Table 1. Patient characteristics by epoetin-beta dosage group

Patent population	Epoetin-beta dosage groups			
	9000 IU	18000 IU	36000 IU	
Randomly assigned patients (*n*)	28	29	29	
Patients evaluated for safety (*n*)	28	27	28	
Patients evaluated for efficacy (PPS) (*n*)	22	24	23	
Characteristic	9000 IU (*n* = 22)	18000 IU (*n* = 24)	36000 IU (*n* = 23)	*P*
Age (year)				
Mean ± SD	60.5 ± 16.6	63.0 ± 11.9	61.9 ± 11.7	0.828
Min–Max	22–79	31–76	34–77	
Weight (kg)				
Mean ± SD	53.5 ± 8.7	50.9 ± 7.3	55.1 ± 11.5	0.316
Min–Max	36.1–69	38.8–66.9	34.8–87.5	
Sex male/female (*n*)	13/9	13/11	14/9	0.890
Disease				
Lung cancer (*n*)	11	13	11	0.907
Malignant lymphoma (*n*)	11	11	12	
de novo/relapse (*n*)	17/5	19/5	18/5	0.988
Performance Status 0/1/2 (*n*)	10/11/1	11/12/1	10/12/1	1.000
RBC transfusion before the study (*n*)	5	2	1	0.130
Hemoglobin (g/dl)				
Mean ± SD	10.1 ± 1.3	10.0 ± 1.5	10.2 ± 1.0	0.914
Min–Max	7.4–12.2	7.4–13.2	8.1–11.7	
Serum EPO concentration (mIU/ml)				
Mean ± SD	43.3 ± 38.1	46.8 ± 43.9	30.4 ± 18.4	0.259
Min–Max	13.1–173	14.4–170	7.0–103	
Serum transferrin saturation (%)				
Mean ± SD	31.1 ± 15.9	25.4 ± 11.5	25.5 ± 13.8	0.287
Min–Max	9.4–77.8	10.1–48.0	6.9–77.4	

SD, standard deviation; Min, minimum; Max, maximum; RBC, red blood cell; EPO, erythropoietin.

in the 36000 IU group experienced deep vein thrombosis, which was evaluated as unrelated to epoetin-beta. When the thrombosis was found, anemia had not improved (baseline hemoglobin level was 9.9 g/dl and that at the onset of thrombosis was 9.2 g/dl); therefore, deep vein thrombosis was considered to be due to prolonged immobility brought on by aggravated malignant lymphoma and PS.

Severe adverse events were reported for 12 patients and were judged by the investigators as unrelated to the administration of epoetin-beta. Of the adverse events, 65 events in 23 patients (27.7%) were considered related to epoetin-beta. The incidence of these events was similar between the three dosage groups (Table 3). An increase of serum ALT was observed in one patient (3.6%) in the 9000 IU group, two (7.4%) in the 18000 IU group and two (7.1%) in the 36000 IU group. Hypertension or an increase of blood pressure was observed in one patient (3.6%) in the 9000 IU group, three (11.1%) in the 18000 IU group and one (3.6%) in the 36000 IU group. Drug administration was discontinued in one of these patients due to hypertension. No tendency was found in the onset time of hypertension, nor in changes of hemoglobin from baseline at the time hypertension occurred.

Anti-erythropoietin antibody was not detected in any patient, but pure red cell aplasia (PRCA) was reported in one malignant lymphoma (Angioimmunoblastic T-cell Lymphoma) patient over a year after this trial. In this patient, neutralizing anti-erythropoietin antibody was not detected even after PRCA was diagnosed.

Table 2. Chemotherapy regimens used by PPS population during the study

Chemotherapy regimens	Epoetin-beta dosage groups		
	9000 IU ($n = 22$)	18 000 IU ($n = 24$)	36 000 IU ($n = 23$)
Malignant lymphoma			
(R)CHOP	5	6	9
(R)EPOCH	2	3	2
ESHAP	0	2	0
Other regimens	4	0	1
Lung cancer			
Platinum + Paclitaxel	4	2	2
Platinum + Irinotecan	1	4	3
Platinum + Etoposide	3	2	1
Platinum + Vinorelbine	1	2	1
Other regimens	2	3	4

PPS, Per Protocol Set; (R)CHOP, (Rituximab) + Cyclophosphamide + Doxorubicin + Vincristine + Prednisolone; (R)EPOCH, (Rituximab) + Etoposide + Doxorubicin + Vincristine + Cyclophosphamide + Prednisolone; ESHAP, Etoposide + Methylprednisolone + High Dose Ara C (Cytarabine) + Cisplatin.

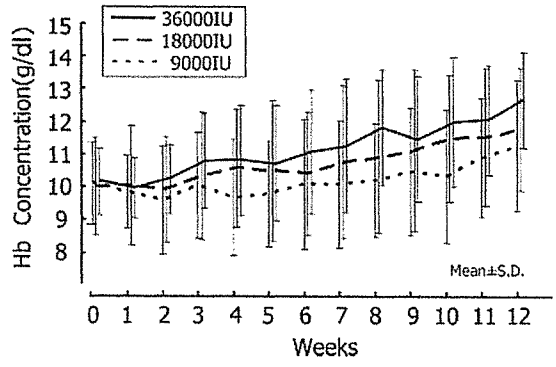

Figure 1. Mean weekly hemoglobin levels for Per Protocol Set population by epoetin-beta dosage Group. Hb, hemoglobin; SD, standard deviation.

