21. Kennedy TC, McWilliams A, Edell E, et al. Bronchial Intraepithelial Neoplasia/Early Central Airways Lung Cancer: ACCP Evidence-Based Clinical Practice Guidelines (2nd edn). *Chest*. 2007;132: 221–233.
22. Auerbach O, Stout AP, Hammond EC, et al. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *N Engl J Med*. 1961;265:253–267.
23. Jeremy George P, Banerjee AK, Read CA, et al. Surveillance for the detection of early lung cancer in patients with bronchial dysplasia. *Thorax*. 2007;62:43–50 [Epub July 6, 2006].
24. Goya T, Asamura H, Yoshimura H, et al. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese lung cancer registry study. *Lung Cancer*. 2005;50:227–234 [Epub August 2, 2005].
25. Sibley GS. Radiotherapy for patients with medically inoperable Stage I nonsmall cell lung carcinoma: smaller volumes and higher doses—a review. *Cancer*. 1998;82:433–438.
26. Mehta M, Scrimger R, Mackie R, et al. A new approach to dose escalation in non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys*. 2001;49:23–33.
27. Lagerwaard FJ, Senan S, van Meerbeeck JP, et al. Has 3-D conformal radiotherapy (3D CRT) improved the local tumour control for stage I non-small cell lung cancer? *Radiother Oncol*. 2002;63:151–157.
28. Le QT, Loo BW, Cotrutz C, et al. Results of a phase I dose-escalation study using single-fraction stereotactic radiotherapy for lung tumors. *J Thorac Oncol*. 2006;1:802–809.

British Journal of Cancer (2008) 98, 693–696

www.bjcancer.com

Short Communication

Randomised phase II trial of irinotecan plus cisplatin *vs* irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

I Sekine*[,1], H Nokihara[1], K Takeda[2], Y Nishiwaki[3], K Nakagawa[4], H Isobe[5], K Mori[6], K Matsui[7], N Saijo[3] and T Tamura[1]

[1]Division of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, Tokyo, Japan; [2]Department of Clinical Oncology, Osaka City General Hospital, Osaka, Japan; [3]Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwa, Japan; [4]Department of Medical Oncology, Kinki University School of Medicine, Sayama, Japan; [5]Department of Pulmonary Disease, National Hospital Organization Hokkaido Cancer Center, Sapporo, Japan; [6]Department of Thoracic Diseases, Tochigi Prefectural Cancer Center, Utsunomiya, Japan; [7]Department of Internal Medicine, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Japan

Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n=55$) or without ($n=54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

British Journal of Cancer (2008) **98,** 693–696. doi:10.1038/sj.bjc.6604233 www.bjcancer.com
Published online 5 February 2008

Keywords: small-cell lung cancer; chemotherapy; irinotecan; etoposide; three drug combination

Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P=0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003).

*Correspondence: Dr I Sekine, Division of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan; E-mail: isekine@ncc.go.jp
Received 15 October 2007; revised 2 January 2008; accepted 9 January 2008; published online 5 February 2008

The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0\times10^3\,\mu l^{-1}$, neutrophil count $\geqslant 2.0\times10^3\,\mu l^{-1}$, haemoglobin $\geqslant 9.5\,g\,dl^{-1}$, platelet count $\geqslant 100\times10^3\,\mu l^{-1}$, total serum bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, hepatic transaminases $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant 1.2\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, and $PaO_2\geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

Clinical Studies

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1–3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0×10^3 μl^{-1}. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}\text{)} = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP ($n=54$)	IPE ($n=55$)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP ($n=54$) No. (%)	IPE ($n=55$) No. (%)
Number of cycles delivered		
6[a]	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment[†]		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

[†]$P = 0.013$ by χ^2 test. [a]Protocol violation.

patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P=0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P=0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P=0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P=0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen[a]
	IP ($n=54$) Median (range)	IPE ($n=55$) Median (range)	IPE ($n=30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

[a]From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n=54$)			IPE ($n=55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)*
Neutropaenia	17	11	28 (52)	24	28	52 (95)*
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)†
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)‡
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

*$P<0.001$; †$P<0.01$; and ‡$P=0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P=0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P=0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P=0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

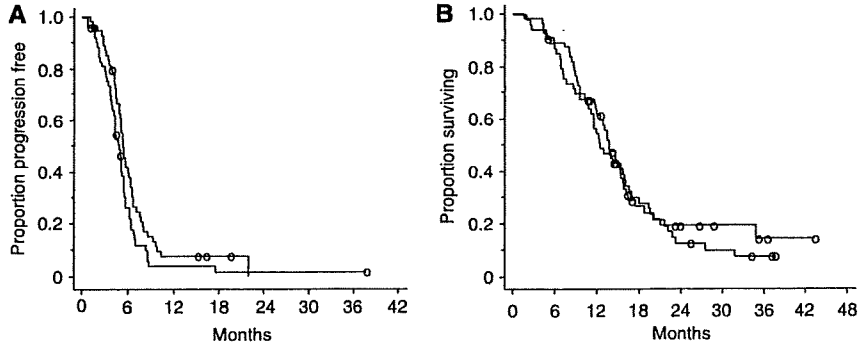

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of 60 mg m^{-2} on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of 30 mg m^{-2} on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

ACKNOWLEDGEMENTS

This study was supported, in part, by Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan. We thank the following doctors for their care for patients and valuable suggestion and comments on this study: Takahiko Sugiura, Aichi Cancer Center; Yoshinobu Ohsaki, Asahikawa Medical College; Shinzo Kudoh, Osaka City University Medical School; Makoto Nishio, Cancer Institute Hospital; Hiroshi Chiba, Kumamoto Community Medical Center; Koichi Minato, Gunma Prefectural Cancer Center; Naoyuki Nogami, Shikoku Cancer Center; Hiroshi Ariyoshi, Aichi Cancer Center Aichi Hospital; Takamune Sugiura, Rinku General Medical Center; Akira Yokoyama, Niigata Cancer Center Hospital; and Koshiro Watanabe, Yokohama Municipal Citizen's Hospital. We also thank Fumiko Koh, Yuko Yabe and Mika Nagai for preparation of the paper.

REFERENCES

Armitage P, Berry G, Matthews J (2002) Survival analysis. In *Statistical Methods in Medical Research*, Armitage P, Berry G, Matthews J (eds), pp 568–590. Oxford: Blackwell Science Ltd

Fukuoka M, Furuse K, Saijo N, Nishiwaki Y, Ikegami H, Tamura T, Shimoyama M, Suemasu K (1991) Randomized trial of cyclophosphamide, doxorubicin, and vincristine *vs* cisplatin and etoposide *vs* alternation of these regimens in small-cell lung cancer. *J Natl Cancer Inst* **83**: 855–861

Hanna N, Bunn Jr PA, Langer C, Einhorn L, Guthrie Jr T, Beck T, Ansari R, Ellis P, Byrne M, Morrison M, Hariharan S, Wang B, Sandler A (2006) Randomized phase III trial comparing irinotecan/cisplatin with etoposide/cisplatin in patients with previously untreated extensive-stage disease small-cell lung cancer. *J Clin Oncol* **24**: 2038–2043

Hryniuk WM, Goodyear M (1990) The calculation of received dose intensity. *J Clin Oncol* **8**: 1935–1937

Jackman DM, Johnson BE (2005) Small-cell lung cancer. *Lancet* **366**: 1385–1396

Liu PY, Dahlberg S, Crowley J (1993) Selection designs for pilot studies based on survival. *Biometrics* **49**: 391–398

Loehrer Sr PJ, Ansari R, Gonin R, Monaco F, Fisher W, Sandler A, Einhorn LH (1995) Cisplatin plus etoposide with and without ifosfamide in extensive small-cell lung cancer: a Hoosier Oncology Group study. *J Clin Oncol* **13**: 2594–2599

Mavroudis D, Papadakis E, Veslemes M, Tsiafaki X, Stavrakakis J, Kouroussis C, Kakolyris S, Bania E, Jordanoglou J, Agelidou M, Vlachonicolis J, Georgoulias V (2001) A multicenter randomized clinical trial comparing paclitaxel–cisplatin–etoposide *vs* cisplatin–etoposide as first-line treatment in patients with small-cell lung cancer. *Ann Oncol* **12**: 463–470

Niell HB, Herndon II JE, Miller AA, Watson DM, Sandler AB, Kelly K, Marks RS, Perry MC, Ansari RH, Otterson G, Ellerton J, Vokes EE, Green MR (2005) Randomized phase III intergroup trial of etoposide and cisplatin with or without paclitaxel and granulocyte colony-stimulating factor in patients with extensive-stage small-cell lung cancer: Cancer and Leukemia Group B Trial 9732. *J Clin Oncol* **23**: 3752–3759

Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* **346**: 85–91

Pujol JL, Daures JP, Riviere A, Quoix E, Westeel V, Quantin X, Breton JL, Lemarie E, Poudenx M, Milleron B, Moro D, Debieuvre D, Le Chevalier T (2001) Etoposide plus cisplatin with or without the combination of 4′-epidoxorubicin plus cyclophosphamide in treatment of extensive small-cell lung cancer: a French Federation of Cancer Institutes multicenter phase III randomized study. *J Natl Cancer Inst* **93**: 300–308

