitinib were assessed using a χ^2 test. The differences in TTP and overall survival (OS) according to the patient subgroups were compared using Kaplan-Meier curves and log-rank tests. Multivariate analyses using logistic regression models and Cox proportional hazard models were performed to assess the association between the biomarkers and clinical outcome while adjusting for the baseline patient characteristics. All analyses were performed using the SPSS statistical package (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Patient Characteristics

The patient characteristics are listed in Table 1. All of the patients were Japanese. The proportions of women (39%), never smokers (47%), and patients with adenocarcinoma (94%) in this study were higher than those in a database of more than 1,000 patients with advanced or recurrent NSCLC treated at our hospital during the four most recent years (27%, 27%, and 73%, respectively). Twenty-two patients (33%) had been included in our phase II trial for first-line gefitinib therapy for patients with recurrent NSCLC, and the others had been treated with gefitinib in clinical practice settings. The operations for primary NSCLC were performed between February 1994 and August 2003, and the median time from the operations to the start of gefitinib was 2.3 years (range, 0.6 to 9.1 years).

Table 1. Patient Characteristics

	Patients (n = 66)	
	No.	%
Age, years		
Median	65	
Range	32-80	
Sex		
Female	26	39
Male	40	61
Smoking history*		
Never smokers	31	47
Former smokers	12	18
Current smokers	23	35
Histologic diagnosis		
Adenocarcinoma	62	94
Papillary/acinar/BAC/solid†	30/18/9/5	45/27/14/8
Ad/AwBF/BwFI/PBAC	15/45/2/0	23/68/3/0
Squamous cell carcinoma	3	5
Pleomorphic carcinoma	1	2
Performance status		
0/1	22/28	33/42
2/3	12/4	18/6
Prior chemotherapy regimens		
0	37	56
1	14	21
≥ 2	15	23

Abbreviations: BAC, bronchioloalveolar carcinoma; Ad, adenocarcinoma without BAC features; AwBF, adenocarcinoma with BAC features; BwFI, BAC with focal invasion; PBAC, pure BAC.
*Never smokers were defined as subjects who have never had a smoking habit, and former smokers were defined as subjects who had stopped smoking at least 1 year before diagnosis.
†Dominant subtype.

Clinical Outcome

Sixty-four patients had measurable lesions at the start of gefitinib administration. CR and PR were observed in two and 32 patients, respectively. MR was observed in three of nine patients with SD. Twenty-one patients had PD, including six patients who died before the first follow-up imaging studies. Two patients had only unmeasurable bone lesions at baseline; one patient showed rapid symptom improvement and continued to receive gefitinib therapy without progression for 13.8+ months, whereas the other

patient developed new lesions and died on day 71. These patients were included in the analysis as a responder and a nonresponder, respectively. The overall response rate was 53%. Forty-one patients died, and the median follow-up time for the 25 survivors was 14.6 months (range, 10.3 to 32.3 months). Eleven patients were still receiving gefitinib without progression at the time of the analysis. The median TTP and the median survival time (MST) for all patients were 5.2 and 16.3 months, respectively.

EGFR *and* ERBB2 *Mutations*

Forty-three mutations in the *EGFR* tyrosine kinase domain were detected in 39 (59%) of the 66 patients. All the mutations detected in this study are shown in Table 2. Twenty patients had deletional mutations in exon 19, and 17 patients had missense mutations (L858R) in exon 21. In exons 18 and 20, five types of missense mutations were detected. Two of them (G719S and G719C) occurred at a codon considered to be a third hotspot.[6,7,9-12] The others (L703V, E709K, and S768I) were detected in patients who also had mutations at the hotspots. Because these mutations were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. No somatic mutations were detected in exons 22 to 24. Silent single nucleotide polymorphisms were identified at nucleotides 2361 (G/A; Q787Q), 2370 (G/A; T790T), and 2457 (G/A; V819V) in exon 20, and at nucleotide 2709 (C/T; T903T) in exon 23, but the association between these polymorphisms and the somatic mutations was not observed. In this study, no mutations and no polymorphisms were detected in exons 18 to 24 of *ERBB2*.

All 43 mutations were detected in LCM samples, but 11 (26%) of these mutations were not detected in the bulk tumor samples. In 13 patients, LCM was performed at separate areas with different histologic subtypes, but no heterogeneity was identified; the same mutations were detected in nine patients, and no mutations were detected in four patients. Mutational analyses of synchronous double lung cancers were performed in two patients; one patient had a tumor with wild-type *EGFR* and a more invasive tumor with L858R + S768I, and the other patient had a tumor with a 9-bp deletion (del L747-E749) and a more invasive tumor with a 15-bp deletion (del E746-T751insA) + L703V.

Among the 39 patients with *EGFR* mutations, the proportion of mutant alleles ranged from 29% to 94%. Nineteen patients showed a BH pattern and 20 patients showed an MD pattern.

EGFR *Copy Number*

The *EGFR* copy number in the laser-captured tumor cells ranged from 1.27 to 31.2 per cell, and increased *EGFR* copy numbers (≥ 3.0 per cell) were observed in 29 patients (44%). The relation between the copy number and the proportion of mutant alleles is shown in Figure 1. Increased copy numbers were observed more frequently in patients with *EGFR* mutations than in patients with wild-type *EGFR* (56% [22 of 39 patients] *v* 26% [seven of 27 patients]; $P = .014$). High copy numbers (≥ 6.0 per cell) were observed only in patients with an MD pattern of mutations. The copy number and the proportion of mutant alleles among patients with *EGFR* mutations was positively correlated (Spearman correlation coefficient = 0.643; $P < .001$), implying that the mutant alleles were selectively amplified in patients with an MD pattern. One patient with an MD pattern had a tumor with only approximately one copy per cell, indicating a hemizygous mutation with a loss of wild-type allele. No alterations in the gene copy number were observed in normal lung tissues.

Table 2. *EGFR* Mutations Detected in the Present Study

Exons	Amino Acids	Nucleotides	No. of Patients
19	del E746-A750	del 2235-2249	12
	del E746-A750	del 2236-2250	5
	del E746-T751insA	del 2237-2251	1
	del L747-E749	del 2239-2247	1
	del E746-S752insV	del 2237-2255 + ins T	1
21	L858R	T → G at 2573	17
18	G719S	G → A at 2155	1
	G719C	G → T at 2155	1
	L703V	C → G at 2107	1*
	E709K	G → A at 2125	1†
20	S768I	G → T at 2303	2‡

Abbreviations: *EGFR*, epidermal growth factor receptor; del, deletion; ins, insertion.
*A patient with del E746-T751insA.
†A patient with L858R.
‡A patient with L858R and a patient with G719C.

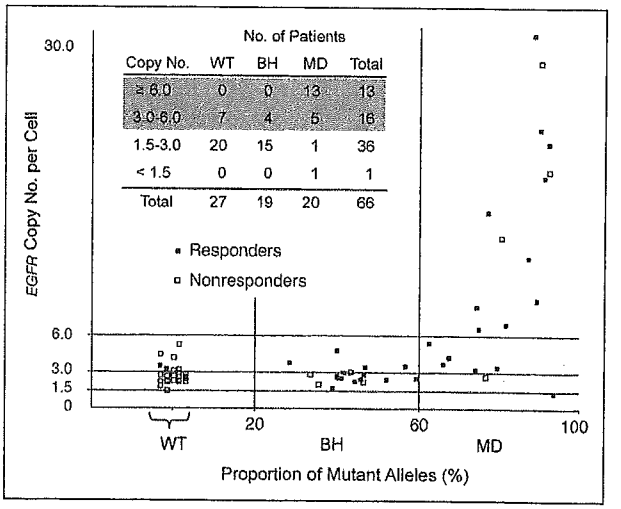

Fig 1. Relation between the epidermal growth factor receptor (*EGFR*) copy number and the proportion of mutant alleles. WT, patients with wild-type *EGFR*; BH, patients with a balanced heterozygous pattern of *EGFR* mutations; MD, patients with a mutant-allele-dominant pattern of *EGFR* mutations.