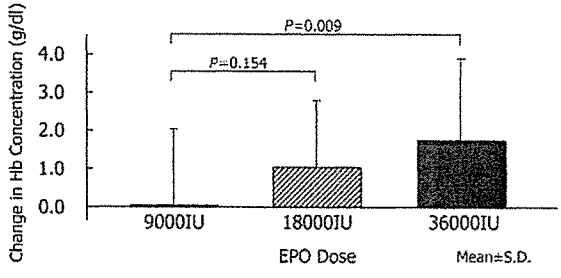

Figure 2. Mean change in hemoglobin level from baseline to last observation (at 12 weeks or 4 weeks after the beginning of a final-course of chemotherapy) by epoetin-beta dosage group (Per Protocol Set population). Hb, hemoglobin; EPO, epoetin-beta; SD, standard deviation.

DISCUSSION

Recently, results of several clinical studies have demonstrated the efficacy and safety of weekly rhEPO for the treatment of cancer-related anemia (10,12,15,16). In a large, nonrandomized, community-based study reported by Gabrilove et al. (10), once-weekly dosing of epoetin-alfa was as effective as three-times weekly dosing in increasing hemoglobin levels and improving QOL. Cazzola et al. (12) reported a randomized study of epoetin-beta that compared the efficacy and tolerability of 30 000 IU once-weekly with the conventional 10 000 IU three-times weekly regimen in patients with lymphoproliferative malignancies. Between these two dosing regimens, there was no significant difference in time-adjusted area under the hemoglobin curve and increase in hemoglobin. Two randomized phase III studies using 40 000 IU once-weekly epoetin-alfa also support the use of epoetin-alfa as an ameliorative agent for cancer-related anemia (15,16).

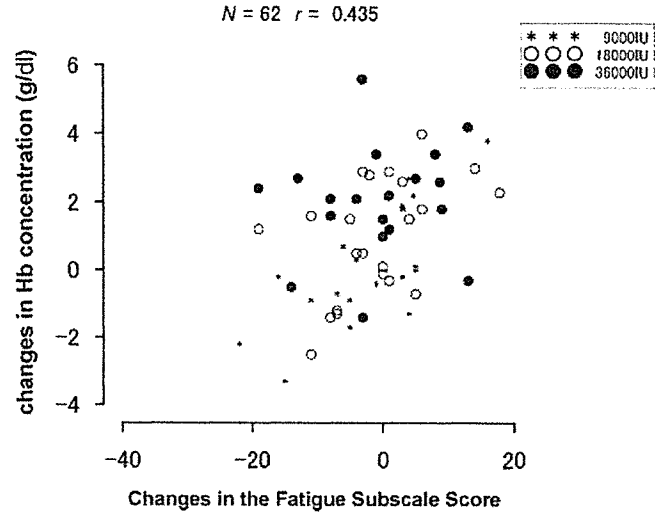

Figure 3. Correlation between change in hemoglobin levels and change in the Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale scores at 7–11 weeks (Per Protocol Set population). Hb, hemoglobin.

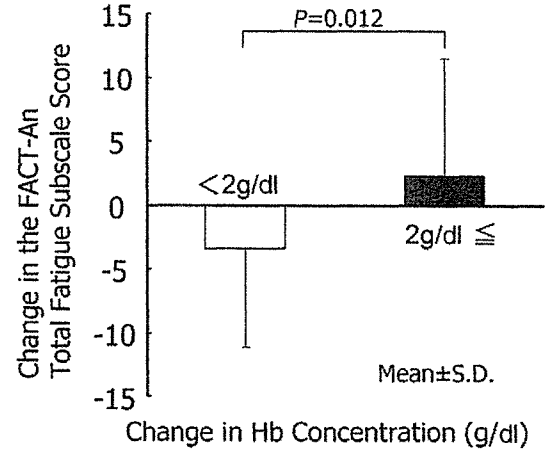

Figure 4. Change in mean Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale score between baseline and 7–11 weeks by change in hemoglobin level (change in hemoglobin of ≥2 g/dl or <2 g/dl from baseline). FACT-An, Functional Assessment of Cancer Therapy–Anemia; Hb, hemoglobin; SD, standard deviation.

Table 3. Incidence of most common adverse events by epoetin-beta dosage group (safety population)

	Epoetin-beta dosage groups							
	9000 IU (n = 28)		18 000 IU (n = 27)		36 000 IU (n = 28)		All patients (n = 83)	
	No.	%	No.	%	No.	%	No.	%
Adverse events (incidence > 20%, any grade)								
Leukopenia	23	82.1	24	88.9	23	82.1	70	84.3
Neutropenia	20	71.4	19	70.4	15	53.6	54	65.1
Nausea	15	53.6	19	70.4	16	57.1	50	60.2
Thrombocytopenia	17	60.7	13	48.1	15	53.6	45	54.2
Anorexia	18	64.3	17	63.0	8	28.6	43	51.8
Fever	10	35.7	6	22.2	12	42.9	28	33.7
Vomiting	8	28.6	9	33.3	11	39.3	28	33.7
Malaise	9	32.1	10	37.0	7	25.0	26	31.3
Increased ALT	7	25.0	8	29.6	10	35.7	25	30.1
Diarrhea	8	28.6	10	37.0	6	21.4	24	28.9
Lymphopenia	13	46.4	6	22.2	5	17.9	24	28.9
Fatigue	8	28.6	7	25.9	8	28.6	23	27.7
Increased AST	5	17.9	6	22.2	9	32.1	20	24.1
Increased LDH	3	10.7	11	40.7	6	21.4	20	24.1
Constipation	5	17.9	6	22.2	6	21.4	17	20.5
Adverse events related to epoetin beta (incidence > 3%, any grade)								
Total number of patients	9	32.1	8	29.6	6	21.4	23	27.7
Total number of events	16		32		17		65	
Hypertension/increased blood pressure	1	3.6	3	11.1	1	3.6	5	6.0
Increased ALT	1	3.6	2	7.4	2	7.1	5	6.0

ALT, alanine aminotransferase.