Roth BJ, Johnson DH, Einhorn LH, Schacter LP, Cherng NC, Cohen HJ, Crawford J, Randolph JA, Goodlow JL, Broun GO, Omura GA, Greco FA (1992) Randomized study of cyclophosphamide, doxorubicin, and vincristine *vs* etoposide and cisplatin *vs* alternation of these two regimens in extensive small-cell lung cancer: a phase III trial of the Southeastern Cancer Study Group. *J Clin Oncol* **10**: 282–291

Sekine I, Nishiwaki Y, Noda K, Kudoh S, Fukuoka M, Mori K, Negoro S, Yokoyama A, Matsui K, Ohsaki Y, Nakano T, Saijo N (2003) Randomized phase II study of cisplatin, irinotecan and etoposide combinations administered weekly or every 4 weeks for extensive small-cell lung cancer (JCOG9902-DI). *Ann Oncol* **14**: 709–714

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**: 205–216

Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 248 (2007) 292–298
CANCER Letters
www.elsevier.com/locate/canlet

Down-regulation of survivin by ultraviolet C radiation is dependent on p53 and results in G_2–M arrest in A549 cells

Masato Ikeda [a], Isamu Okamoto [a,*], Kenji Tamura [b], Taroh Satoh [a], Kimio Yonesaka [a], Masahiro Fukuoka [a], Kazuhiko Nakagawa [a]

[a] *Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan*
[b] *Department of Medical Oncology, Kinki University School of Medicine Nara Hospital, Nara, Japan*

Received 27 May 2006; received in revised form 5 July 2006; accepted 2 August 2006

Abstract

Deregulation of survivin expression is implicated in tumorigenesis. To examine the regulation of survivin expression in response to DNA damage, we exposed A549 human lung cancer cells to ultraviolet C (UVC) radiation, which induces DNA single-strand breakage. UVC irradiation induced G_2–M arrest that was accompanied by accumulation of p53 and subsequent down-regulation of survivin. Depletion of p53 by RNA interference prevented the UVC-induced down-regulation of survivin. Furthermore, depletion of survivin resulted in G_2–M arrest, suggesting that down-regulation of survivin by p53 contributes to the p53-dependent G_2–M checkpoint triggered by DNA damage.

Keywords: Survivin; p53; RNA interference; G_2–M arrest; Ultraviolet C

1. Introduction

Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, is thought to play an important role in regulation of both apoptosis and cell division [1,2]. It is present in only small amounts in terminally differentiated normal cells but is overexpressed in almost all types of human malignancy [3–8]. Such overexpression of survivin is associated with poor prognosis in affected individuals, an increased rate of tumor recurrence, and resistance to certain anticancer agents and radiation [9,10].

The expression of survivin is regulated in a cell cycle-dependent manner. The promoter of the survivin gene possesses features typical of genes that are expressed at G_2–M phase of the cell cycle. Indeed, survivin is most abundant in cells at G_2–M and associates with the mitotic spindle of dividing cells [2]. Survivin interacts with Aurora B and inner centromere protein (INCENP), and the complex of Aurora B–INCENP–survivin monitors the integrity of the mitotic spindle [11]. It has been suggested that survivin controls the elimination by apoptosis of cells with an improperly formed mitotic spindle [3,12]. Overexpression of survivin in cancer may overcome cell cycle checkpoints and thereby facilitate aberrant progression of

* Corresponding author. Tel.: +81 72 366 0221; fax: +81 72 360 5000.
E-mail address: okamoto@dotd.med.kindai.ac.jp (I. Okamoto).

0304-3835/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2006.08.005

transformed cells through mitosis. Although deregulation of survivin expression is an important event in tumorigenesis, the molecular mechanisms of survivin regulation are not fully understood.

The tumor suppressor p53 blocks progression of cells through the cell cycle or induces apoptosis [13,14]. Following its induction in response to DNA damage, p53 up-regulates the expression of various genes that contribute to cell cycle arrest, DNA repair, or apoptosis. It also negatively regulates the expression of a separate set of genes [15–18]. The functional loss of wild-type p53 has been shown to be associated with up-regulation of survivin expression in human cancers [19–21]. We have previously shown that the amounts of survivin mRNA and protein in cell lines positive for wild-type p53 decreased markedly after induction of p53 by adriamycin, which causes DNA double-strand breakage [22]. However, no such down-regulation of survivin was apparent in cell lines with mutated or null p53 alleles. These observations have suggested that p53 negatively regulates the expression of survivin in response to DNA damage.

In the present study, we show that exposure of p53-positive A549 human lung cancer cells to ultraviolet C (UVC) radiation, which induces DNA single-strand breakage, resulted in down-regulation of survivin expression after the induction of p53. Depletion of p53 by RNA interference (RNAi) prevented this down-regulation of survivin in cells exposed to UVC. Furthermore, RNAi-mediated depletion of survivin resulted in growth arrest in G_2–M phase of the cell cycle. These findings suggest that negative regulation of survivin by p53 contributes to the p53-dependent G_2–M checkpoint.

2. Materials and methods

2.1. Cell culture and irradiation

A549 cells were provided by Tohoku University (Miyagi, Japan). The cells were cultured under a humidified atmosphere of 5% CO_2 at 37 °C in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum. Each batch of cells was discarded after 20 generations, and new batches were obtained from frozen stocks. Cells were exposed to UVC (30 J/m^2) with a Hoefer UVC 500 Ultraviolet Crosslinker (Amersham Pharmacia Biotech, Piscataway, NJ).

2.2. Immunoblot analysis

Cells were harvested by exposure to trypsin–EDTA, washed with phosphate-buffered saline (PBS), and lysed in a solution containing 30 mM HEPES, 1% Triton X-100, 10% glycerol, 5 mM $MgCl_2$, 25 mM NaF, 1 mM EDTA, and 10 mM NaCl. Equal amounts of lysate protein were fractionated by SDS–polyacrylamide gel electrophoresis at 100 V for 80 min at room temperature. The separated proteins were transferred to a nitrocellulose membrane, which was then probed for 2 h at room temperature with various primary antibodies, including affinity-purified rabbit polyclonal anti-survivin (R&D Systems, Minneapolis, MN), mouse monoclonal anti-p53 (Santa Cruz Biotechnology, Santa Cruz, CA), and affinity-purified rabbit polyclonal anti-β-actin (Sigma–Aldrich, St. Louis, MO). Immune complexes were detected with horseradish peroxidase-conjugated goat antibodies to rabbit immunoglobulin G (Amersham Biosciences, Little Chalfont, UK) or sheep antibodies to mouse immunoglobulin G (Santa Cruz Biotechnology) and with a chemiluminescence detection system (Perkin-Elmer, Boston, MA).

2.3. Flow cytometry

Cells were harvested, washed with PBS, fixed with 70% methanol, washed again with PBS, and stained with propidium iodide (0.05 mg/ml) in a solution containing 0.1% Triton X-100, 0.1 mM EDTA, and RNase A (0.05 mg/ml). The stained cells ($\sim 1 \times 10^5$) were than analyzed for DNA content with a flow cytometer (FACScaliber; Becton–Dickinson).

2.4. RNAi

Small interfering RNA (siRNA) duplexes specific for survivin or p53 mRNAs were synthesized by Dharmacon Research (Lafayette, CO) with the use of 2′-ACE protection chemistry. The survivin siRNA corresponded to nucleotides 206–224 of the coding region (GenBank Accession No. NM001168), whereas the p53 siRNA corresponded to nucleotides 775–793 of the coding region. BLAST searches of the human genome database were performed to ensure that the siRNA sequences would not target other gene transcripts. Cells in the exponential phase of growth were plated at a density of 3×10^4 cells per well in 12-well culture plates, cultured for 24 h, and then transfected with siRNA (300 nM) with the use of Oligofectamine in OPTI-MEM (Invitrogen, Carlsbad, CA). Control cells were treated with a scrambled siRNA duplex (Dharmacon).

2.5. Statistical analysis

Data are presented as means ± SD and were analyzed by Student's two-tailed t test (Stat View; SAS Institute, Cary, NC). A p value of <0.05 was considered statistically significant.

3. Results

3.1. UVC radiation inhibits A549 cell proliferation and induces G_2–M arrest

To evaluate the effect of UVC on A549 cell proliferation, we counted the number of viable cells at various times after irradiation. UVC treatment resulted in a 70% reduction in the number of viable cells compared with that for untreated cells at 48 h and a 60% reduction at 72 h (Fig. 1A). Flow cytometric analysis of cell cycle distribution revealed that this inhibition of cell proliferation by UVC was accompanied by an approximately twofold increase in the proportion of cells in G_2–M at 24 h (25.8% versus 13.4%), at 48 h (17.1% versus 7.9%) and at 72 h (12.3% versus 6.1%) compared with untreated cells (Fig. 1B), whereas irradiation had no marked effect on the sub-G_1 (apoptotic) population. These data indicated that treatment of A549 cells with UVC results in growth arrest at the G_2–M phase of the cell cycle.

3.2. UVC exposure induces p53 up-regulation followed by survivin down-regulation

Given that p53 mediates cell cycle arrest at the G_2–M transition in response to DNA damage and that we recently showed that down-regulation of survivin expression follows the accumulation of p53 in cells subjected to DNA double-strand breakage [22], we next examined whether survivin and p53 are functionally linked in A549 cells treated with UVC, which induces DNA single-strand breakage. Immunoblot analysis revealed that the abundance of p53 was increased 6 h after UVC exposure, reached a peak at 24 h, and then gradually returned to basal levels by 72 h (Fig. 2). In contrast, the amount of survivin began to decline at 48 h and its down-regulation was more pronounced at 72 h.