EGFR *Mutations,* EGFR *Copy Number, and Clinical Outcome*

The tumor responses to gefitinib according to the mutational status of *EGFR* are shown in Table 3. The response rates of patients with mutant and wild-type *EGFR* were 82% and 11%, respectively ($P < 10^{-7}$). Seven patients with *EGFR* mutations were nonresponders; three patients had PD at 0.3 (early death), 2.3, and 2.3 months, and four patients had SD. Three of the four patients with SD had MR (TTP, 2.5, 5.2, and 6.9 months), and the other patient continued to receive gefitinib therapy without progression for 24.2 months, whereas all SD tumors with wild-type *EGFR* progressed within 5 months without MR. Meanwhile, three patients with wild-type *EGFR* exhibited PR, and two of these patients were still receiving gefitinib therapy without progression at 10.9+ and 21.1+ months. The Kaplan-Meier plots of TTP and OS according to the presence of the *EGFR* mutations are shown in Figures 2 and 3, respectively. Patients with *EGFR* mutations had a significantly longer TTP and OS compared with those with wild-type *EGFR*.

Univariate analyses were performed to assess the correlations among patient characteristics, *EGFR* mutations, *EGFR* copy number, and clinical outcome (Tables 4 and 5). The response rates were significantly higher in women, never/former smokers, and patients with BAC features and were marginally higher in patients with a papillary-dominant subtype. The response rates among these subgroups were approximately consistent with the rates of *EGFR* mutations. An increased *EGFR* copy number was also significantly associated with a higher response rate and a longer TTP.

The results of multivariate analyses among 62 patients with adenocarcinoma are shown in Table 6. The presence of *EGFR* mutations was strongly associated with a higher response rate, a longer TTP, and a longer OS. An increased *EGFR* copy number was also a significant or marginally significant predictor of a higher response rate and a longer TTP. These results did not change substantially if any combinations of variables were included in the models.

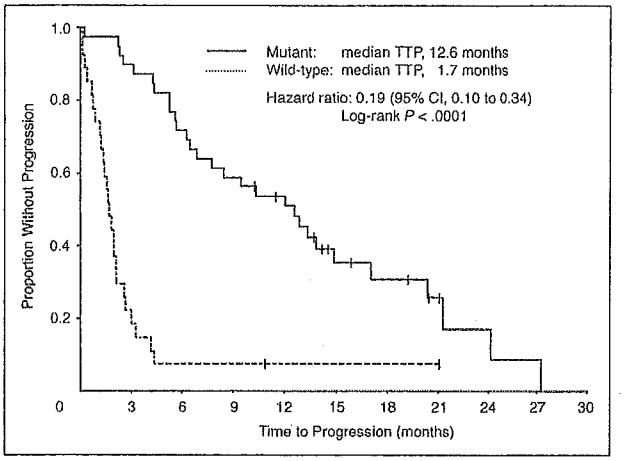

Fig 2. Kaplan-Meier plot of time to progression (TTP) according to epidermal growth factor receptor (*EGFR*) mutation status.

Among patients with wild-type *EGFR*, TTP was significantly longer in patients with increased *EGFR* copy numbers (median, 3.0 *v* 1.4 months; log-rank $P = .021$), and both of the two long-term responders had tumors with moderately increased *EGFR* copy numbers (3.20 and 3.45/cell). Among patients with *EGFR* mutations, TTP and OS were not significantly different according to the types of mutations, the presence of additional mutations, the proportion of mutant alleles, or the *EGFR* copy number (data not shown).

DISCUSSION

This study strongly implies that the mutational status of *EGFR* is a major determinant of gefitinib sensitivity in patients with NSCLC. The response rate was 82%, the median TTP was 12.6 months, and the MST was 20.4 months in gefitinib-treated patients with *EGFR*-mutant NSCLC. *EGFR* mutations might be a good prognostic factor independent of treatment, but these remarkable results suggest a

Table 3. *EGFR* Mutations and Tumor Response to Gefitinib

	Responders		Nonresponders			Responders/Total Patients	Response Rates (%)
	CR	PR	MR	SD	PD		
Mutant	2	30*	3	1	3†	32/39	82
DEL	0	18*	2	0	0	18/20	90
L858R	2	11	1	1	2†	13/17	76
G719	0	1	0	0	1	1/2	50
Wild-type	0	3	0	5	19	3/27	11
Total	2	33	3	6	22	35/66	53

Abbreviations: *EGFR*, epidermal growth factor receptor; CR, complete response; PR, partial response; MR, minor response; SD, stable disease without MR; PD, progressive disease; DEL, deletional mutations in exon 19; G719, G719S, or G719C.
*Including a clinical responder without measurable lesions.
†Including a patient who had no measurable lesions at baseline.

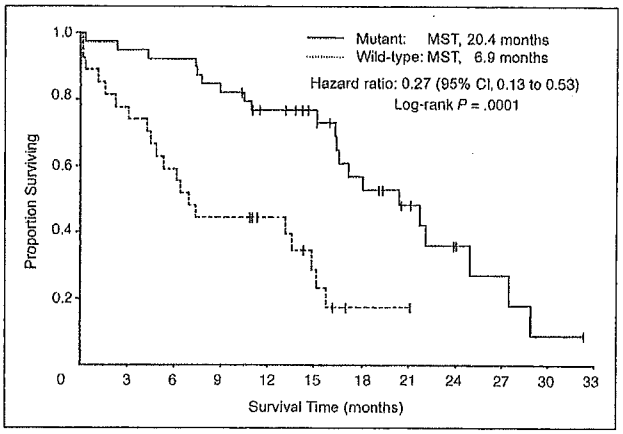

Fig 3. Kaplan-Meier plot of overall survival according to epidermal growth factor receptor (*EGFR*) mutation status. MST, median survival time.

survival benefit from gefitinib therapy in patients with *EGFR* mutations. Four of seven nonresponders with *EGFR* mutations also seemed to experience some clinical benefits because they had MR or a long SD (≥ 6 months). Among nine patients with SD, MR, or a long SD was observed only in patients with *EGFR* mutations. Although the sample size was too small to draw a firm conclusion, this finding suggests that *EGFR* mutations are also associated with clinical benefits in SD.

Table 4. *EGFR* Mutations Among Patient Subgroups

	EGFR Mutations		
	No. of Patients	%	*P*
Total	39/66	59	
Sex			.18
Female	18/26	69	
Male	21/40	53	
Smoking history			.003†
Never smokers	21/31	68	
Former smokers	10/12	83	
Current smokers	8/23	35	
Histologic diagnosis			—
Adenocarcinoma	38/62	61	
Squamous cell carcinoma	0/3	0	
Pleomorphic carcinoma	1/1	100	
Dominant subtype*			.059‡
Papillary	22/30	73	
Acinar	10/18	56	
BAC	5/9	56	
Solid	1/5	20	
BAC features*			.002
Yes	34/47	72	
No	4/15	27	

Abbreviations: *EGFR*, epidermal growth factor receptor; BAC, bronchioloalveolar carcinoma.
*Only patients with adenocarcinoma (n = 62).
†Comparison between never/former smokers and current smokers.
‡Comparison between patients with papillary-dominant adenocarcinoma and patients with other adenocarcinoma.

The *EGFR* mutations detected in this study were concentrated in three hotspots, deletions around codons 747 to 749, L858R, and G719S (or G719C), similar to the results of previous reports.[6-13] Some genetic variations existed among these mutations. Together with one of the hotspot mutations, additional missense mutations in exons 18 or 20 were detected in four patients. Among the 39 patients with *EGFR* mutations, an MD pattern was observed in 20 patients. Because the *EGFR* copy number in their tumor cells increased as the proportion of mutant alleles increased, this pattern was assumed to be caused not by homozygous mutations but by the selective amplification of the mutant alleles. Because one patient had a hemizygous mutation without amplification, the loss of wild-type alleles was also thought to be responsible for the pattern. The moderately increased copy number in patients with a BH pattern or wild-type *EGFR* can be explained by *EGFR* amplification and/or polysomy of chromosome 7.