This is the first prospective randomized dose-finding study of once-weekly epoetin-beta in anemic cancer patients treated with chemotherapy. The study demonstrated that the mean increase in hemoglobin level in the 36 000 IU group was significantly higher than that in the 9000 IU group, while the mean increase in hemoglobin level in the 18 000 IU group was not significantly higher than that in the 9000 IU group. In the present study, epoetin-beta 36 000 IU once-weekly administration showed the same efficacy (an increase in hemoglobin level) as a 200 IU/kg thrice-weekly regimen studied in lung cancer patients receiving chemotherapy (6). It is noteworthy to point out that once-weekly epoetin-beta can be conveniently used in an outpatient-based chemotherapy regimen.

FACT-An is one of the widely used QOL assessment tools, which comprises the FACT-General and a 20-item Anemia subscale, 13 items of which make up a Fatigue subscale. Many reports indicated that chemotherapy-induced anemia increased the ease of a patient becoming fatigued and resulted in decreased patient QOL (17–19). The administration of 36 000 IU epoetin-beta did not significantly improve the patients' Fatigue subscale score in spite of increased hemoglobin levels. As a primary goal of the study was to determine a recommended dose of epoetin-beta, the study design was not planned and did not have adequate statistical power to determine whether epoetin-beta would improve the Fatigue subscale scores. According to the results of the study by Hedenus et al. (20), patients with the lowest baseline Fatigue subscale scores (baseline scores of <24) reported the largest improvement in Fatigue subscale scores after the treatment with darbepoetin alfa. In contrast, patients with baseline Fatigue subscale scores of >36 did not show any improvement. In the subset analysis of our study, among the patients with baseline Fatigue subscale scores of ≤36, a mean improvement in the Fatigue subscale scores at 7–11 weeks were −1.8 for the 9000 IU group, +1.9 for the 18 000 IU group and +4.3 for the 36 000 IU group (36 000 IU versus 9000 IU $P = 0.183$). Our data also demonstrated a significant correlation between change in Fatigue subscale score and change in hemoglobin level and showed that the patients who responded

with a hemoglobin increase of ≥2 g/dl showed significant improvements in the Fatigue subscale scores. In conjunction with these findings, the administration of epoetin-beta may not be beneficial for the patients with relatively high hemoglobin levels and/or less symptomatic even in an anemic state. Thus, the actual hemoglobin level for initiation of epotein beta will be critical for its optimal use. The ASCO/ASH clinical practice guideline in 2002 recommends the use of rhEPO for chemotherapy-associated anemia patients with the hemoglobin level of ≤10 g/dl and that the use of rhEPO for patients with the hemoglobin level of 10–12 g/dl should be determined by clinical circumstances (21).

Most of the adverse events in the present study were considered to be related to concomitant chemotherapy, and the incidence of side effects was similar among the three dosage groups. Two large randomized studies (22,23) targeting higher hemoglobin levels raised concerns about the safety of rhEPO, because of the increased thrombovascular events and worsening survival of cancer patients. In our study, one patient in the 36 000 IU group experienced deep vein thrombosis, which was evaluated as unrelated to epoetin-beta. Stimulated tumor growth is another possible mechanism for worsened survival in the rhEPO studies. A meta-analysis of 27 randomized trials of rhEPO showed suggestive but inconclusive evidence for improved overall survival in patients who received rhEPO (8). Further large scale randomized studies are necessary to confirm the effect of rhEPO on tumor outcome and overall survival.

In conclusion, once-weekly epoetin-beta 36 000 IU for 12 weeks was well tolerated and significantly increased hemoglobin levels in anemic cancer patients receiving chemotherapy. Therefore, the weekly dose of 36 000 IU epoetin-beta was determined as a recommended dose for a subsequent randomized, placebo-controlled, phase III study in Japan.

Part of the results in this report was contributed as Abstract No. 8169 at the 2004 American Society of Clinical Oncology Annual Meeting.