To determine whether p53 negatively regulates survivin expression, we examined the effect of UVC radiation on the abundance of survivin in cells depleted of p53 by RNAi. In cells transfected with a control (scrambled) siRNA or in nontransfected cells, the abundance of p53 was increased at 18 h after UVC exposure and the amount of

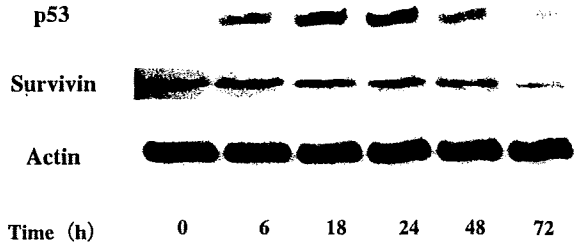

Fig. 2. Effects of UVC on the abundance of p53 and survivin in A549 cells. Total cellular protein extracted at the indicated times after exposure of cells to UVC (30 J/m^2) was subjected to immunoblot analysis with antibodies to p53, to survivin, or to β-actin (loading control). Data are representative of three independent experiments.

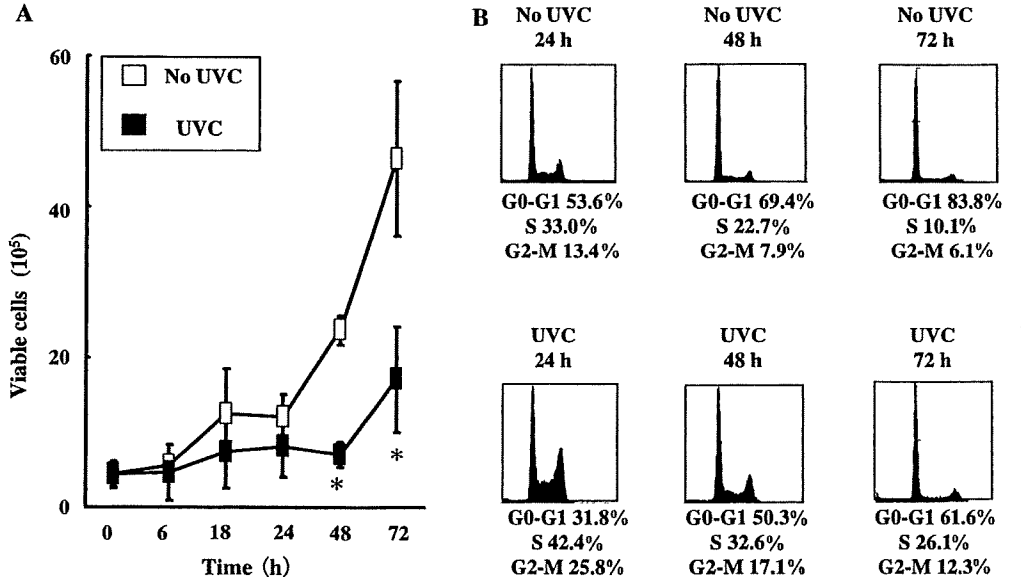

Fig. 1. Effects of UVC on the proliferation and cell cycle distribution of A549 cells. (A) Cell proliferation was evaluated by counting the number of viable cells by trypan blue staining at the indicated times after UVC irradiation (30 J/m^2). Data are means ± SD of values from three independent experiments. *$p < 0.05$ versus the corresponding value for cells not exposed to UVC. (B) Cell cycle distribution was analyzed by propidium iodide staining and flow cytometry at 24, 48 h and 72 h after UVC exposure. The percentages of cells at various stages of the cell cycle are indicated, and the data are representative of three independent experiments.

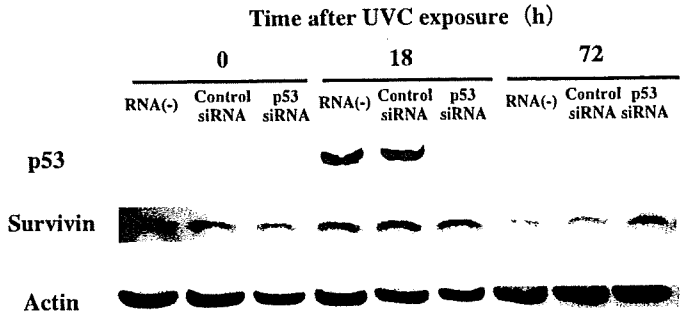

Fig. 3. Effect of UVC on the abundance of survivin in A549 cells depleted of p53 by RNAi. Cells were transfected (or not) with an siRNA specific for p53 mRNA or with a control (scrambled) siRNA, exposed to UVC (30 J/m^2), and subjected to immunoblot analysis with antibodies to p53, to survivin, or to β-actin at the indicated times after irradiation. Data are representative of three independent experiments.

survivin was decreased at 72 h (Fig. 3). In contrast, in cells transfected with an siRNA specific for p53 mRNA, UVC failed to increase p53 expression and had no effect on the level of survivin. These results thus indicated that induction of p53 by exposure of cells to UVC is necessary for down-regulation of survivin.

3.3. Ablation of survivin inhibits cell proliferation and induces G_2–M arrest

We next examined the effects of UVC irradiation in cells depleted of survivin by RNAi. The abundance of survivin was greatly reduced in cells transfected with an siRNA specific for survivin mRNA compared with that in nontransfected cells or cells transfected with a control (scrambled) siRNA (Fig. 4A). Cell proliferation (as evaluated from viable cell number) was also inhibited by 60% or 70% in cells subjected to transfection with the survivin siRNA for 48 or 72 h, respectively, compared with that apparent in nontransfected cells (Fig. 4B). The viable cell count was not affected by transfection with the control siRNA. Flow cytometry revealed that transfection of A549 cells with the survivin siRNA resulted in a marked increase in the proportion of cells in G_2–M at 48 and 72 h compared with that apparent for nontransfected cells or cells transfected with the control siRNA (Fig. 4C and D). There was no difference in the proportion of sub-G_1 cells among the three treatment groups.

4. Discussion

Several genes whose products play a role in control of the G_2–M transition of the cell cycle, including stathmin, Map4, cyclin B1, Cdc2, and Cdc25c, have been shown to be negatively regulated by p53 [15–18]. Repression of the expression of these genes in response to DNA damage requires wild-type p53 and contributes to a DNA damage-induced G_2–M checkpoint [23,24]. Survivin, a member of the IAP family of proteins, is maximally expressed at G_2–M and physically associates with microtubules of the mitotic spindle [2]. Previous studies have suggested that the expression of survivin is also subject to negative regulation by p53 [25–27], but the mechanism of such regulation has been unclear. We have now shown that exposure of the human lung cancer cell line A549 to UVC, which induces DNA single-strand breakage, resulted in the induction of endogenous p53 and a subsequent decrease in survivin expression. These observations are consistent with those of our previous study showing that survivin expression is repressed subsequent to p53 accumulation in cells treated with adriamycin [22], which induces DNA double-strand breakage. To investigate the possible role of p53 in the down-regulation of survivin induced by DNA damage, we depleted A549 cells of p53 by RNAi. Prevention of endogenous p53 accumulation in cells irradiated with UVC was found to block the repression of survivin expression, providing direct evidence that p53 is required for this effect of UVC. These data thus constitute further support for the notion that the survivin gene is a target of negative regulation by p53 in response to DNA damage.

The time course of survivin protein repression following UVC (DNA single-strand breakage)-induced p53 accumulation was almost identical to that observed in the cells having DNA double-strand breakage [22]. These results suggest that p53-dependent survivin suppression in response to these two types of DNA damage may share the common mechanisms at transcriptional level. Hoffmann et al. proposed that direct binding of p53 to a consensus binding site in the survivin gene promoter mediates transcriptional repression of the