Among the patients with *EGFR* mutations, three patients had PD and eight of the other 36 patients had tumor regrowth within 6 months. This suggests the presence of other factors associated with intrinsic or acquired resistance to gefitinib. Although any genetic alterations of *EGFR*-mutant tumors at the time of primary surgery were not significantly associated with clinical outcome, that might be because further alterations occurred after the primary surgery or after gefitinib administration. Recently, a secondary mutation (C → T at nucleotide 2369; T790M) in exon 20 was detected in patients with *EGFR*-mutant NSCLC who had tumor regrowth during gefitinib therapy after exhibiting an initial response to the agent; this mutation was thought to be associated with acquired resistance.[28,29] To elucidate the determinants and the mechanism of resistance to gefitinib, genetic analyses of tumor samples obtained after gefitinib treatment are needed.

In this study, three (11%) of the 27 patients with wild-type *EGFR* responded to gefitinib. Various explanations for this result are possible: (1) the mutational analyses of the responders were false-negative, (2) the *EGFR* mutations occurred in their tumors after the primary surgery, (3) the recurrent tumors originated from a source other than the analyzed tumor cells, or (4) other determinants of gefitinib sensitivity were present.

The results of multivariate analyses suggest that the *EGFR* copy number is another independent predictor of gefitinib sensitivity. It is noteworthy that an increased *EGFR* copy number was observed in two of the three responders with wild-type *EGFR*, and was significantly associated with a longer TTP among patients with wild-type *EGFR*. Because patients with *EGFR* mutations had favorable clinical outcome regardless of *EGFR* copy numbers, the impact of increased copy numbers on *EGFR*-mutant NSCLC was unclear. In the overall population, an increased *EGFR* copy number was significantly associated with a higher response

Table 5. Clinical Outcome Among Patient Subgroups (univariate analyses)

	Response Rate			Time to Progression		Overall Survival	
	No.	%	*P*	Median (months)	Log-Rank *P*	Median (months)	Log-Rank *P*
Total	66	53		5.2		16.3	
Sex			.033		.35		.30
Female	26	69		6.2		16.5	
Male	40	43		3.3		15.1	
Smoking history			.007		.026		.37
Never/former smokers	43	65		6.9		16.4	
Current smokers	23	30		2.6		15.1	
Dominant subtype*			.070		.28		.65
Papillary	30	67		7.7		16.4	
Others	32	44		4.2		15.7	
BAC features*			.012		.12		.19
Yes	47	64		6.5		16.5	
No	15	27		2.1		15.7	
Performance status			.77		.012		< .0001
0-1	50	52		5.2		17.1	
2-3	16	56		3.1		6.1	
EGFR mutations			< .0001		< .0001		.0001
Yes	39	82		12.6		20.4	
No	27	11		1.7		6.9	
EGFR copy number			.005		.038		.33
≥ 3.0	29	72		9.4		16.4	
< 3.0	37	38		2.6		15.7	

Abbreviation: BAC, bronchioloalveolar carcinoma; *EGFR*, epidermal growth factor receptor.
*Only patients with adenocarcinoma (n = 62).

rate and a longer TTP, but not with a longer OS, which might be because an increased copy number had an unfavorable impact on prognosis, as suggested by another study.[15] In chronic myeloid leukemia, as well as *BCR-ABL* mutations that were structurally corresponding to T790M in *EGFR*, an increased *BCR-ABL* gene copy number was reported as a determinant of resistance to imatinib, a BCR-ABL tyrosine kinase inhibitor.[30] Therefore, we should consider the possibility that an increased *EGFR* copy number is associated with not only sensitivity but also resistance to gefitinib.

Among adenocarcinomas, the presence of BAC features was significantly associated with gefitinib sensitivity and *EGFR* mutations, but the BAC component was relatively small in most of the responders. The dominant subtype associated with a higher response rate was not BAC but papillary; both of the two patients with BwFI had PD, and all three patients with pure papillary adenocarcinoma without BAC features had PR. The association between pathologic features and gefitinib sensitivity or *EGFR* mutations is also the subject of further investigation.

Table 6. Univariate and Multivariate Analyses of the Association Between Biomarkers and Clinical Outcome in Patients With Lung Adenocarcinoma (n = 62)

	Odds Ratios for Response		Hazard Ratios for TTP		Hazard Ratios for OS	
	Univariate	Multivariate*	Univariate	Multivariate*	Univariate	Multivariate*
EGFR mutations, yes *v* no	31.0	27.9	0.21	0.13	0.30	0.16
95% CI	7.2 to 134	3.7 to 209	0.11 to 0.38	0.06 to 0.29	0.15 to 0.62	0.06 to 0.39
P	< .001	.001	< .001	< .001	.001	< .001
EGFR copy number, ≥ 3.0 *v* < 3.0	4.0	4.6	0.57	0.42	0.80	0.59
95% CI	1.4 to 12	0.84 to 25	0.32 to 1.0	0.21 to 0.84	0.42 to 1.5	0.26 to 1.4
P	.011	.079	.050	.014	.49	.22

Abbreviations: TTP, time to progression; OS, overall survival; *EGFR*, epidermal growth factor receptor.
*In the multivariate analyses, age (continuous variable), sex (women *v* men), smoking history (never/former smokers *v* current smokers), dominant subtype (papillary *v* others), bronchioloalveolar carcinoma features (yes *v* no), performance status (0 to 1 *v* 2 to 3), prior chemotherapy (yes *v* no), *EGFR* mutations (yes *v* no), and *EGFR* copy number (≥ 3.0 *v* < 3.0) were included as factors.

In never/former smokers, both the *EGFR* mutation rate and the response rate were significantly higher than in current smokers. We speculate that *EGFR* mutations occur equally throughout the entire population, regardless of smoking history, and account for smoking-unrelated carcinogenesis. Because many other genetic alterations, like *KRAS* mutations, occur and induce lung adenocarcinoma more frequently in smokers, the *EGFR* mutation rate seems to be relatively lower in smokers with lung adenocarcinoma.

The response rate of 53% and the *EGFR* mutation rate of 59% observed in this study were higher than previously reported rates. These results can partially be attributed to the fact that the physicians tended to select patients with characteristics known to be predictive for gefitinib sensitivity: women, never-smokers, and patients with adenocarcinoma. Consequently, this cohort was not necessarily representative of unselected NSCLC populations in Japan. However, other recent studies have also shown relatively high frequencies (32% to 55%) of *EGFR* mutations in Japanese or East Asian patients with lung adenocarcinoma who underwent surgical resection.[7,9-11,13] The reason why such somatic mutations occur selectively in East Asian people remains unknown. Environmental or genetic factors common among East Asian populations should be investigated to answer this question.

Recently, no significant survival benefit of gefitinib was reportedly observed in the initial analysis of the IRESSA Survival Evaluation in Lung Cancer (ISEL) trial, a phase III trial comparing gefitinib monotherapy to a placebo as a second- or third-line treatment for patients with advanced NSCLC.[31] Because subgroup analyses of the trial suggested survival benefits in never smokers or Asian patients, the selection of patients is thought to be crucial when considering gefitinib treatment. Because the present study showed that the *EGFR* mutation status is a major determinant of gefitinib sensitivity, mutational analyses in patients with advanced NSCLC should be considered before deciding on a course of treatment.

In this study, we performed LCM and direct sequencing using methanol-fixed surgical specimens to obtain high-quality data. If we had analyzed only bulk tumor samples without LCM, nine of the 39 patients with *EGFR* mutations would have been misjudged as having wild-type *EGFR*. Thus such procedures with LCM are presently recommended for the detection of *EGFR* mutations. However, obtaining appropriate tumor samples is often difficult in patients with advanced NSCLC, and performing LCM and direct sequencing in all patients is not practical. Thus more practical methods for detecting the major *EGFR* mutations using small tumor samples contaminated with normal tissue should be developed and validated.

Other than *EGFR* mutations, some candidate predictive biomarkers have been studied. The *EGFR* copy number is the leading candidate, and it can also be detected by FISH. Practicality and accuracy should be assessed comparing FISH and quantitative real-time PCR. The impact of *ERBB2* mutations on clinical outcome remains to be investigated because we could not detect any mutations in *ERBB2* in the present study. Protein expression analyses by IHC are easier to perform than the genetic analyses, but their significance is still controversial. Further studies are required to evaluate the predictive values of these biomarkers and to determine whether they are independent predictors of gefitinib sensitivity or surrogate markers of *EGFR* mutations.