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References

1. Bron D, Meuleman N, Mascaux C. Biological basis of anemia. *Semin Oncol* 2001;28(2 suppl 8):1–6.
2. Sasaki H, Bothner B, Dell A, Fukuda M. Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA. *J Biol Chem* 1987; 262:12059–76.
3. Okamoto H, Saijo N, Shinkai T, Eguchi K, Sasaki Y, Tamura T, et al. Chemotherapy-induced anemia in patients with primary lung cancer. *Ann Oncol* 1992;3:819–24.
4. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. *J Natl Cancer Inst* 1999;91:1616–34.
5. Caro JJ, Salas M, Ward A, Goss G. Anemia as an independent prognostic factor for survival in patients with cancer. *Cancer* 2001;91:2214–21.
6. Kunikane H, Watanabe K, Fukuoka M, Saijo N, Furuse K, Ikegami H, et al. Double-blind randomized control trial of the effect of recombinant human erythropoietin on chemotherapy-induced anemia in patients with non-small cell lung cancer. *Int J Clin Oncol* 2001;6:296–301.
7. Seidenfeld J, Piper M, Flamm C, Hasselblad V, Armitage JO, Bennett CL, et al. Epoetin treatment of anemia associated with cancer therapy: a systematic review and meta-analysis of controlled clinical trials. *J Natl Cancer Inst* 2001;93:1204–14.
8. Bohlius J, Langensiepen S, Schwarzer G, Seidenfeld J, Piper M, Bennett C, et al. Recombinant human erythropoietin and overall survival in cancer patients: results of a comprehensive meta-analysis. *J Natl Cancer Inst* 2005;97:489–98.
9. Littlewood TJ, Bajetta E, Nortier JW, Vercammen E, Rapoport B. Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: results of a randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 2001;19:2865–74.
10. Gabrilove JL, Cleeland CS, Livingston RB, Sarokhan B, Winer E, Einhorn LH. Clinical evaluation of once-weekly dosing of epoetin alfa in chemotherapy patients: improvements in hemoglobin and quality of life are similar to three-times weekly dosing. *J Clin Oncol* 2001;19:2875–82.
11. Demetri GD, Kris M, Wade J, Degos L, Cella D. Quality-of-life in chemotherapy patients treated with epoetin alfa is independent of disease response or tumor type: results from a prospective community oncology study. *J Clin Oncol* 1998;16:3412–25.
12. Cazzola M, Beguin Y, Kloczko J, Spicka I, Coiffier B. Once-weekly epoetin-beta is highly effective in treating anaemic patients with lymphoproliferative malignancy and defective endogenous erythropoietin production. *Br J Haematol* 2003;122:386–93.
13. Yoshimura A, Kobayashi K, Fumimoto H, Fujiki Y, Eremenco S, Kudoh S. Cross-cultural validation of the Japanese Functional Assessment of Cancer Therapy-Anemia (FACT-An). *J Nippon Med Sch* 2004;71:314–22.
14. Dunnett CW, Tamhane AC. Multiple testing to establish superiority/equivalence of a new treatment compared with kappa standard treatments. *Stat Med* 1997;16:2489–506.
15. Witzig TE, Silberstein PT, Loprinzi CL, Sloan JA, Novotny PJ, Mailliard JA, et al. Phase III, randomized, double-blind study of epoetin alfa compared with placebo in anemic patients receiving chemotherapy. *J Clin Oncol* 2005;23:2606–17.
16. Chang J, Couture F, Young S, Mcwatters KL, Lau CY. Weekly epoetin alfa maintains hemoglobin, improves quality of life, and reduces transfusion in breast cancer patients receiving chemotherapy. *J Clin Oncol* 2005;23:2597–605.
17. Boogaerts M, Coiffier B, Kainz C, Epoetin beta QOL Working Group. Impact of epoetin-beta on quality of life in patients with malignant disease. *Br J Cancer* 2003;88:988–95.
18. Cella D, Zagari MJ, Vandoros C, Gagnon DD, Hurtz HJ, Nortier JW. Epoetin alfa treatment results in clinically significant improvements in quality of life on anemic cancer patients when referenced to the general population. *J Clin Oncol* 2003;21:366–73.
19. Osterborg A, Brandberg Y, Molostova V, Iosava G, Abdulkadyrov K, Hedenus M, et al. Randomized, double-blind, placebo-controlled trial of recombinant human erythropoietin, epoetin-beta in hematologic malignancies. *J Clin Oncol* 2002;20:2486–94.
20. Hedenus M, Adriansson M, San Miguel J, Kramer MH, Schipperus MR, Juvonen E, et al. Efficacy and safety of darbepoetin alfa in anaemic patients with lymphoproliferative malignancies: a randomized, double-blind, placebo-controlled study. *Br J Haematol* 2003;122:394–403.
21. Rizzo JD, Lichtin AE, Woolf SH, Seidenfeld J, Bennett CL, Cella D, et al. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. *J Clin Oncol* 2002;20:4083–107.
22. Henke M, Laszig R, Rube C, Schafer U, Haase KD, Schilcher B, et al. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet* 2003;362:1255–60.
23. Leyland-Jones B. BEST Investigators and Study Group. Breast cancer trial with erythropoietin terminated unexpectedly. *Lancet Oncol* 2003;4:459–60.

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Association of polymorphisms in the *MTH1* gene with small cell lung carcinoma risk