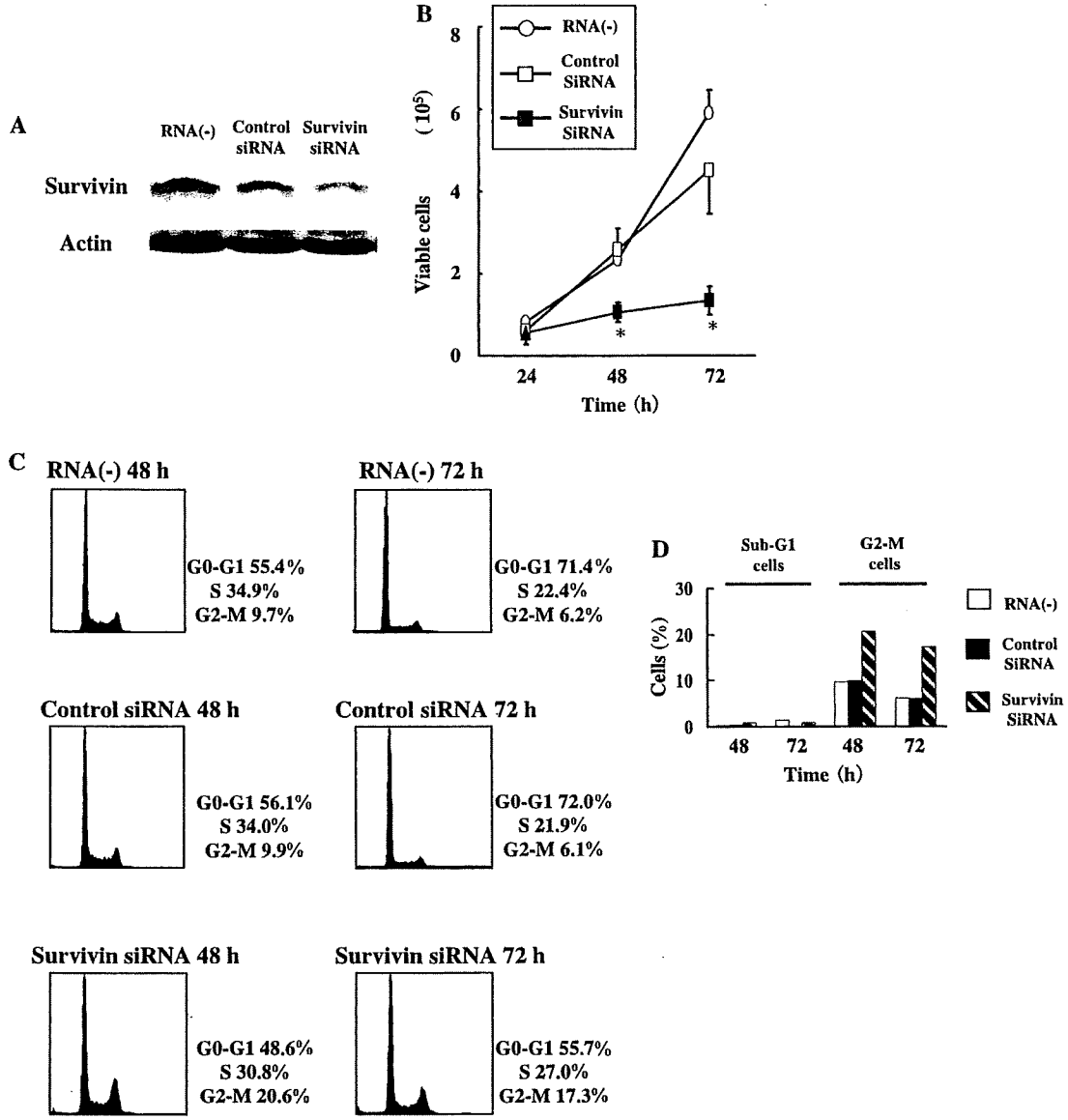

Fig. 4. Effects of survivin depletion by RNAi on the proliferation and cell cycle distribution of A549 cells. (A) Cells were transfected (or not) with a siRNA specific for survivin mRNA or with a control siRNA and were then subjected to immunoblot analysis with antibodies to survivin or to β-actin. Data are representative of three independent experiments. (B) Cells transfected for 24, 48, or 72 h as in (A) were evaluated for cell proliferation by counting the number of viable cells as revealed by staining with trypan blue. Data are means ± SD of values from three independent experiments. $*p < 0.05$ versus the corresponding value for nontransfected cells or cells transfected with the control siRNA. (C) The cell cycle distribution of cells transfected for 48 or 72 h as in (A) was determined by flow cytometry. The percentages of cells at various stages of the cell cycle are indicated. Data are representative of three independent experiments. (D) The percentages of sub-G_1 and G_2–M cells in the experiment shown in (C).

survivin gene by p53 [25]. In contrast, Mirza et al. suggested that chromatin deacetylation in the survivin promoter might contribute to p53-dependent repression of survivin gene expression in the absence of direct binding of p53 to the promoter DNA [26]. In the present study, repression of survivin expression was apparent 24 h after endogenous p53 accumulation, consistent with the results of our previous study [22]. This delay suggests that the mechanism of transcriptional inhibition of the survivin gene by p53 may be indirect. The repression of Cdc2 gene expression by p53 is mediated by a member of the E2F family of transcription factors subsequent to up-regulation of p21 and dephosphorylation of pRB family proteins [17]. However, UV-induced accumulation of p53 and subsequent

down-regulation of survivin have been observed in mouse embryonic fibroblasts derived from p21-null mice [29], suggesting that the ability of p53 to repress survivin gene expression is independent of its ability to up-regulate p21. The molecular mechanism by which p53 induces repression of survivin gene expression in response to DNA damage thus requires further investigation.

To examine the biological consequences of survivin gene repression in cells subjected to DNA damage, we depleted A549 cells of survivin by RNAi. Depletion of survivin resulted in growth arrest in G_2–M phase of the cell cycle, consistent with previous observations [28–31]. Survivin was originally proposed to perform an antiapoptotic function, but this issue remains controversial [29,32]. Indeed, several lines of evidence suggest that survivin plays an important role in regulation of mitotic events [11]. The chromosomal passenger complex (CPC), which consists of Aurora B, INCENP, and survivin, contributes to chromosome segregation and cytokinesis [33]. Depletion or inhibition of survivin or of the other proteins of the CPC thus results in mitotic arrest [30,34]. Furthermore, G_2–M arrest induced by survivin ablation was found to occur in $p53^{+/+}$ cells but not in $p53^{-/-}$ cells, implicating survivin in the p53-dependent G_2–M checkpoint that is essential for maintenance of genomic integrity [29]. Together, these various observations suggest that p53-induced repression of survivin expression in response to DNA damage may lower the threshold for apoptosis in cells in which the p53-dependent G_2–M checkpoint has been activated. Survivin repression following DNA damage may play critical role in deciding if lethal damaged cells die before DNA repair is completed, or if they will have the opportunity to repair and survive. Further characterization of the regulation of survivin in response to DNA damage may provide the basis for potential new approaches to cancer treatment that couple standard cytotoxic DNA-damaging agents with survivin-targeted therapy.

Acknowledgement

We thank Erina Hatashita and Yuki Yamada for technical assistance.

References

[1] G. Ambrosini, C. Adida, D.C. Altieri, A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma, Nat. Med. 3 (1997) 917–921.

[2] F. Li, D.C. Altieri, Transcriptional analysis of human survivin gene expression, Biochem. J. 344 (1999) 305–311.

[3] F. Li, E.J. Ackermann, C.F. Bennett, A.L. Rothermel, J. Plescia, S. Tognin, A. Villa, P.C. Marchisio, D.C. Altieri, Pleiotropic cell-division defects and apoptosis induced by interference with survivin function, Nat. Cell Biol. 1 (1999) 461–466.

[4] M. Monzo, R. Rosell, E. Felip, J. Astudillo, J.J. Sanchez, J. Maestre, C. Martin, A. Font, A. Barnadas, A. Abad, A novel anti-apoptosis gene: re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers, J. Clin. Oncol. 17 (1999) 2100–2104.

[5] C. Adida, D. Berrebi, M. Peuchmaur, M. Reyes-Mugica, D.C. Altieri, Anti-apoptosis gene, survivin, and prognosis of neuroblastoma, Lancet 351 (1998) 882–883.

[6] A. Islam, H. Kageyama, N. Takada, T. Kawamoto, H. Takayasu, E. Isogai, M. Ohira, K. Hashizume, H. Kobayashi, Y. Kaneko, A. Nakagawara, High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma, Oncogene 19 (2000) 617–623.

[7] H. Kawasaki, D.C. Altieri, C.D. Lu, M. Toyoda, T. Tenjo, N. Tanigawa, Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer, Cancer Res. 58 (1998) 5071–5074.

[8] H. Meng, C.D. Lu, Y.L. Sun, D.J. Dai, S.W. Lee, N. Tanigawa, Expression level of wild-type survivin in gastric cancer is an independent predictor of survival, World J. Gastroenterol. 10 (2004) 3245–3250.

[9] N. Zaffaroni, M.G. Daidone, Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions, Drug Resist. Update 5 (2002) 65–72.

[10] K. Asanuma, R. Moriai, T. Yajima, A. Yagihashi, M. Yamada, D. Kobayashi, N. Watanabe, Survivin as a radioresistance factor in pancreatic cancer, Jpn. J. Cancer Res. 91 (2000) 1204–1209.

[11] R. Honda, R. Korner, E.A. Nigg, Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis, Mol. Biol. Cell 14 (2003) 3325–3341.

[12] F. Li, G. Ambrosini, E.Y. Chu, J. Plescia, S. Tognin, P.C. Marchisio, D.C. Altieri, Control of apoptosis and mitotic spindle checkpoint by survivin, Nature 396 (1998) 580–584.

[13] R.V. Sionov, Y. Haupt, The cellular response to p53: the decision between life and death, Oncogene 18 (1999) 6145–6157.

[14] B. Vogelstein, D. Lane, A.J. Levine, Surfing the p53 network, Nature 408 (2000) 307–310.

[15] J. Ahn, M. Murphy, S. Kratowicz, A. Wang, A.J. Levine, D.L. George, Down-regulation of the stathmin/Op18 and FKBP25 genes following p53 induction, Oncogene 18 (1999) 5954–5958.

[16] S.A. Innocente, J.L. Abrahamson, J.P. Cogswell, J.M. Lee, p53 regulates a G2 checkpoint through cyclin B1, Proc. Natl. Acad. Sci. USA 96 (1999) 2147–2152.

[17] W.R. Taylor, A.H. Schonthal, J. Galante, G.R. Stark, p130/E2F4 binds to and represses the cdc2 promoter in response to p53, J. Biol. Chem. 276 (2001) 1998–2006.