In conclusion, this study indicates that *EGFR* mutations and increased copy numbers predict better clinical outcome in patients with NSCLC treated with gefitinib. Further research and clinical trials are needed to incorporate these markers into clinical practice appropriately.

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We thank Yukihiro Yoshida, MD; Shunichi Watanabe, MD; Kenji Suzuki, MD; Hisao Asamura, MD; and Ryosuke Tsuchiya, MD, for providing surgical specimens and helpful advice, and Chizu Kina, Chie Hirama, Sanae Kobayashi, Yasuko Kuwahara, Go Maeno, Sachiyo Mimaki, Yoko Odaka, Shizuka Shinohara, Takahiro Taniguchi, and Mineko Ushiama for LCM and DNA analysis. We also thank Setsuo Hirohashi, MD, for his invaluable direction and support of the study.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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CORRESPONDENCE

Erlotinib in Lung Cancer

TO THE EDITOR: Shepherd and colleagues (July 14 issue)[1] report that erlotinib prolongs survival in non–small-cell lung cancer, as compared with placebo, after the failure of first-line or second-line chemotherapy. One disturbing aspect of this trial is that some patients underwent only one prior chemotherapy regimen before randomization. These same authors previously reported that docetaxel is superior to best supportive care after first-line chemotherapy.[2] Subsequent studies have confirmed the efficacy of docetaxel and shown that pemetrexed achieves similar results.[3] Did Shepherd and colleagues think that random assignment to placebo after the failure of first-line chemotherapy was ethically justifiable? The only patients for whom one could justify the assignment to placebo were those with a performance status of 3, who made up only 8.6 percent of all patients. Contrary to the authors' claim that inclusion of a placebo group was ethical, we believe that some patients were denied a therapeutic option known to be effective. Furthermore, the overall survival in the erlotinib group was inferior to that in published results with docetaxel and pemetrexed, suggesting that erlotinib should be used as third-line chemotherapy.

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Dr. Nabhan reports being an investigator in a study that is sponsored by Sanofi-Aventis.

1. Shepherd FA, Rodrigues Pereira JR, Ciuleanu T, et al. Erlotinib in previously treated non–small-cell lung cancer. N Engl J Med 2005; 353:123-32.
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TO THE EDITOR: Shepherd et al. and Tsao et al.[1] (July 14 issue) report an important study (BR.21) showing a survival benefit of erlotinib, but the results of the molecular analysis confused us. Recent East Asian studies[2-4] have strongly suggested that the mutational status of the epidermal growth factor receptor (EGFR) is the major determinant of tumor response and survival in patients with non–small-cell lung cancer who are treated with gefitinib, another EGFR tyrosine kinase inhibitor. Response rates among patients with an EGFR mutation were consistently higher than 80 percent in those studies. However, in the BR.21 study, the response rate among such patients was only 16 percent, and mutational status had no significant effect on survival, although the EGFR copy number correlated with responsiveness and survival. In our study,[2] the EGFR copy number was associated with gefitinib sensitivity, but we consider it to be a surrogate marker for EGFR mutations, rather than a true determinant.

THIS WEEK'S LETTERS

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These discrepancies may be due to differences in the ethnic background of the populations, the drugs, the study design, and, most important, the accuracy of the molecular analyses. To avoid fruitless controversy, standard methods for analyzing EGFR mutations and copy number should be established.

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TO THE EDITOR: Tsao and colleagues suggest that EGFR mutations were not valuable in predicting a benefit of erlotinib in the BR.21 trial. We believe that the mutation data in their report are inconclusive, for several reasons.

First, in Europe and North America,[1,2] the frequency of mutations is approximately 10 percent; Tsao et al. report mutations in more than 20 percent of the tumors. Second, only 47 percent of the mutations reported were drug-sensitive exon 19 deletions and L858R substitutions; these make up approximately 90 percent of the EGFR mutations in aggregate in the published data.[3] Third, the remaining cases showed "novel variant" mutations whose somatic nature was not established and that were not adequately confirmed. Fourth, these novel mutations were predominantly nucleotide transitions (92 percent), suggesting they were artifacts generated in the polymerase chain reaction (PCR).[4]

Finally, of the 427 patients treated with erlotinib, only 19 who had EGFR mutations could be evaluated. Among these 19 patients, only 8 had tumors with the well-established, drug-sensitive EGFR mutations. At our institution, 33 patients who had tumors containing one of these two common mutations have received erlotinib or gefitinib, and of these, 32 patients (97 percent) have had a response according to the Response Evaluation Criteria in Solid Tumors; the aggregate published response rate for both drugs and mutations is nearly 80 percent.

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DRS. SHEPHERD AND SEYMOUR REPLY: Patients entering the BR.21 trial after first-line chemotherapy were considered by their doctors not to be suitable candidates for second-line chemotherapy. Physicians had to attest to this, and reasons were recorded and monitored. Thus, these patients could not be compared with patients who participated in the trials cited by Drs. Nabhan and Bitran. We think, therefore, as did ethics review boards and regulatory authorities, that the inclusion of a placebo-control group was ethical, since further chemotherapy was not an option and alternative systemic treatments were unavailable.

It is inappropriate to compare the results of the BR.21, TAX 317,[1] and JMEI[2] trials, since their patient populations differed considerably. One third of the patients in the BR.21 study had a performance status of between 2 and 3 or 3, as compared with 25 percent of those in the TAX 317 trial and 12 percent of those in the JMEI study. Survival shortens with each successive chemotherapy regimen. In JMEI and TAX 317, 100 percent and 75 percent of patients, respectively, had undergone only one regimen, as compared with 50 percent of the patients in the BR.21 trial. These imbalances in prognostic factors alone could result in shorter survival, independent of treatment.

With regard to patients who were not eligible

for second-line chemotherapy, we think that EGFR inhibitor therapy is ethical on the basis of the BR.21 trial. Whether it should be considered electively for patients who are otherwise suitable candidates for chemotherapy awaits the results of an ongoing study comparing docetaxel with gefitinib.

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1. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000;18:2095-103.
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DR. TSAO AND COLLEAGUES REPLY: Of 177 tumor samples analyzed in the BR.21 trial, 21 samples (from 20 patients) were exon 19 deletions or L858R substitutions. This rate per patient of 11 percent for classic mutations is similar to that in other reports involving non-Asian patients.[1,2] The response rate among patients who could be evaluated who had classic mutations was 25 percent (two of eight). Although the rate is lower than that among Asian patients, it probably falls within the confidence interval of other series involving non-Asian patients who did not have adenocarcinoma.

The patients in the BR.21 trial who had classic mutations did not derive a greater survival benefit from erlotinib (hazard ratio for death, 0.67) than those with novel mutations (hazard ratio, 0.65) or those with wild-type EGFR (hazard ratio, 0.73). In the Tarceva Responses in Conjunction with Taxol and Carboplatin (TRIBUTE) trial,[3] 29 of 274 (11 percent) of the samples contained mutations (86 percent were classic mutations). Patients who had mutations had longer progression-free survival ($P<0.001$) and overall survival ($P<0.001$) than those who did not have mutations, regardless of the type of treatment (chemotherapy with or without erlotinib); the benefit of erlotinib was statistically nonsignificant. Among patients in the placebo group, those with classic mutations had a longer median survival than those with wild-type or novel EGFR variants (9.1, 3.5, and 3.5 months, respectively). This suggests that classic EGFR mutations have a prognostic influence that is independent of treatment and that the superior survival reported for mutation-positive patients in uncontrolled studies may not have been due to heightened sensitivity to the EGFR inhibitor.

Dr. Pao and colleagues suggested that novel variants are PCR artifacts caused by formalin fixation. The probability of the appearance of PCR artifacts correlates inversely with the number of cells used for the PCR.[1] However, we found novel mutations more frequently in large biopsy or resection specimens (61 percent) than in small biopsy specimens (41 percent). Chou et al.[4] also identified several new mutations (V689M, N700D, S720P, V765A, T783A, and G863D) in formalin-fixed tumors from patients who had a response, and the one patient in our series who had a complete response had a transition mutation (V742A[T→C]).

The role of mutations in patients with lung cancer receiving EGFR inhibitors is still evolving. We elected to publish all our mutation results and encourage others to do so as well. Only in this way will sufficient numbers accrue for all mutations to permit clinical correlation. We agree with Takano and Ohe that standard methods for EGFR-mutation analysis and copy number should be established. It is premature to say that EGFR-inhibitor therapy should not be prescribed for patients who do not have EGFR mutations.