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Fifty single-nucleotide polymorphisms (SNPs) associated with amino acid changes in 36 genes involved in diverse DNA repair pathways were assessed for associations with risk for small cell lung carcinoma (SCLC) by a case–control study consisting of 211 SCLC cases and 685 controls. An SNP, Val83Met, in the *MTH1* (mutT homolog 1) gene encoding a triphosphatase that hydrolyzes pro-mutagenic oxidized nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP, showed the strongest and a significant association with SCLC risk [odds ratio (OR) = 1.6, 95% confidence interval (CI): 1.2–2.2, $P = 0.004$], while three other SNPs in the *TP53, BLM* and *SNM1* genes, respectively, also showed marginal associations ($0.05 < P < 0.1$). Another SNP, which causes a nucleotide change in the 5′-UTR of *MTH1* transcripts leading to alternative translation initiation, was additionally examined and the SNP also showed a significant association (OR = 1.7, 95% CI: 1.2–2.3, $P = 0.002$). The two SNPs in the *MTH1* gene were in linkage disequilibrium, and the OR for carrying a copy of the haplotype consisting of both the risky SNP alleles was 2.0 (95% CI: 1.2–3.2, $P = 0.002$). The present results indicate that inter-individual differences in MTH1 activities due to SNPs are involved in susceptibility to SCLC.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world, and is comprised of a group of four histologically distinct types: adenocarcinoma (ADC), squamous cell carcinoma (SQC), large cell carcinoma (LCC) and small cell lung carcinoma (SCLC) (1). SCLC accounts for ~20% of all lung cancer cases and has clinical and biological characteristics distinct from non-small cell lung carcinoma (NSCLC). More than 90% of patients at the time of diagnosis are stage III or stage IV owing to its early and wide dissemination. Although, in most cases tumors initially respond to chemotherapy, >95% of patients eventually die from the cancer. Accordingly, the prognosis of patients with SCLC is poor, and 5-year survival of SCLC is <10% (1–3). Thus, SCLC is the most aggressive type of lung cancer. Genes responsible for the susceptibility to SCLC have been searched for to establish novel and efficient ways of preventing the disease. On the basis of the fact that smoking contributes to SCLC development, polymorphisms in metabolic genes encoding enzymes that activate or detoxify carcinogens in tobacco smoke are being studied for association with SCLC risk by case–control studies. Up to the present, a few metabolic genes, such as *CYP1A1, CYP2A6* and *GSTM1*, have been found to be associated with SCLC risk (4–7). Thus, it is possible that polymorphisms in several metabolic genes are involved in SCLC susceptibility.

Polymorphisms in DNA repair genes have been considered to be involved in susceptibility to cancers, since they are thought to cause inter-individual differences in the capacity for preventing mutagenesis (8–12). In fact, single-nucleotide polymorphisms (SNPs) in several DNA repair genes have been shown to be associated with the risk for several types of cancers (12,13). Carcinogens in cigarette smoke are thought to cause a variety of pro-mutagenic DNA adducts, including benzo[*a*]pyrene-diol-epoxide (BPDE) and 8-hydroxyguanine (8OHG), which are repaired by nucleotide excision repair (NER) and base excision repair (BER) (12). Lung cancer patients were indicated to have lower activities of NER and BER than healthy individuals (9,14). Mice deficient in BER were reported to predispose to lung cancer (15). These results support the fact that inter-individual variations of DNA repair activity are involved in lung cancer susceptibility. We recently identified 50 non-synonymous (associated with amino acid change) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; LCC, large cell carcinoma; *MTH1*, mutT homolog 1; NSCLC, non-small cell lung carcinoma; OR, odds ratio; SCLC, small cell lung carcinoma; SNPs, single-nucleotide polymorphisms; SQC, squamous cell carcinoma.

[†]These authors contributed equally to this work.

integrity (13) (see Table II). These 50 SNPs were examined for association with NSCLC risk in a case–control study consisting of 752 ADC cases, 250 SQC cases and 685 controls, and four of them, LIG4-Ile658Val, TP53-Arg72Pro, POLI-Thr706Ala and REV1-Phe257Ser, were found associated with NSCLC risk. The results suggested that polymorphisms in genes involved in a variety of DNA repair pathways contribute to NSCLC susceptibility. However, to our knowledge, association of SNPs in DNA repair genes with SCLC risk has not been extensively investigated; therefore, their involvements in SCLC susceptibility is unknown. Thus, in the present study, allele distributions for 50 SNPs in 36 DNA repair genes were examined in 211 SCLC cases to investigate association of the SNPs with SCLC risk. Furthermore, DNA repair genes commonly or specifically involved in susceptibility to SCLC and NSCLC were investigated by comparing the present results with our previous results on NSCLC.

Subjects and method

Case–control study

All cases and controls were Japanese. The cases consisted of 211 SCLC patients of four hospitals located in the Kanto area of Japan (i.e. Tokyo and surrounding prefectures) from 1999 to 2004. The hospitals were the National Cancer Center Hospital (NCCH) (113 patients), the National Cancer Center Hospital East (NCCHE) (81 patients), the National Nishigunma Hospital (NNGH) (16 patients) and the Gunma Prefecture Cancer Center Hospital (1 patient). All SCLC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. All the cases were diagnosed by cytological and/or histological examinations according to WHO classification (16). From each individual, a 10 or 20 ml whole-blood sample was obtained. Genomic DNAs for all the cases and the controls were isolated from the samples, and 10 ng of genomic DNA was subjected to genotyping by pyrosequencing as described previously (13). Information on the primer sequences and conditions for PCR were described previously (13).

Genotypes for the 50 SNPs of 685 controls had been determined by the same method as used in the present study (13). The information on the controls was described previously (13). Briefly, the controls consisted of patients of two hospitals, NCCH and NNGH, in which SCLC cases were enrolled, and 302 healthy volunteers of Keio University, located in Tokyo. All of the control subjects were selected with a criterion of no history of any cancer.

Smoking history of cases and controls was obtained via interview using a questionnaire. Smoking habit was expressed by pack-years, which was defined as the number of cigarette packs smoked daily multiplied by years of smoking, both in current smokers and former smokers. Smokers were defined as those who had smoked regularly for 12 months or longer at any time in their life, while non-smokers were defined as those who had not. The study was approved by the institutional review boards of the National Cancer Center, the Nishigunma Hospital, the Gunma Prefecture Cancer Center and Keio University.