[18] R. Zhao, K. Gish, M. Murphy, Y. Yin, D. Notterman, W.H. Hoffman, E. Tom, D.H. Mack, A.J. Levine, Analysis of p53-regulated gene expression patterns using oligonucleotide arrays, Genes Dev. 14 (2000) 981–993.

[19] C.D. Lu, D.C. Altieri, N. Tanigawa, Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas, Cancer Res. 58 (1998) 1808–1812.
[20] A.I. Sarela, C.S. Verbeke, J. Ramsdale, C.L. Davies, A.F. Markham, P.J. Guillou, Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma, Br. J. Cancer 86 (2002) 886–892.
[21] J. Nakano, C.L. Huang, D. Liu, M. Ueno, S. Sumitomo, H. Yokomise, survivin gene expression is negatively regulated by the p53 tumor suppressor gene in non-small cell lung cancer, Int. J. Oncol. 27 (2005) 1215–1221.
[22] K. Yonesaka, K. Tamura, T. Kurata, T. Satoh, M. Ikeda, M. Fukuoka, K. Nakagawa, Small interfering RNA targeting survivin sensitizes lung cancer cell with mutant p53 to adriamycin, Int. J. Cancer 118 (2005) 812–820.
[23] C. Badie, J.E. Itzhaki, M.J. Sullivan, A.J. Carpenter, A.C. Porter, Repression of CDK1 and other genes with CDE and CHR promoter elements during DNA damage-induced G_2/M arrest in human cells, Mol. Cell. Biol. 20 (2000) 2358–2366.
[24] W.R. Taylor, G.R. Stark, Regulation of the G2/M transition by p53, Oncogene 20 (2001) 1803–1815.
[25] W.H. Hoffman, S. Biade, J.T. Zilfou, J. Chen, M. Murphy, Transcriptional repression of the anti-apoptotic survivin gene by wild type p53, J. Biol. Chem. 277 (2002) 3247–3257.
[26] A. Mirza, M. McGuirk, T.N. Hockenberry, Q. Wu, H. Ashar, S. Black, S.F. Wen, L. Wang, P. Kirschmeier, W.R. Bishop, L.L. Nielsen, C.B. Pickett, S. Liu, Human survivin is negatively regulated by wild-type p53 and participates in p53-dependent apoptotic pathway, Oncogene 21 (2002) 2613–2622.
[27] M. Kappler, H. Taubert, F. Bartel, K. Blumke, M. Panian, H. Schmidt, J. Dunst, M. Bache, Radiosensitization, after a combined treatment of survivin siRNA and irradiation, is correlated with the activation of caspases 3 and 7 in a wt-p53 sarcoma cell line, but not in a mt-p53 sarcoma cell line, Oncol. Rep. 13 (2005) 167–172.
[28] M. Kappler, M. Bache, F. Bartel, M. Kotzsch, M. Panian, P. Wurl, K. Blumke, H. Schmidt, A. Meye, H. Taubert, Knockdown of survivin expression by small interfering RNA reduces the clonogenic survival of human sarcoma cell lines independently of p53, Cancer Gene Ther. 11 (2004) 186–193.
[29] E. Beltrami, J. Plescia, J.C. Wilkinson, C.S. Duckett, D.C. Altieri, Acute ablation of survivin uncovers p53-dependent mitotic checkpoint functions and control of mitochondrial apoptosis, J. Biol. Chem. 279 (2004) 2077–2084.
[30] A. Carvalho, M. Carmena, C. Sambade, W.C. Earnshaw, S.P. Wheatley, Survivin is required for stable checkpoint activation in taxol-treated HeLa cells, J. Cell Sci. 116 (2003) 2987–2998.
[31] S.M. Lens, R.M. Wolthuis, R. Klompmaker, J. Kauw, R. Agami, T. Brummelkamp, G. Kops, R.H. Medema, Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension, EMBO J. 22 (2003) 2934–2947.
[32] J.C. Reed, The Survivin saga goes in vivo, J. Clin. Invest. 108 (2001) 965–969.
[33] M.A. Bolton, W. Lan, S.E. Powers, M.L. McCleland, J. Kuang, P.T. Stukenberg, Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation, Mol. Biol. Cell 13 (2002) 3064–3077.
[34] S. Hauf, R.W. Cole, S. LaTerra, C. Zimmer, G. Schnapp, R. Walter, A. Heckel, J. van Meel, C.L. Rieder, J.M. Peters, The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint, J. Cell Biol. 161 (2003) 281–294.

Japanese Journal of Clinical Oncology Advance Access published October 16, 2009

Jpn J Clin Oncol 2009
doi:10.1093/jjco/hyp137

Original Article

Cisplatin and Etoposide Chemotherapy Combined with Early Concurrent Twice-daily Thoracic Radiotherapy for Limited-disease Small Cell Lung Cancer in Elderly Patients

Kunio Okamoto[1], Isamu Okamoto[1], Ken Takezawa[1], Izumi Tachibana[2], Masahiro Fukuoka[3], Yasumasa Nishimura[2] and Kazuhiko Nakagawa[1]

[1]Department of Medical Oncology, Kinki University School of Medicine, [2]Department of Radiation Oncology, Kinki University School of Medicine and [3]Department of Medical Oncology, Kinki University School of Medicine, Sakai Hospital, Osaka, Japan

Received August 7, 2009; accepted September 13, 2009

Objective: The optimal management of elderly patients with limited-disease small cell lung cancer (LD-SCLC) has not been established.
Methods: The records of elderly (≥70 years of age) patients with LD-SCLC who had been treated with etoposide and cisplatin chemotherapy with early concurrent twice-daily thoracic radiotherapy (TRT) were reviewed retrospectively.
Results: Of the 25 elderly patients with LD-SCLC identified, 12 (48%) individuals received etoposide–cisplatin chemotherapy with early concurrent twice-daily TRT. The main toxicities of this treatment regimen were hematologic, with neutropenia of Grade 4 being observed in all patients and febrile neutropenia of Grade 3 in eight patients during the first cycle of chemoradiotherapy. The toxicity of TRT was acceptable, with all patients completing the planned radiotherapy within a median of 29 days (range, 19–33). No treatment-related deaths were observed. The median progression-free survival and overall survival times were 14.2 months (95% confidence interval, 4.3–18.2) and 24.1 months (95% confidence interval, 11.3–27.2), respectively.
Conclusions: Etoposide–cisplatin chemotherapy with early concurrent twice-daily TRT was highly myelotoxic in elderly patients with LD-SCLC, although no treatment-related deaths were observed in our cohort. Prospective studies are required to establish the optimal schedule and dose of chemotherapy and TRT in such patients.

Key words: elderly – small cell lung cancer – chemoradiotherapy – cisplatin – etoposide – concurrent thoracic radiotherapy

INTRODUCTION

Small cell lung cancer (SCLC) accounts for 10–15% of all lung cancer cases, with individuals aged 70 years or older constituting up to 25–40% of the SCLC patients (1,2). Limited-disease (LD) SCLC is a disease that is confined to one hemithorax and its regional lymph nodes and which can be encompassed by a single radiation therapy port. About 30–40% of all SCLC patients present with LD-SCLC (1,2). The proportion of elderly SCLC patients continues to increase with the growing geriatric population (1,3).

The combination of radiotherapy and chemotherapy, specifically etoposide and cisplatin chemotherapy with early concurrent twice-daily thoracic radiotherapy (TRT), is now regarded as the standard treatment for LD-SCLC (4). However, many clinical trials of potential new treatments for LD-SCLC have excluded elderly patients for various reasons, such as the presence of concomitant chronic illness,

For reprints and all correspondence: Isamu Okamoto, Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. E-mail: chi-okamoto@dotd.med.kindai.ac.jp

a decline in organ function that may interfere with drug clearance and possible decreased bone marrow tolerance to myelosuppressive agents (5). The optimal management of elderly patients with LD-SCLC has therefore not been defined to date.

We have now performed a retrospective analysis to evaluate patient characteristics as well as treatment delivery, toxicity and antitumor efficacy for elderly individuals (70 years or older) with LD-SCLC who were treated with etoposide and cisplatin chemotherapy and early concurrent twice-daily TRT.

PATIENTS AND METHODS

We retrospectively evaluated the records of elderly (≥70 years) patients with LD-SCLC who were treated at Kinki University School of Medicine from January 2003 to December 2008. All patients had a pathological diagnosis of SCLC. LD-SCLC was defined as cancer that is confined to one hemithorax including contralateral mediastinal and hilar lymph nodes as well as ipsilateral or bilateral supraclavicular lymph nodes, but excluding malignant pleural effusion. Response evaluation was assessed after completion of treatment on the basis of the Response Evaluation Criteria in Solid Tumors (RECIST). Laboratory testing and toxicities were graded weekly during the whole treatment according to the National Cancer Institute—Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3). Progression-free survival time was measured from the date of initiation of treatment to the date of disease progression. Overall survival time was measured from the date of initiation of treatment to death or to the time that the patient was last known to be alive. After completion of all treatment, patients were followed up at 1- to 2-month intervals until the time of progression or death. Median progression-free survival time and overall survival time were estimated by the Kaplan–Meier method.