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Expert Opinion

1. Introduction
2. Chemoradiotherapy for non-small cell lung cancer
3. Chemoradiotherapy for small cell lung cancer
4. Conclusion
5. Expert opinion

Monthly Focus: Oncologic

Chemoradiotherapy for lung cancer

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Chemoradiotherapy is a standard treatment for both unresectable locally advanced non-small cell lung cancer and limited-stage small cell lung cancer. Cisplatin-based chemotherapy with concurrent thoracic radiotherapy yields a 5-year survival rate of ~ 15% for patients with unresectable locally advanced non-small cell lung cancer. The state-of-the-art treatment for limited-stage small cell lung cancer is four cycles of chemotherapy with cisplatin plus etoposide combined with early concurrent twice-daily thoracic irradiation and prophylactic cranial irradiation after complete remission. A 5-year survival rate of ~ 25% is expected among patients treated for limited-stage small cell lung cancer. The incorporation of new agents, including target-based drugs, is one of the most promising strategies for improving the survival of patients.

Keywords: chemoradiotherapy, fractionation, non-small cell lung cancer, small cell lung cancer, target-based drug

Expert Opin. Pharmacother. (2005) 6(16):2793-2804

1. Introduction

Lung cancer has been the most common cancer worldwide since 1985; as of 2002, 1.35 million new cases have been reported, representing 12.4% of all new cancers. It was also the most common cause of death from cancer with 1.18 million deaths: 17.6% of the world total [1]. Lung cancer remains a highly lethal disease. Survival at 5 years measured by the Surveillance Epidemiology and End Results (SEER) programme in the US was 15%: the best recorded rate at the population level. Average survival in Europe is 10%, which is not much better than the 8.9% observed in developing countries [1]. Lung cancer in both men and women continues to be the most common fatal cancer in the US. In 2005 lung cancer is expected to account for 31 and 27% of all deaths from cancer in men and women, respectively, in the US [2]. Nearly 60,000 patients died of lung cancer in 2004, and mortality continues to rise in Japan. In particular, the number of elderly lung cancer patients in Japan is increasing. Lung cancer is the leading cause of cancer death in men and is anticipated to become the leading cause of cancer deaths in women in Japan.

Of lung cancer patients ~ 15 – 20% have small cell lung cancer (SCLC); the remaining patients typically have non-small cell lung cancer (NSCLC), such as adenocarcinoma, squamous cell carcinoma or large cell carcinoma. Surgery is the most effective curative treatment for early-stage NSCLC; however, only 30% of patients with NSCLC receive a curative resection [3]. Platinum-based chemotherapy offers a survival benefit and symptom relief for patients with metastatic NSCLC, and the combination of cisplatin-containing chemotherapy with thoracic radiotherapy is presently the standard treatment for patients with unresectable locally advanced NSCLC [4]. Of patients with unresectable locally advanced NSCLC ~ 15% could be cured by concurrent chemoradiotherapy [5]. Most patients with SCLC are not considered to be candidates for surgery. Combination chemotherapy consisting of cisplatin plus etoposide and concurrent twice-daily thoracic radiotherapy has yielded a 5-year survival rate of ~ 25% in limited-stage (LD) patients [6-8]. Chemoradiotherapy plays a very important

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Table 1. Survival after concurrent chemoradiotherapy for unresectable locally advanced NSCLC and resectable pN2 NSCLC after surgery.

Study	JCOG 9202 [11]	JCOG 9209 [12]
Objective	Unresectable	Resectable, pN2
Treatment	Cisplatin + vindesine + mitomycin Concurrent RT	Surgery with/ without induction cisplatin + vindesine
No. of patients	160	62
No. of institutions	27	18
c-stage		
IIIA/IIIB (%)	29/71	98/2
N0-1/N2/N3 (%)	12/54/34	0/98/2
Survival rate		
2 year (%)	35	36
3 year (%)	22	25
5 year (%)	16	17

JCOG: Japan Clinical Oncology Group; NSCLC: Non-small cell lung cancer.

role in the treatment of both patients with unresectable locally advanced NSCLC and patients with LD-SCLC.

2. Chemoradiotherapy for non-small cell lung cancer

2.1 Patient selection

Patients with stage IIIA or IIIB NSCLC without pleural effusion, pericardiac effusion and/or pleural dissemination are candidates for chemoradiotherapy. Only selected patients with stage IIIA NSCLC are candidates for surgery [9]. Surgery after induction chemotherapy for cytologically proven N2 NSCLC did not improve either overall survival or progression-free survival compared with thoracic radiotherapy [10]. Chemoradiotherapy for unresectable locally advanced NSCLC has achieved a long-term survival rate comparable to that of resectable N2 NSCLC after surgery (Table 1) [11,12]. Patients receiving chemoradiotherapy should have a good performance status and adequate organ function. Only few data exist about the feasibility of chemoradiotherapy in patients with poor performance status. If a patient receives radiotherapy with a radiation field including the contralateral hilum and > 50% of the lung, the patient should be excluded from radiotherapy. Pre-existing pulmonary fibrosis as identified on plain chest X-ray films is a very strong risk factor for treatment-related death after thoracic radiotherapy because of pneumonitis [13,14]. Thus, patients with pulmonary fibrosis identified on plain chest X-ray films should be excluded from radiotherapy.

2.2 Chemoradiotherapy versus radiotherapy alone or chemotherapy alone

A meta-analysis of 1780 cases in 11 randomised trials showed that cisplatin-containing chemoradiotherapy was significantly superior to radiotherapy alone in terms of survival [4]. Other meta-analyses have also demonstrated the survival superiority of chemoradiotherapy compared with radiotherapy alone, for patients with unresectable locally advanced NSCLC [15,16]. On the other hand, Kubota *et al.* reported that the addition of radiotherapy to chemotherapy for locally advanced NSCLC significantly improved the 2- and 3-year survival rates compared with chemotherapy alone [17]. Sculier *et al.* reported the results of a randomised Phase III trial that compared further chemotherapy and chest irradiation as a consolidation treatment after the achievement of a response to induction chemotherapy in patients with non-metastatic unresectable NSCLC [18]. No significant difference in survival or response duration was seen, but chest irradiation was associated with a significantly greater duration of local control than chemotherapy. Thus, the combination of cisplatin-containing chemotherapy with thoracic radiotherapy has been considered the standard treatment for patients with unresectable locally advanced NSCLC.

2.3 Timing of chemotherapy and radiotherapy

Randomised Phase III trials to compare the sequence schedule of chemoradiotherapy with concurrent chemoradiotherapy have been conducted by the Japan Clinical Oncology Group (JCOG) and by the Radiation Therapy Oncology Group (RTOG) (Table 2) [11,19]. In the JCOG trial, 320 patients with unresectable locally advanced NSCLC were randomised and received chemotherapy with cisplatin, vindesine and mitomycin followed by radiotherapy (sequential arm) or concurrent chemoradiotherapy (concurrent arm). The response rate for the concurrent arm was significantly higher (84%) than that of the sequential arm (66%) ($p = 0.0002$). The median survival duration was significantly longer in patients receiving concurrent therapy (16.5 months) compared with those receiving sequential therapy (13.3 months; $p = 0.03998$). The 2-, 3-, 4- and 5-year survival rates in the concurrent group (34.6, 22.3, 16.9 and 15.8%, respectively) were better than those in the sequential group (27.4, 14.7, 10.1 and 8.9%, respectively). The concurrent approach yielded a significantly higher response rate and enhanced survival duration compared with the sequential approach [11]. Similar results were reported by the RTOG trial. Survival was significantly superior in the concurrent arm, with a median survival time (MST) of 17 months and a 4-year survival rate of 21%, than in the sequential arm, with 14.6 months and 12%, respectively ($p = 0.046$). This report also demonstrated a long-term survival benefit of the concurrent delivery of cisplatin-based chemotherapy with thoracic radiotherapy, compared with the sequential delivery of these therapies [19]. In these trials, acute toxicities such as myelosuppression and oesophagitis were greater among patients in the concurrent arm than in the sequential arm. Based on these Phase III trials, concurrent chemoradiotherapy seems to result in a better survival than sequential therapy.