Statistical analysis

Differences in the allele distributions for the 50 SNPs between the cases and controls were tested by the χ^2-test. Hardy–Weinberg equilibrium (HWE) tests were performed using the TFPGA software (http://bioweb.usu.edu/mpmbio/). Calculation of the D' and r^2 values and haplotype estimation were undertaken using the EM algorithm. The strength of association of *MTH1* (mutT homolog 1) genotypes and haplotypes with SCLC risk was measured as crude odds ratios (ORs), and ORs were adjusted for gender, age (49, 50–59, 60–69, 70) and smoking dosage (pack-years: 0, 1–49, 50) with 95% confidence intervals (CIs) by unconditional logistic regression analysis (17). ORs for carrying a copy of a haplotype were also calculated by the bootstrap method with 5000 resampling. All the statistical analyses were performed using the SAS version 9 software (SAS Institute, NC, USA).

Results

We conducted a case–control study consisting of 211 SCLC cases and 685 controls (Table I). The SCLC cases consisted of patients enrolled in four hospitals in Tokyo and surrounding prefectures. The 685 controls consisted of patients/outpatients and healthy volunteers without a history of cancer enrolled in two hospitals and a university in the same area. Most of the SCLC cases were males and had a smoking habit, as has been reported (18,19). Therefore, the SCLC cases showed a higher fraction of males and smokers than the controls, and the mean smoking dosage of the SCLC cases was larger than that of the controls.

All the 685 controls were genotyped for the 50 SNPs with a success rate of 99.98% in our previous study (13). The 211 SCLC cases were genotyped for the same 50 SNPs in the present study, and the success rate was 99.94% (Table II). The allele distribution in the SCLC cases was compared with that in the 685 controls. None of the 50 SNPs deviated from HWE in cases and controls ($P \geq 0.05$). A significant difference in the allele distribution between the controls and cases was observed in one of the 50 SNPs, MTH1-Val83Met (OR for the MTH1-83Met allele = 1.6, 95% CI: 1.2–2.2, $P = 0.004$) (Table II). Marginally significant ($0.5 \leq P < 0.1$) allele differentiations were observed in three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met. Allele distributions for the other 46 SNPs were not significantly or were marginally significantly different between the controls and cases.

The relative risks of the genotypes for the four SNPs, which showed significant or marginally significant allele differentiations, were calculated as crude and adjusted ORs. Heterozygotes, homozygotes for the MTH1-83Met allele and carriers of the allele showed significantly increased

Table I. SCLC cases and controls for case–control study

Subject	Institution[a]	No.	Gender (%)		Age	Smoking habit (%)			Pack-years of smokers
			Male	Female	(Mean ± SD)	Non-smoker	Smoker	Unknown	(Mean ± SD)
Case	NCCH	113	88 (78)	25 (22)	61 ± 10	8 (7)	105 (93)	0 (0)	62 ± 31
	NCCHE	81	68 (84)	13 (16)	65 ± 8	0 (0)	77 (95)	4 (5)	57 ± 30
	NNGH[b]	17	16 (94)	1 (6)	68 ± 8	0 (0)	17 (100)	0 (0)	55 ± 25
	Total	211	172 (82)	39 (18)	63 ± 9	8 (4)	199 (94)	4 (2)	59 ± 30
Control	NCCH	242	129 (53)	113 (47)	60 ± 16	138 (57)	102 (42)	2 (1)	36 ± 32
	NNGH	141	100 (71)	41 (29)	65 ± 14	46 (33)	91 (65)	4 (3)	46 ± 35
	KEIO	302	254 (84)	48 (16)	48 ± 10	202 (67)	94 (31)	6 (2)	22 ± 20
	Total	685	483 (71)	202 (29)	55 ± 13	386 (56)	287 (42)	12 (2)	35 ± 31

[a]NCCH, National Cancer Center Hospital; NCCHE, National Cancer Center Hospital East; NNGH, National Nishigunma Hospital; KEIO, Keio university.
[b]Including a case of Gunma Prefecture Cancer Center Hospital.

Table II. Allele frequencies of 50 SNPs in 36 DNA repair genes in controls and cases