RESULTS

Patient Characteristics

Of the 170 SCLC patients treated between 2003 and 2008, 48 individuals were diagnosed with LD-SCLC and 25 of these individuals were 70 years of age or older. Among these 25 patients, 12 (48%) elderly patients with LD-SCLC received etoposide and cisplatin chemotherapy with early concurrent twice-daily TRT. The characteristics of these 12 patients are shown in Table 1. They included eight men and four women as well as seven individuals aged between 70 and 74 years and five aged 75 years or older. All the patients were in good general condition, although they had some complications. The remaining 13 patients' characteristics are shown in Table 2. Two of the 13 elderly patients with LD-SCLC were treated with chemotherapy and sequential TRT, and 1 patient was treated with etoposide–carboplatin and concurrent TRT. Chemotherapy alone was administered in 4 of the 13 patients. Two patients were subjected to surgery followed by chemotherapy. Four patients did not receive intensive therapy.

Treatment Delivery

The treatment plan consisted of an initial cycle of concurrent chemoradiotherapy followed by three cycles of consolidation chemotherapy (Table 3). All patients received the same chemotherapy regimen of cisplatin at 40–80 mg/m^2 on day 1 combined with etoposide at 80–100 mg/m^2 on days 1–3.

Table 1. Characteristics of the study cohort

Patient	Age/sex	TNM stage	PS	Complications	Smoking history
1	70/M	T2N1M0	1	HT	20/day × 50 years
2	70/M	T3N1M0	0	Berger disease, old TB	40/day × 50 years
3	71/M	T3N2M0	0	DM, bladder cancer	20/day × 50 years
4	71/M	T1N2M0	1	Harada disease	20/day × 50 years
5	72/F	T2N2M0	1	HT, old TB, asthma, one kidney	20/day × 35 years
6	72/M	T1N2M0	0	HT, hyperlithuria	10/day × 50 years
7	73/M	T1N2M0	1	HT	25/day × 60 years
8	76/M	T2N1M0	0	None	20/day × 50 years
9	77/F	T3N0M0	1	Deafness	15/day × 57 years
10	78/M	T3N0M0	0	DM, ASO, old TB	20/day × 58 years
11	79/F	T2N2M0	1	None	None
12	79/F	T1N2M0	0	HT	5/day × 50 years

PS, Eastern Cooperative Oncology Group performance status; HT, hypertension; TB, tuberculosis; DM, type 2 diabetes mellitus; ASO, arteriosclerosis obliterans.

Table 2. Characteristics of patients who did not received EP with concurrent TRT

Patient	Age/sex	TNM stage	PS	Complications	Treatment	Reason[a]
1	70/M	T4N2M0	1	HT, renal dysfunction	CE and sequential TRT	Complication
2	70/M	T1N0M0	1	HT, DM	Surgery	Physician's decision
3	71/M	T3N2M0	1	HT	Best supportive care	Patient's refusal
4	72/M	T2N1M0	1	DM, renal dysfunction	CE and concurrent TRT	Complication
5	74/M	T3N2M0	1	HT, renal dysfunction	CE and sequential TRT	Complication
6	74/M	T2N1M0	2	DM, IP, chronic renal failure, dialysis, old TB	Chemotherapy	Complication
7	75/M	T3N2M0	3	HCC, chronic HCV	Best supportive care	Complication
8	77/M	T2N1M0	2	renal dysfunction, dementia	Chemotherapy	Complication
9	78/M	T1N1M0	1	SSS, HT, DM	Chemotherapy	Physician's decision
10	81/M	T2N2M0	1	renal dysfunction	Chemotherapy	Patient's refusal
11	82/M	T1N2M0	1	HT	Surgery	Physician's decision
12	84/M	T2N0M0	2	HT	Best supportive care	Patient's refusal
13	84/M	T2N0M0	2	HT, asthma, heart failure, cerebral infarction	Best supportive care	Complication

EP, etoposide and cisplatin; TRT, thoracic radiotherapy; CE, carboplatin and etoposide; IP, interstitial pneumonia; HCC, hepatic cancer; HCV, hepatitis C virus; SSS, sick sinus syndrome.
[a]The reason for not to select the combination therapy of etoposide and cisplatin with early concurrent TRT.

Twice-daily TRT was performed with X-rays at 6–10 MV and with an interval of at least 6 h and a total dose of 45 Gy (1.5 Gy bid) over 3 weeks. TRT was initiated on day 1 of the first cycle of chemotherapy. All patients completed the TRT protocol, with the days of irradiation ranging from 19 to 33 (median of 29). Reasons for a delay in TRT included febrile neutropenia of Grade 3 in eight patients and leukopenia of Grade 4 in three patients. All patients proceeded to consolidation chemotherapy. However, five patients (42%) did not complete the planned three cycles of consolidation chemotherapy because of the development of pneumonitis of Grade 3 in one patient, a decline in renal function in one patient, suspected invasive aspergillosis in one patient and refusal by two patients. A dose reduction was necessary in seven patients because of the development of febrile neutropenia of Grade 3 in three patients, leukopenia of Grade 4 in two patients and nausea–vomiting of Grade 3 in two patients. The actual dose intensities of cisplatin and etoposide were 13.7 mg/m^2/week (68.7% of the planned dose intensity) and 52.4 mg/m^2/week (69.9% of the planned dose intensity), respectively.

Toxicities

Reported toxicities during the concurrent chemoradiotherapy are listed in Table 4. Leukopenia and neutropenia of Grade 3 or 4 were observed in all patients (100%), and eight patients (67%) had febrile neutropenia of Grade 3. Thrombocytopenia of Grade 3 or 4 was apparent in three patients (25%), with one patient requiring platelet transfusion. Reported toxicities during the consolidation chemotherapy are listed in Table 5. Leukopenia and neutropenia of Grade 3 or 4 were observed in 8 (67%) and 11 (92%) patients, respectively, and 4 patients (33%) developed febrile neutropenia of Grade 3. Anemia and thrombocytopenia of Grade 3 or 4 were each observed in four patients (33%). The major non-hematologic toxicity observed during the entire treatment period was nausea–vomiting. None of the patients developed esophagitis of Grade 3 or 4, but one patient manifested radiation pneumonitis of Grade 3 during consolidation chemotherapy. There were no treatment-related deaths.

Response and Survival

All 12 patients were evaluated for progression-free survival and overall survival. With a median follow-up time of 23.1 months (ranged, 7.2–45.0 months), six patients were still alive. An objective tumor response was observed in all patients: a complete response (CR) in five patients and a partial response in seven patients (Table 3). Prophylactic cranial irradiation was not routinely administered and delivered to three patients who achieved CR after completion of the planned treatment. The median progression-free survival time was 14.2 months, and the median overall survival time was 24.1 months.

Pattern of Relapse

Seven of the 12 patients relapsed, 3 with local regional failure inside the radiation field and 4 with distant failure. Among the latter four patients, three individuals manifested metastases in the brain as the sole site and the remaining individual had both local and distant failure including the liver.

Table 3. Treatment details and outcome for the study cohort

Patient	Regimen (mg/m^2)	Total no. of cycles	Dose reduction in consolidation chemotherapy	DI of P (mg/m^2/week)	RDI of P (%)	DI of E (mg/m^2/week)	RDI of E (%)	Duration of TRT (days)	V20 (%)	Response	PFS (months)	Survival time (months)
1	E(100) + P(40)[a] + TRT	2	No	5.0	25.0	37.5	50.0	23	21	CR	14.0+	14.0+
2	E(100) + P(80) + TRT	4	Yes	17.2	86.0	73.7	98.2	19	25	CR	7.3+	7.3+
3	E(100) + P(80) + TRT	4	Yes	17.8	89.1	68.7	91.6	27	35	CR	10.7+	10.7+
4	E(100) + P(80) + TRT	4	Yes	15.7	78.4	61.6	82.1	30	13	PR	9.3	22.2
5	E(100) + P(80) + TRT	2	No	9.5	47.5	35.6	47.5	29	20	PR	4.3	11.4
6	E(100) + P(80) + TRT	4	No	19.6	98.2	73.7	98.2	29	30	PR	18.2	48.1+
7	E(100) + P(80) + TRT	4	No	17.2	86.2	64.6	86.2	26	27	PR	13.1	26.1+
8	E(80)[b] + P(80) + TRT	3	Yes	11.4	56.9	39.4	52.5	30	21	PR	8.3	17.1
9	E(100) + P(60)[a] + TRT	4	Yes	14.4	71.8	61.0	81.4	30	25	CR	20.6+	20.6+
10	E(100) + P(80) + TRT	4	Yes	13.1	65.5	49.1	65.5	28	NA	PR	14.4	16.5
11	E(100) + P(80) + TRT	2	Yes	7.9	39.5	30.5	40.6	33	26	PR	3.9	14.8
12	E(100) + P(80) + TRT	2	No	9.9	49.6	37.2	49.6	29	22	CR	14.1	27.2

DI, dose intensity; P, cisplatin; RDI, relative dose intensity; E, etoposide; V20, the percentage of lung volume receiving >20 Gy; PFS, progression-free survival; CR, complete response; +, without event; PR, partial response; NA, not available.
[a]Dose reduction because of a decline in renal function.
[b]Dose reduction because of physician's decision.