There are some limitations to the generalisation of the results of these trials because old-generation cisplatin-based

Table 2. Randomised trials of sequential versus concurrent chemoradiotherapy.

Author	Treatment	N	MST (months)	2-year survival	4-year survival	p value
Furuse [11]	CDDP + VDS + MMC sequential TRT	158	13.3	27.4	8.9 (5 years)	0.3998
	CDDP + VDS + MMC concurrent TRT	156	16.6	34.6	15.8 (5 years)	
Curran [19]	CDDP + VBL sequential TRT	610 (total)	14.6	32	12	-
	CDDP + VBL concurrent TRT		17.0	35	21	0.046
	CDDP + ETOP concurrent TRT (twice daily)		15.2	34	17	0.296
Fournel [29]	CDDP + VNR sequential TRT	101	14.5	26	14	0.24
	CDDP + ETOP concurrent TRT followed by CDDP + VNR	100	16.3	39	21	
Zatloukal [30]	CDDP + VNR sequential TRT	50	12.9	14.3	9.5 (3 years)	0.023
	CDDP + VNR concurrent TRT	52	16.6	34.2	18.6 (3 years)	

CDDP: Cisplatin; ETOP: Etoposide; MMC: Mitomycin; MST: Mean survival time; TRT: Thoracic radiotherapy; VBL: Vinblastine; VDS: Vindesine; VNR: Vinorelbine.

combination chemotherapies were used in these trials: cisplatin, vindesine plus mitomycin or cisplatin plus vinblastine [11,19]. These old-generation cisplatin-based chemotherapies could be combined with concurrent radiotherapy using a full dose. Several new anticancer agents were developed in the 1990s, such as irinotecan, paclitaxel, docetaxel, gemcitabine and vinorelbine [20-24]. The combination of platinum and these new agents is more effective than the old-generation combination chemotherapy for metastatic NSCLC [23,24]; however, these new agents cannot be combined with concurrent radiotherapy at the full dose [25-28]. A French cooperative group conducted a Phase III trial to compare sequential versus concurrent chemoradiotherapy for unresectable NSCLC [29]. The sequential arm consisted of three cycles of cisplatin plus vinorelbine followed by thoracic radiotherapy. The concurrent arm consisted of two cycles of cisplatin plus etoposide with concurrent thoracic radiotherapy followed by two cycles of cisplatin plus vinorelbine. A total of 205 patients were enrolled in this trial. The MST was 14.5 months for the sequential arm and 16.3 months for the concurrent arm. The 2-year survival rates were 26 and 39%, respectively [29]. Whereas concurrent therapy tended to be more favourable, the difference was not statistically significant ($p = 0.24$). Zatloukal *et al.* reported the results of a randomised study of concurrent versus sequential chemoradiotherapy with cisplatin and vinorelbine in locally advanced NSCLC [30]. The concurrent chemoradiotherapy arm demonstrated significant benefits in terms of response rate, overall survival and time to progression over the sequential chemoradiotherapy arm. However, they used a reduced dose of vinorelbine in both the concurrent and sequential arms. No data from Phase III trials comparing sequential full-dose, new-generation chemotherapy with concurrent reduced-dose, new-generation chemotherapy are available.

2.4 Fractionation

Radical radiotherapy for NSCLC is most commonly given in daily fractions, Monday to Friday, for a total dose of 60 – 70 Gy over 6 – 8 weeks [31,32]. Novel fractionation schedules have been explored, with the aim of improving local tumour control and survival without increasing late morbidity (Table 3). In hyperfractionated radiotherapy, the dose per fraction is reduced and the total dose is increased to give improved tumour control without increased late morbidity. The clinical trials of RTOG used hyperfractionated radiotherapy, 1.2 Gy/fraction b.i.d. for a total of 69.6 Gy [33]. However, this hyperfractionation schedule did not offer significant benefits when compared with conventional radiotherapy plus chemotherapy [34,35]. Schild *et al.* reported the results of a Phase III study that compared split-course accelerated hyperfractionated radiotherapy (AHFRT), at 1.5 Gy/fraction b.i.d. (60 Gy), with standard radiotherapy (STDRT) at 2 Gy/fraction/day (60 Gy) combined with concurrent chemotherapy

Table 3. Once-daily versus multiple-daily radiotherapy for unresectable NSCLC.

Author	Chemotherapy	Radiotherapy	N	MST (months)	2-year survival (%)	5-year survival (%)	p values
Sause [34,35]	None	2 Gy/day; 60 Gy 5 days/week continuous	163	11.4	21	5	-
	CDDP + VBL induction	2 Gy/day; 60 Gy 5 days/week, continuous	164	13.2	32	8	0.04
	None	1.2 Gy b.i.d.; 69.6 Gy 5 days/week continuous (HFRT)	163	12.0	24	6	NR
Schild [36]	CDDP + ETOP concurrent	2 Gy/day; 60 Gy 5 days/week continuous	117	14	37	13	
							0.4
	CDDP + ETOP concurrent	1.5 Gy b.i.d.; 60 Gy 5 days/week split (AHFRT)	117	15	40	20	
Saunders [37,38]	None	2 Gy/day; 60 Gy 5 days/week continuous	225	NR	20	NR	
							0.004
	None	1.5 Gy t.i.d., 54 Gy 7 days/week continuous (CHART)	338	NR	29	NR	
Belani [42]	CBDCA + PTX induction	2 Gy/day; 64 Gy 5 days/week continuous	56	14.9	34	NR	
							0.28
	CBDCA + PTX induction	1.5 – 1.8 – 1.5 Gy/day; 57.6 Gy 5 days/week continuous (HART)	56	20.3	44	NR	

AHFRT: Accelerated hyperfractionated radiotherapy; CBDCA: Carboplatin; CDDP: Cisplatin; CHART: Continuous hyperfractionated accelerated radiotherapy; ETOP: Etoposide; HART: Hyperfractionated accelerated radiation therapy; HFRT: Hyperfractionated radiotherapy; MST: Median survival time; NR: Not reported; NSCLC: Non-small cell lung cancer; PTX: Paclitaxel; VBL: Vinblastine; VNR: Vinorelbine.

[36]. The toxicity, tumour control and survival rates were similar with AHFRT and STDRT. JCOG retrospectively compared STDRT and AHFRT using data from six JCOG clinical trials [5]. AHFRT did not show a clear tendency to improve the survival of the patients with locally advanced NSCLC. Twice-daily fractionations at doses of 1.2 or 1.5 Gy/fraction were not superior, compared with standard once-daily fractionation, in patients with locally advanced NSCLC.

More recently, continuous hyperfractionated accelerated radiotherapy (CHART) and hyperfractionated accelerated radiation therapy (HART) have been investigated [37-43]. CHART consisted of 36 small fractions of 1.5 Gy given three-times daily, yielding 54 Gy administered on only 12 consecutive days, including the weekend. CHART, compared with conventional radiotherapy, provided a significant improvement in the survival of patients with NSCLC [37,38]; however, this result was obtained from randomised Phase III trials of radiotherapy alone. No randomised trials of chemoradiotherapy using CHART have been reported. HART consisted of a total dose of 57.6 Gy in 36 fractions delivered over 15 days using three-times daily fractions with a 4-h interval between fractions and an 8-h interval between on-cord fields [40-43]. Patients were not treated on weekends. The results of a Phase III study comparing standard thoracic radiotherapy with HART after induction chemotherapy for patients with unresectable NSCLC were reported by the Eastern Cooperative Oncology Group (ECOG) [42]; however, the study was closed prematurely because of poor patient accrual. Nevertheless, induction chemotherapy of carboplatin plus paclitaxel followed by HART resulted in an acceptable toxicity profile and a provocative efficacy, with a median survival of 20.3 months, in contrast to a median survival of 14.9 months in the standard thoracic radiotherapy arm [42]. Ishikura *et al.* reported the results of a pilot study of HART following induction cisplatin and vinorelbine for stage III NSCLC [43]. A total of 30 patients were enrolled in the study. The overall objective response rate was 83%, and the MST was 24 months. The 2- and 3-year survival rates were 50 and 32%, respectively [43]. Further investigations of CHART or HART with chemotherapy are warranted.