DNA repair	Gene	SNP	Amino acid change	dbSNP ID	Minor allele frequency[a]		
					Control[b]	Case	
BER	*PARP/ADPRT*	T2444C	Val762Ala	rs1805412	0.40	0.37	
		A2978G	Lys940Arg	rs1136471	0.05	0.04	
	APEX/APE1	A395G	Ile64Val	rs2307486	0.04	0.05	
		T649G	Asp148Glu	rs3136820	0.38	0.41	
	MBD4	G1212A	Glu346Lys	rs140693	0.35	0.36	
	MTH1/NUDT1	G273A	Val83Met	rs4866	0.09	0.15	($P = 0.004$)
	OGG1	C2243G	Ser326Cys	rs1052133	0.48	0.46	
	XRCC1	C685T	Arg194Trp	rs1799782	0.33	0.30	
		G944A	Arg280His	rs25489	0.09	0.08	
		G1301A	Arg399Gln	rs25487	0.25	0.24	
NER	*XPG/ERCC5*	C3507G	His1104Asp	rs17655	0.42	0.46	
	CSB/ERCC6	G1275A	Gly399Asp	rs2228528	0.45	0.43	
	XPC	A2655C	Lys939Gln	rs2228001	0.40	0.38	
	XPD/ERCC2	G1615A	Asp312Asn	rs1799793	0.04	0.04	
		A2932C	Lys751Gln	rs1052559	0.05	0.05	
Mismatch repair	*MLH1*	A676G	Ile219Val	rs1799977	0.05	0.03	
	MLH3	C2645T	Pro844Leu	rs175080	0.18	0.16	
		C2939T	Thr942Ile	rs17102999	0.05	0.06	
	MSH2	C91T	Thr8Met	rs17217716	0.02	0.02	
	MSH3	A3122G	Thr1036Ala	rs26279	0.24	0.27	
	MSH6	G203A	Gly39Glu	rs1042821	0.32	0.31	
DNA double-strand break repair	*BRCA2*	A1342C	Asn372His	rs144848	0.22	0.21	
	SNM1/KIAA0086	C1867G	His317Asp	rs3750898	0.26	0.30	($P = 0.08$)
	LIG4	A2245G[c]	Ile658Val	rs2232641	0.04	0.06	
	NBS1	C605G	Gln185Glu	rs1805794	0.50	0.46	
	RAD51L3/RAD51D	G501A	Arg165Gln	rs4796033	0.04	0.03	
	RAD54L	A551G	Lys151Glu	rs2295466	0.02	0.01	
	RINT-1	G33C	Glu4Gln	rs818620	0.07	0.09	
	XRCC3	C1075T	Thr241Met	rs861539	0.09	0.09	
DNA damage response	*TP53*	G466C[c]	Arg72Pro	rs1042522	0.33	0.38	($P = 0.097$)
DNA polymerase	*POLD1*	G409A	Arg119His	rs1726801	0.20	0.22	
	POLH/XPV/RAD30	A1840G	Lys535Glu	-	0.03	0.04	
	POLI/RAD30B	A2180G[c]	Thr706Ala	rs8305	0.25	0.24	
	POLL	C1683T	Arg438Trp	rs3730477	0.01	0.012	
	REV1	T982C[c]	Phe257Ser	rs3087386	0.33	0.32	
	A1330G	Asn373Ser	rs3087399	0.04	0.04		
	POLZ/REV3	C4259T	Thr1146Ile	rs462779	0.35	0.37	
Other pathways	*BLM*	C967T	Thr298Met	rs28384991	0.09	0.12	($P = 0.09$)
		G4035A	Val1321Ile	rs7167216	0.04	0.04	
	FANCA	G827A	Ala266Thr	rs17232400	0.03	0.03	
		G1080A	Arg350Gln	rs17233497	0.01	0.01	
		A1532G	Ser501Gly	rs2239359	0.17	0.16	
		A2457G	Asp809Gly	rs7195066	0.03	0.03	
		C3294T	Ser1088Phe	rs7190823	0.02	0.02	
	FANCE	G451T	Arg89Leu	-	0.01	0.00	
		G1213A	Arg343Gln	-	0.04	0.04	
	FANCF	A983G	Lys324Glu	-	0.003	0.002	
	FANCG/XRCC9	C1382T	Thr297Ile	rs2237857	0.12	0.13	
	WRN	C2573T	Thr781Ile	rs17847568	0.03	0.03	
		T4330C	Cys1367Arg	rs1346044	0.09	0.07	

[a]*P*-values by χ^2-test against the control population are shown, when they are <0.1.
[b]Determined in our previous study (12).
[c]Significantly associated with SQC and/or ADC risks in our previous study (12).

ORs, when homozygotes for the 83Val allele were used as a reference, respectively (Table III). On the other hand, ORs of genotypes for the remaining three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met, did not show significant increases or decreases in SCLC cases (data not shown); therefore, these SNPs were not further investigated in the present study.

The *MTH1* gene, whose SNP, Val83Met, showed a significant association as described above, encodes a triphosphatase that hydrolyzes oxidized purine nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP (20). The activity of the MTH1-83Met protein was reported to be more thermolabile than that of the MTH1-83Val protein (20–22). The mitochondrial translocation of the MTH1-83Met protein was reported to be less efficient than that of the MTH1-83Val protein (23). Thus, it was suggested that the MTH1-83Met protein is less active than the MTH1-83Val protein. Previously, another SNP was found in a non-coding exon of *MTH1* (i.e. the T/C SNP in exon 2) 7.0 kb distal to the MTH1-Val83Met SNP, and the C allele in exon 2 leads to the production of an additional translation start site, resulting in the production of a longer MTH polypeptide in addition to commonly produced MTH polypeptides (21). This T/C SNP of the *MTH1* gene was

Table III. MTH1 genotypes and SCLC risk

SNP	Genotype	No. of controls (%)	No. of cases (%)	Crude OR (95% CI, P)	Adjusted OR[a](95% CI, P
Val83Met	Val/Val	558 (82)	154 (73)	Reference	Reference
	Val/Met	117 (17)	53 (25)	1.6 (1.1–2.4, 0.009)	1.7 (1.1–2.7, 0.03)
	Met/Met	6 (1)	4 (2)	2.4 (0.7–8.7, 0.2)	6.5 (1.3–32.1, 0.02)
	Val/Met + Met/Met	123 (18)	57 (27)	1.7 (1.2–2.4, 0.005)	1.8 (1.2–2.9, 0.01)
T/C in exon 2	T/T	560 (82)	154 (73)	Reference	Reference
	T/C	118 (17)	53 (25)	1.6 (1.1–2.4, 0.009)	1.7 (1.1–2.7, 0.03)
	C/C	3 (0)	4 (2)	4.8 (1.1–21.9, 0.04)	15.7 (2.5–100.6, 0.004)
	T/C + C/C	121 (17)	57 (27)	1.7 (1.2–2.5, 0.004)	1.9 (1.2–3.0, 0.008)

[a]Adjusted for gender, age and smoking dosage.

not included in our 50 SNP set, because it was located in a non-coding exon. However, the above result prompted us to genotype this SNP in the same SCLC and control subjects. Since genotype data for the MTH1-Val83Met SNP were obtained from 681 of the 685 controls and all 211 cases, the genotypes for the T/C SNP were also determined for the same 681 controls and the 211 SCLC cases. Significant allele differentiations were also observed in the T/C SNP (OR for the C allele = 1.7, 95% CI: 1.2–2.3, $P = 0.002$). ORs of the heterozygotes, homozygotes for the exon 2-C allele and carriers of the allele were also significantly increased, when homozygotes for the exon 2-T allele were used as a reference, respectively (Table III).