Table 4. Toxicities during concurrent chemoradiotherapy

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4 (%)
Leukopenia	0	0	2	10	100
Neutropenia	0	0	0	12	100
Anemia	2	1	0	0	0
Thrombocytopenia	0	2	2	1	25
Febrile neutropenia	—	—	8	0	67
Nausea–vomiting	2	2	2	0	17
Esophagitis	1	3	0	0	0
Appetite loss	5	2	2	0	17

Table 5. Toxicities during consolidation chemotherapy

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4 (%)
Leukopenia	0	2	4	4	67
Neutropenia	0	0	2	9	92
Anemia	2	4	3	1	33
Thrombocytopenia	2	2	2	2	33
Febrile neutropenia	—	—	4	0	33
Nausea–vomiting	2	5	2	0	17
Appetite loss	4	1	1	0	8
Radiation pneumonitis	3	0	1	0	8

DISCUSSION

Two meta-analyses have shown that the combined modality of chemotherapy and TRT improves the survival of individuals with LD-SCLC in comparison with chemotherapy alone (6,7). The schedule, dose and fractionation of TRT have been extensively investigated in patients with LD-SCLC in several randomized controlled trials (8,9). On the basis of two pivotal Phase III trials (10,11), etoposide and cisplatin chemotherapy with early concurrent twice-daily TRT is currently considered the standard treatment for patients with LD-SCLC. An age-specific subset analysis of one of these Phase III trials (11), in which patients received etoposide–cisplatin with early concurrent TRT, showed that the survival outcomes for individuals aged 70 years or older were similar to those of their younger counterparts, although the elderly patients experienced greater toxicity, in particular hematologic toxicity (12). However, given that the patients in this subgroup analysis were assigned either once- or twice-daily TRT, the significance of early concurrent twice-daily TRT in the management of elderly patients with LD-SCLC has remained undefined. No specific Phase III trial of elderly patients with LD-SCLC has been reported. We therefore retrospectively analyzed the feasibility and antitumor efficacy of etoposide–cisplatin chemotherapy with early concurrent

twice-daily TRT for treatment of LD-SCLC in patients aged 70 years or older.

The median overall survival time of 24.1 months in our cohort is similar to that described for non-elderly patients with LD-SCLC in previous studies (10,11). This favorable survival outcome may be attributable to the strict selection of elderly patients in good general condition; all 12 patients in the present study had normal organ function, an Eastern Cooperative Oncology Group performance status of 0 or 1 and no severe co-morbidity. Given that the elderly are more likely to have reduced organ function as well as concomitant morbidities or medications, the general condition of elderly SCLC patients is worse than that of younger patients (1). Among LD-SCLC patients, increasing age was found to be significantly associated with a lower likelihood of receiving combined chemoradiotherapy (7). Indeed, in the present study, only 12 (48%) of the 25 identified elderly patients with LD-SCLC were treated with etoposide–cisplatin and early concurrent twice-daily TRT.

Despite the strict selection of patients, highly treatment-related toxicity was observed in our cohort. The major adverse events were hematologic toxicities, with neutropenia of Grade 4 being apparent in all patients (100%) and febrile neutropenia of Grade 3 in eight patients (67%) during the first cycle of concurrent chemoradiotherapy. The previous analysis of the outcome of elderly patients in the Phase III study in which individuals received etoposide–cisplatin chemotherapy with early concurrent once- or twice-daily TRT found statistically significant differences not only in the incidence of hematologic toxicity (Grade 4 or 5: 61% in younger patients vs. 84% in patients aged 70 years or older, $P < 0.01$) but also in that of treatment-related deaths (1% vs. 10%, respectively, $P = 0.01$) (12). Although no treatment-related deaths were observed in the present study, severe hematologic toxicity was consistent with that in this foregoing analysis (12). In addition, maintenance of the optimal dose intensity of chemotherapy was difficult in our cohort because of frequent dose reductions or treatment delays due to hematologic or infection-related toxicities. Indeed, the actual dose intensity was <70% of the planned dose intensity for both etoposide and cisplatin in the present study, a value much smaller than that for non-elderly patients in a previous Phase III study (>90% for both agents) (10). On the other hand, the toxicity of radiotherapy was acceptable in our study, with all patients completing TRT within a median of 29 days (range, 19–33). None of our patients developed radiation esophagitis of Grade 3 or higher. With regard to pulmonary complications, one patient developed radiation pneumonitis of Grade 3. A recent meta-analysis of randomized trials in which patients with LD-SCLC were treated with chemoradiotherapy reported that the time between the first day of chemotherapy and the last day of radiotherapy was an important prognostic factor for LD-SCLC, with the survival advantage being more pronounced if the TRT was completed in <30 days (13). In the present study, a shorter time to completion of TRT may also be associated with our favorable survival outcome. However, elderly patients with LD-SCLC must be carefully selected and monitored during treatment because of the increased potential for the development of treatment-related morbidity and mortality.

The optimal therapeutic strategy for elderly patients with LD-SCLC remains a matter of debate. Despite the highly treatment-related toxicity, patients in our cohort derived a survival benefit with no treatment-related deaths, suggesting that the full-dose chemoradiotherapy may represent a valid option for 'fit' elderly patients with adequate organ function. Since the general condition of elderly patients varies widely from patients to patients, prospective evaluation and definition of 'fit' elderly patients who are candidates for full-dose chemoradiotherapy are important. Research is also needed to develop modified chemoradiotherapy regimens that are less toxic for the elderly. A modified chemotherapy schedule designed to reduce toxicity for elderly patients with LD-SCLC was evaluated in a Phase II trial, with two cycles of a chemotherapy regimen (oral etoposide and carboplatin) combined with early concurrent twice-daily TRT being found to have acceptable toxicity and to produce promising results, with a 5-year survival rate of 13% (14). A recent Phase III trial specifically designed for elderly or poor-risk patients with extensive-disease SCLC found that split doses of cisplatin plus etoposide (cisplatin at 25 mg/m^2 and etoposide at 80 mg/m^2 on days 1–3) could be safely administered and were effective (15). Such split-dose chemotherapy might also be suitable for the treatment of patients with LD-SCLC. We are currently conducting a clinical trial to evaluate the feasibility of etoposide at 80 mg/m^2 and cisplatin at 25 mg/m^2 on days 1–3 with early concurrent twice-daily TRT for elderly patients with LD-SCLC.

The overall findings of the present study suggest that administration of full-dose chemotherapy and early concurrent twice-daily TRT is highly myelotoxic for elderly patients with LD-SCLC. Development and assessment of modified treatment regimens with reduced toxicity are thus warranted for such patients.

Conflict of interest statement

None declared.

References

1. Sekine I, Yamamoto N, Kunitoh H, Ohe Y, Tamura T, Kodama T, et al. Treatment of small cell lung cancer in the elderly based on a critical literature review of clinical trials. *Cancer Treat Rev* 2004;30:359–68.
2. Gridelli C, Langer C, Maione P, Rossi A, Schild SE. Lung cancer in the elderly. *J Clin Oncol* 2007;25:1898–907.
3. Morita T. A statistical study of lung cancer in the annual of pathological autopsy cases in Japan, from 1958 to 1997, with reference to time trends of lung cancer in the world. *Jpn J Cancer Res* 2002;93:15–23.
4. Socinski MA, Bogart JA. Limited-stage small-cell lung cancer: the current status of combined-modality therapy. *J Clin Oncol* 2007;25:4137–45.

5. Talarico L, Chen G, Pazdur R. Enrollment of elderly patients in clinical trials for cancer drug registration: a 7-year experience by the US Food and Drug Administration. *J Clin Oncol* 2004;22:4626–31.
6. Pignon JP, Arriagada R, Ihde DC, Johnson DH, Perry MC, Souhami RL, et al. A meta-analysis of thoracic radiotherapy for small-cell lung cancer. *N Engl J Med* 1992;327:1618–24.
7. Ludbrook JJ, Truong PT, MacNeil MV, Lesperance M, Webber A, Joe H, et al. Do age and comorbidity impact treatment allocation and outcomes in limited stage small-cell lung cancer? A community-based population analysis. *Int J Radiat Oncol Biol Phys* 2003;55:1321–30.
8. Bonner JA, Sloan JA, Shanahan TG, Brooks BJ, Marks RS, Krook JE, et al. Phase III comparison of twice-daily split-course irradiation versus once-daily irradiation for patients with limited stage small-cell lung carcinoma. *J Clin Oncol* 1999;17:2681–91.
9. Schild SE, Bonner JA, Shanahan TG, Brooks BJ, Marks RS, Geyer SM, et al. Long-term results of a phase III trial comparing once-daily radiotherapy with twice-daily radiotherapy in limited-stage small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2004;59:943–51.
10. Turrisi AT, 3rd, Kim K, Blum R, Sause WT, Livingston RB, Komaki R, et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med* 1999;340:265–71.
11. Takada M, Fukuoka M, Kawahara M, Sugiura T, Yokoyama A, Yokota S, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with cisplatin and etoposide for limited-stage small-cell lung cancer: results of the Japan Clinical Oncology Group Study 9104. *J Clin Oncol* 2002;20:3054–60.
12. Yuen AR, Zou G, Turrisi AT, Sause W, Komaki R, Wagner H, et al. Similar outcome of elderly patients in intergroup trial 0096: cisplatin, etoposide, and thoracic radiotherapy administered once or twice daily in limited stage small cell lung carcinoma. *Cancer* 2000;89:1953–60.
13. De Ruysscher D, Pijls-Johannesma M, Bentzen SM, Minken A, Wanders R, Lutgens L, et al. Time between the first day of chemotherapy and the last day of chest radiation is the most important predictor of survival in limited-disease small-cell lung cancer. *J Clin Oncol* 2006;24:1057–63.
14. Jeremic B, Shibamoto Y, Acimovic L, Milisavljevic S. Carboplatin, etoposide, and accelerated hyperfractionated radiotherapy for elderly patients with limited small cell lung carcinoma: a phase II study. *Cancer* 1998;82:836–41.
15. Okamoto H, Watanabe K, Kunikane H, Yokoyama A, Kudoh S, Asakawa T, et al. Randomised phase III trial of carboplatin plus etoposide vs split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small-cell lung cancer: JCOG 9702. *Br J Cancer* 2007;97:162–9.