2.5 Selection of anticancer agents

In the 1980s to early 1990s, old-generation cisplatin-based chemotherapy, such as cisplatin plus etoposide, cisplatin plus vindesine, cisplatin plus vinblastin or cisplatin, vindesine plus

Table 4. Randomised Phase II study of chemoradiotherapy for unresectable NSCLC (CALGB94 31) [26].

No. of patients	Induction CT	RT (66Gy) + CT	CR (%)	RR (%)	MST (months)	3-year survival (%)
62	Gem 1250 mg/m^2 on days 1, 8, 22 and 29; CDDP 80 mg/m^2 on days 1 and 22	Gem 600 mg/m^2 on days 43, 50, 64 and 71; CDDP 80 mg/m^2 on days 43 and 64	13	74	18.3	28
58	PTX 225 mg/m^2 on days 1 and 22; CDDP 80 mg/m^2 on days 1 and 22	PTX 135 mg/m^2 on days 43 and 64; CDDP 80 mg/m^2 on days 43 and 64	33	67	14.8	19
55	VNR 25 mg/m^2 on days 1, 8, 15, 22 and 29; CDDP 80 mg/m^2 on days 1 and 22	VNR 15 mg/m^2 on days 43, 50, 64 and 71; CDDP 80 mg/m^2 on days 43 and 64	29	73	17.7	23

CDDP: Cisplatin; CR: Complete response; CT: Chemotherapy; Gem: Gemcitabine; MST: Median survival time; PTX: Paclitaxel; RR: Response rate; RT: Radiotherapy; VNR: Vinorelbine.

mitomycin, were commonly used in chemoradiotherapy according to sequential or concurrent schedules for the treatment of locally advanced NSCLC [5,11,19]. In the 1990s, several new anticancer agents were developed, including irinotecan, paclitaxel, docetaxel, gemcitabine and vinorelbine [20-24]. Most of these new agents have different mechanisms of action from those of the old-generation agents. A full dose of the old-generation combination chemotherapy could be combined with concurrent radiotherapy [11,19]. When combining new-generation chemotherapy and thoracic radiotherapy, however, either reduced-dose chemotherapy with concurrent thoracic radiotherapy or full-dose chemotherapy followed by sequential radiotherapy must be used [25-28]. Full-dose, old-generation combination chemotherapy combined with concurrent radiotherapy and reduced-dose, new-generation chemotherapy combined with concurrent thoracic radiotherapy have not yet been compared. Very few reports have compared chemotherapy regimens with concurrent thoracic radiotherapy. To evaluate the use of the new drugs, gemcitabine, paclitaxel and vinorelbine, in combination with cisplatin in patients with unresectable locally advanced NSCLC, the Cancer and Leukaemia Group B (CALGB) conducted a randomised Phase II study of two cycles of induction chemotherapy followed by two additional cycles of the same drugs with concomitant radiotherapy (Table 4) [26]. A total of 175 patients received four cycles of cisplatin 80 mg/m^2 on days 1, 22, 43 and 64 with gemcitabine 1250 mg/m^2 on days 1, 8, 22 and 29 and 600 mg/m^2 on days 43, 50, 64 and 71, or paclitaxel 225 mg/m^2 for 3 h on days 1 and 22 and 135 mg/m^2 on days 43 and 64, or vinorelbine at 25 mg/m^2 on days 1, 8, 15, 22 and 29 and at 15 mg/m^2 on days 43, 50, 64 and 71. Radiotherapy was initiated on day 43 at 2 Gy/day for a total dose of 66 Gy. The response rates after completion of radiotherapy were 74, 67 and 73% for the gemcitabine, paclitaxel and vinorelbine arms, respectively. The MSTs were 18.3 (95% confidence interval [CI] 13.8 – 23.6), 14.8 (95% CI 12 – 19.5) and 17.7 months (95% CI 12.4 – 24.7) for the gemcitabine, paclitaxel and vinorelbine arms, respectively [26]. No consistent standard chemotherapy regimens for chemoradiotherapy have been established.

Concomitant low-dose daily or weekly chemotherapies are also used for chemoradiotherapy as a radiosensitiser. Cisplatin or carboplatin have been commonly used in studies to investigate sensitising effects [44-47]. Of the numerous single-platinum studies, only one Phase III study demonstrated a survival benefit for the daily administration of cisplatin with thoracic radiotherapy [44]. Two studies demonstrated prolonged survival with concomitant, platinum-based, multidrug chemotherapy and hyperfractionated radiotherapy [48,49]. No data from large Phase III studies comparing full-dose chemotherapy with low-dose sensitising chemotherapy combined with concurrent radiotherapy for the treatment of locally advanced NSCLC have been reported. CALGB conducted a Phase III study to compare low-dose weekly carboplatin plus paclitaxel with concomitant radiotherapy (arm 1) and induction chemotherapy with full-dose carboplatin plus paclitaxel followed by the same concomitant chemoradiotherapy (arm 2) for stage III NSCLC [50]. A total of 366 patients were entered in the study. The median survival in arm 1 was 11.4 versus 14.0 months in arm 2, and the 1-year survival rates were 48 and 54%, respectively ($p = 0.154$). The median survival achieved in each of the treatment groups was low compared with other recent trials. This result indicated that low-dose weekly carboplatin plus paclitaxel with concomitant radiotherapy may be insufficient for the treatment of stage III NSCLC. Induction chemotherapy with full-dose carboplatin plus paclitaxel, followed by radiotherapy with concomitant low-dose weekly chemotherapy with carboplatin plus paclitaxel, was not superior in terms of survival compared with the same induction chemotherapy followed by radiotherapy alone [51]. Not only do the systemic effect of low-dose weekly or daily chemotherapy, such as carboplatin plus paclitaxel, remain unclear, but so do the radiosensitising effects.

The Southwest Oncology Group (SWOG) conducted a Phase II study of concurrent chemoradiotherapy with cisplatin

plus etoposide followed by consolidation docetaxel in patients with stage IIIB NSCLC [52]. Treatment consisted of cisplatin 50 mg/m^2 on days 1, 8, 29 and 36, etoposide 50 mg/m^2 on days 1 – 5 and 29 – 33, and concurrent thoracic radiotherapy, with a total dose of 61 Gy. Consolidation docetaxel was started 4 – 6 weeks after chemoradiotherapy at an initial dose of 75 mg/m^2. A total of 83 eligible patients were entered in this study. The median survival was 26 months, and the 1-, 2- and 3-year survival rates were 76, 54 and 37%, respectively [52]. Recently, long-term follow-up data revealing a 5-year survival rate of 29% was reported [53]. These results are much better than the results of the previous SWOG trial. To evaluate the feasibility and efficacy of docetaxel consolidation therapy following cisplatin, vinorelbine and concurrent thoracic radiotherapy in patients with unresectable stage III NSCLC, the authors conducted a feasibility study [54]. Among 97 patients the response rate was 82%, the median progression-free survival period was 12.8 months, and the MST was 30.8 months. Although this regimen was effective, the docetaxel consolidation compliance was very poor, with only one third of the patients completing all three cycles of consolidation docetaxel [54]. Phase III trials evaluating docetaxel consolidation have been initiated to validate these results.

2.6 Incorporation of target-based drugs

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors such as gefitinib, erlotinib and cetuximab are one of the most promising kinds of target-based agents for NSCLC [55,56]. Strong preclinical evidence indicates that EGFR inhibition is additive or synergistic with radiotherapy in NSCLC [57-61]. A large, randomised, Phase III trial comparing definitive dose radiotherapy with or without cetuximab in locally advanced head and neck cancer has been completed and reported [62]. The addition of cetuximab to radiotherapy improved locoregional control. More importantly, the MST was prolonged from 28 to 54 months, and the 3-year survival rate was increased from 44 to 57% in the treatment arm receiving radiation plus cetuximab [62]. CALGB 30106 and a multi-institutional Australian Phase I trial have shown that gefitinib can be added to concurrent chemoradiotherapy for stage III NSCLC without excessive toxicity [63,64]. A Phase I trial at the University of Chicago evaluated erlotinib with concurrent chemoradiotherapy in patients with stage III NSCLC [65]. Thus, the combination of gefitinib or erlotinib with chemoradiotherapy is a candidate strategy for improving the survival of patients with unresectable locally advanced NSCLC. JCOG has started a safety and efficacy trial of induction chemotherapy with cisplatin and vinorelbine followed by gefitinib and concurrent thoracic radiotherapy for unresectable locally advanced NSCLC (JCOG 0402-MF). SWOG 0023 is a large, Phase III, randomised trial comparing concurrent chemoradiotherapy and consolidation docetaxel with or without maintenance small-molecule therapy with gefitinib [66]. Unfortunately, SWOG 0023 was closed based on the interim analysis, which showed that the continuation of SWOG 0023 would not have shown a survival benefit for gefitinib. These results may indicate that the maintenance use of gefitinib after induction chemoradiotherapy does not improve the survival of patients with locally advanced NSCLC; however, the incorporation of EGFR tyrosine kinase inhibitors in chemoradiotherapy is still an attractive strategy for locally advanced NSCLC.