OPEN ACCESS Freely available online

PLoS one

Twenty-Seven Years of Phase III Trials for Patients with Extensive Disease Small-Cell Lung Cancer: Disappointing Results

Isao Oze, Katsuyuki Hotta*, Katsuyuki Kiura, Nobuaki Ochi, Nagio Takigawa, Yoshiro Fujiwara, Masahiro Tabata, Mitsune Tanimoto

Department of Respiratory Medicine, Okayama University Hospital, Okayama, Japan

Abstract

Background: Few studies have formally assessed whether treatment outcomes have improved substantially over the years for patients with extensive disease small-cell lung cancer (ED-SCLC) enrolled in phase III trials. The objective of the current investigation was to determine the time trends in outcomes for the patients in those trials.

Methods and Findings: We searched for trials that were reported between January 1981 and August 2008. Phase III randomized controlled trials were eligible if they compared first-line, systemic chemotherapy for ED-SCLC. Data were evaluated by using a linear regression analysis. Results: In total, 52 trials were identified that had been initiated between 1980 and 2006; these studies involved 10,262 patients with 110 chemotherapy arms. The number of randomized patients and the proportion of patients with good performance status (PS) increased over time. Cisplatin-based regimens, especially cisplatin and etoposide (PE) regimen, have increasingly been studied, whereas cyclophosphamide, doxorubicin, and vincristine–based regimens have been less investigated. Multiple regression analysis showed no significant improvement in survival over the years. Additionally, the use of a PE regimen did not affect survival, whereas the proportion of patients with good PS and the trial design of assigning prophylactic cranial irradiation were significantly associated with favorable outcome.

Conclusions and Significance: The survival of patients with ED-SCLC enrolled in phase III trials did not improve significantly over the years, suggesting the need for further development of novel targets, newer agents, and comprehensive patient care.

Citation: Oze I, Hotta K, Kiura K, Ochi N, Takigawa N, et al. (2009) Twenty-Seven Years of Phase III Trials for Patients with Extensive Disease Small-Cell Lung Cancer: Disappointing Results. PLoS ONE 4(11): e7835. doi:10.1371/journal.pone.0007835

Editor: Joseph Najbauer, City of Hope National Medical Center, United States of America

Received June 24, 2009; **Accepted** October 5, 2009; **Published** November 13, 2009

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: khotta@md.okayama-u.ac.jp

Introduction

Lung cancer is a leading cause of cancer-related mortality in many industrialized countries. Small-cell lung cancer (SCLC), which accounts for about 15% of all lung cancer cases, is categorized into two clinical stages: limited disease (LD) and extensive disease (ED). For patients with ED-SCLC, combination chemotherapy is the mainstay of treatment.

In the 1980s, the most widely used combination of drugs for initial treatment of ED-SCLC was cyclophosphamide, doxorubicin, and vincristine (CAV), which produced a median survival time of 9 to 11 months [1]. In the late 1980s, a combination regimen of cisplatin and etoposide (PE) was introduced, and an alternating regimen of PE and CAV has been widely investigated in randomized controlled trials [2].

In 1999, the results of a systemic review indicated a modest improvement over the years in the survival time of patients with ED-SCLC treated with chemotherapy between 1972 and 1994 [3]. This improvement was potentially attributable to (i) introduction of the PE regimen in the late 1980s and (ii) improvements in the supportive care and general management of the patients. However, this included just North American trials and would provide some justification for looking at the world-wide result.

A decade has passed since that systemic review, and recent clinical trials have investigated newer antineoplastic agents such as irinotecan and topotecan. Thus, we performed a literature search to determine whether patient outcomes have improved in the treatment of ED-SCLC.

Materials and Methods

Searching

We searched for trials that were reported between January 1981 and August 2008. To avoid publication bias, we identified both published and unpublished trials through a computer-based search of the PubMed database and abstracts from past conferences of the American Society of Clinical Oncology (1998–2008). We used the following search terms: *lung neoplasm*, *carcinoma*, *small-cell*, *chemotherapy*, and *randomized controlled trial*. The search was guided by a

thorough examination of reference lists from original articles, review articles, relevant books, and the Physician Data Query registry of clinical trials.

Selection

Phase III randomized controlled trials were eligible for inclusion in this study if they compared first-line, systemic chemotherapy for ED-SCLC that contained cytotoxic agents, providing the year of trial initiation. Trials were excluded if they only investigated immunotherapy regimens, or if they enrolled only responders to the initial chemotherapy. Trials initially designed to assess combined-modality treatment, including radiotherapy and surgery concurrently undergone with the initial chemotherapy, were also ineligible, but those optionally designed to conduct these therapies or prophylactic cranial irradiation (PCI) sequentially after the induction chemotherapy were allowed. Some phase III trials incorporated patients with both LD-SCLC and ED-SCLC. These were considered eligible only if survival data for patients with ED-SCLC could be solely obtained. We acknowledge that the definitions for LD-SCLC and ED-SCLC vary somewhat in the different groups compared, and we could not strictly reallocate each patient because we were unable to access the individual patient databases. Instead, we applied the definition described in each original report to this study. If no relevant descriptions were documented, we considered that the definition in that trial would have been based on the guidelines in existence at the time of that trial initiation [4,5]. The control arms in each of the phase III trials were identified based on statements in each trial.

Validity Assessment

To avoid bias in the data abstraction process, four medical oncologists (I.O., N.O., Y.F., and K.H.), one of whom (K.H.) holds a board certificate for medical oncology, independently abstracted the data from the trials and subsequently compared the results. All data were checked for internal consistency, and disagreements were resolved by discussion among the investigators.

Data Abstraction

The following information was obtained from each report: year of trial initiation (i.e., year when the first patient was accrued); number of patients enrolled and randomized; median age of patients; proportion of patients with good performance status (PS); proportion of patients who were male and who had brain metastasis; chemotherapy regimen; definition of ED; description of the administration of sequential thoracic irradiation, surgery, or PCI as one of the trial designs; and median survival time (per treatment arm).

Study Characteristics

All studies included were phase III randomized controlled trials of first-line systemic chemotherapy for ED-SCLC. The study outcomes were median survival time. Variation in study characteristics and clinical heterogeneity between studies were adjusted statistically (see below).

Quantitative Data Synthesis

Data from phase III trials were evaluated by using multiple, stepwise regression analysis (with the following stepping method criteria: probability of F to enter the model, <0.05; to remove from the model, >0.10). The data analyzed included year of trial initiation, use of PE regimen, maximal age of patients, proportion of patients with good PS, proportion of male patients, and definition of PCI settings. These data were used to determine whether each factor had an independent impact on the survival of patients with ED-SCLC who were treated in the phase III studies over time. All P values corresponded to 2-sided tests, and significance was set at $P<0.05$.

Results

Trial Flow/Flow of Included Studies

Figure 1 shows a flow chart of this study. In total, 52 trials for ED-SCLC were identified as a result of the computer-based and manual searches for relevant articles, abstracts, and references

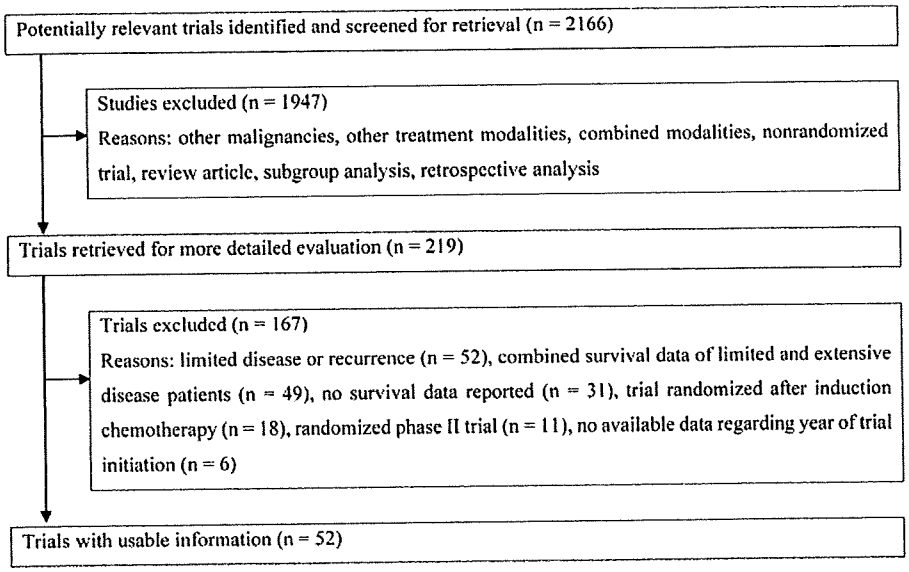

Figure 1. Flow chart showing the progress of trials through the review.
doi:10.1371/journal.pone.0007835.g001