The combination of antiangiogenic agents and radiotherapy is also an attractive strategy. *In vivo* and *in vitro* studies supported that the combination of radiation with an antiangiogenic agent, angiostatin, improved tumour eradication without increasing deleterious effects [67]. Recent Phase III studies demonstrated the survival benefits of the anti-vascular endothelial growth factor antibody bevacizumab in addition to chemotherapy for several kinds of cancer including NSCLC [68]. Thus, a combination of chemoradiotherapy with bevacizumab is also a candidate strategy for improving the survival of patients with unresectable locally advanced NSCLC. Thalidomide is also well known as an antiangiogenic agent. ECOG is conducting a Phase III study of carboplatin, paclitaxel and radiotherapy with or without thalidomide in treating patients with stage III NSCLC (ECOG 3598) based on their pilot study [69].

COX-2 overexpression in lung cancer is a poor prognostic factor and COX-2 inhibitors add to the efficacy of both chemotherapy and radiotherapy. A pilot study has shown the feasibility of celecoxib with docetaxel plus radiation, and consolidation docetaxel plus cisplatin in inoperable stage IIIa and IIIb NSCLC [70]. Celecoxib (400 mg b.i.d.) administration continued as a maintenance therapy over 6 months for patients.

3. Chemoradiotherapy for small cell lung cancer

3.1 Patient selection

SCLC is generally classified into a two-stage system, LD and extensive disease (ED) [71,72]. In the consensus reports of the International Association of Lung Cancer, LD is defined as disease involvement of one haemithorax including ipsilateral pleural effusion and regional lymph nodes including ipsilateral hilar, bilateral mediastinal and bilateral supraclavicular [71,72]. Patients with LD-SCLC, except for those with ipsilateral malignant pleural effusion and ipsilateral pulmonary metastasis, are considered to be candidates for chemoradiotherapy. Patients requiring radiotherapy with a radiation field of > 50% of the lung, or those with pre-existent pulmonary fibrosis identified on plain chest X-ray films, should be excluded from chemoradiotherapy [6,13,14].

3.2 Standard chemoradiotherapy for small cell lung cancer

A meta-analysis including 13 trials and 2140 patients with LD-SCLC demonstrated a survival benefit of chemoradiotherapy, compared with chemotherapy alone [73]. The relative risk of

Table 5. Twice- versus once-daily radiotherapy for limited-stage small cell lung cancer.

Author	Chemotherapy	Radiotherapy	N	MST (months)	5-year survival (%)	p values
Turrisi [8]	CDDP + ETOP x four cycles	1.5 Gy b.i.d.; 45 Gy, 1st cycle continuous	211	23	26	
						0.04
	CDDP + ETOP x four cycles	1.8 Gy/day; 45 Gy, 1st – 2nd cycle continuous	206	19	16	
Bonner [75] Schild [76]	CDDP + ETOP x six cycles	1.5 Gy b.i.d.; 48 Gy, 4th – 5th cycles split	130	20.6	22	
						0.68
	CDDP + ETOP x six cycles	1.8 Gy/day; 50.4 Gy, 4th – 5th cycles continuous	132	20.6	21	

CDDP: Cisplatin; ETOP: Etoposide; MST: Median survival time.

death in the chemoradiotherapy group, compared with the chemotherapy group, was 0.86 (95% CI 0.78 – 0.94; p = 0.001), corresponding to a 14% reduction in the mortality rate. The benefit in terms of overall survival at 3 years was 5.4%. Based on this meta-analysis, chemoradiotherapy is presently regarded as the standard treatment for LD-SCLC. In this meta-analysis, non-platinum-based combination chemotherapies were commonly used, and only a few trials used platinum-based modern chemotherapy. Recently, cisplatin plus etoposide has become widely regarded as a standard chemotherapy for LD-SCLC, particularly because this regimen can be integrated with concurrent thoracic irradiation with acceptable toxicity [74]. Early thoracic irradiation with concurrent cisplatin plus etoposide chemotherapy is the state-of-the-art treatment for LD-SCLC.

A US intergroup trial demonstrated a survival benefit of twice-daily accelerated thoracic radiotherapy over once-daily radiotherapy with cisplatin plus etoposide for LD-SCLC (Table 5) [8]. A total of 417 LD-SCLC patients were randomised to receive a total of 45 Gy of concurrent thoracic radiotherapy, given either twice daily over a 3-week period or once daily over a period of 5 weeks. The median survival was 19 months for the once-daily group and 23 months for the twice-daily group. The 2- and 5-year survival rates were 41 and 16%, respectively, for patients receiving once-daily radiotherapy, and 47 and 26%, respectively, for the twice-daily group (p = 0.04 by the log-rank test) [8]. In contrast, another Phase III trial using split-course twice-daily radiotherapy failed to demonstrate a survival benefit of twice-daily radiotherapy with cisplatin plus etoposide [75,76]. A split radiotherapy schedule seems to diminish the benefit of twice-daily radiotherapy (Table 4).

The brain is one of the most common relapse sites of SCLC. However, the CNS is protected from anticancer drugs by the blood–brain barrier. Several Phase III trials have demonstrated that prophylactic cranial irradiation (PCI) reduces the incidence of brain metastasis in patients with SCLC, but no Phase III trials have demonstrated a survival benefit of PCI for patients with SCLC [77-79]. A meta-analysis using individual data for 987 patients with SCLC in complete remission (CR) who took part in seven trials comparing PCI with no PCI demonstrated a survival benefit [80]. The relative risk of death in the PCI group, compared with the no PCI group, was 0.84 (95% CI 0.73 – 0.97; p = 0.01), corresponding to a 5.4% increase in the rate of survival at 3 years (15.3% in no PCI group versus 20.7% in PCI group). This absolute improvement in 3-year survival (5.4%) was the same as that shown in the meta-analysis comparing chemotherapy with chemoradiotherapy for SCLC [73,80]. Thus, PCI for SCLC, in patients who achieved a CR, has similar power to improve survival as that of thoracic radiotherapy for LD-SCLC.

The state-of-the-art treatment for LD-SCLC is four cycles of combination chemotherapy with cisplatin plus etoposide, combined with early concurrent twice-daily thoracic irradiation 45 Gy. If patients achieve a CR, PCI should be administered. A 5-year survival rate of ~ 25% is expected using this state-of-the-art treatment for LD-SCLC.

3.3 Incorporation of new drugs

JCOG conducted a randomised, multi-centre Phase III study of irinotecan plus cisplatin versus etoposide plus cisplatin for previously untreated ED-SCLC (JCOG 9511) [81]. A total of 154 patients were randomised, 77 into each arm. The MST was 12.8 months in the irinotecan plus cisplatin arm and 9.4 months in the etoposide plus cisplatin arm. The irinotecan plus cisplatin arm showed a significantly better survival, compared with the standard treatment with etoposide plus cisplatin (p = 0.002; unadjusted one-sided log-rank test). Treatment with four cycles of irinotecan plus cisplatin every 4 weeks yielded a highly significant improvement in survival, with less myelosuppression in ED-SCLC patients, over the standard etoposide plus cisplatin treatment [81]. Thus, the incorporation of irinotecan into the treatment of LD-SCLC is considered to be one of the most important strategies for improving the survival of LD-SCLC patients. Concurrent twice-daily thoracic