Since both the case and control subjects in the present case–control study were enrolled in several institutions, it was possible that differences in the institutions lead to the observed allele differentiations due to population stratification. Therefore, we compared allele frequencies for the MTH1-Val83Met and exon 2-T/C SNPs among SCLC cases and controls of each institution (Figure 1). Allele frequencies for the MTH1-Val83Met SNP had been also reported in two other populations consisting of healthy Japanese volunteers (21,24); therefore, the frequencies in those studies were also compared. Frequencies of the 83Met and exon 2-C alleles in any SCLC populations were higher than those in any of the control populations. Allele frequencies for these SNPs were not significantly different among control populations and among case populations ($P > 0.05$ by χ^2-test). We also compared the frequencies of genotypes for the two SNPs, and they were not significantly different among control populations and among case populations, either ($P > 0.05$ by χ^2-test). Thus, it was indicated that the 83Met and exon 2-C alleles were associated with the SCLC risk beyond institutional differences.

Both the SNPs of *MTH1* were found to be in linkage disequilibrium with each other ($D' = 0.97$, $r^2 = 0.91$). Thus, we further evaluated the haplotype differentiation between the SCLC cases and the controls (Table IV). The haplotype consisting of the two risky alleles (i.e., haplotype #2 consisting of the 83Met and C alleles in Table IV) was significantly over-represented in the SCLC population (OR = 1.7, 95% CI: 1.2–2.4, $P = 0.001$), and the OR for haplotype #2 was similar to those for individual 83Met and C allele, respectively. In addition, by taking into account the estimation error of haplotype frequency, crude and adjusted ORs for carrying one copy of haplotype #2 were calculated on the basis of the estimated number of haplotypes for each subject by the bootstrap method, and they were 1.8 (95% CI: 1.2–2.5, $P = 0.0004$) and 2.0 (95% CI: 1.2–3.2, $P = 0.002$), respectively.

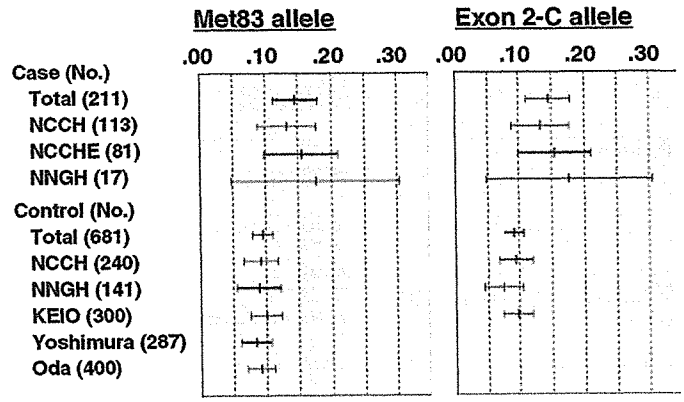

Fig. 1. Frequencies of the MTH-83Met and exon 2-C alleles in cases and controls. Allele frequency is shown with its sampling variations estimated by 95% CI. Frequencies of the MTH-83Met allele in two control populations reported by Yoshimura *et al.* (24) and Oda *et al.* (21) are also shown.

We next assessed the effect of smoking on the contribution of the MTH1-Val83Met and exon 2-T/C SNPs to the SCLC risk. ORs in light (PY < 50) smokers and heavy (PY ≥ 50) smokers were compared (Table V). The number of non-smokers in the case subjects was small (i.e. $N < 10$); therefore, they were excluded from the analysis. Increases of ORs for the 83Met and exon 2-C alleles were more evident in light smokers than in heavy smokers, and the ORs were statistically significant in light smokers but not in heavy smokers. *P*-values for interaction of the Val83Met and exon 2-T/C genotypes on the SCLC risk with smoking were 0.15 and 0.11, respectively. *P*-value for interaction of haplotype #2 on the SCLC risk by smoking was calculated as being 0.095.

Discussion

The *MTH1* gene was cloned as a human homolog for the *Escherichia coli mutT* gene, encoding an enzyme hydrolyzing 8-hydroxy-dGTP, an oxidized dNTP causing A:T to C:G transversion (20). It has been shown that MTH1 protein hydrolyzes not only 8-hydroxy-dGTP but also several other oxidatively damaged dNTPs, such as 2-hydroxy-dATP, thereby preventing multiple mutations including A:T to C:G, G:C to T:A and G:C to A:T mutations (20). *Mth1* nullizygous mice are susceptible to tumor development in lung and other tissues (25). Thus, it has been assumed that inter-individual differences in *MTH1* activity are associated with risks for cancers by causing inter-individual differences

Table IV. Association of MTH1 haplotypes and SCLC risk

Haplotype	SNP		Haplotype frequency		OR (95% CI)	*P*
	Val83Met	T/C in exon 2	Control (95% CI)	Case (95% CI)		
1	Val	T	0.90 (0.89–0.92)	0.85 (0.82–0.89)	Reference	
2	Met	C	0.089 (0.073–0.10)	0.14 (0.11–0.18)	1.7 (1.2-2.4)	0.001
3	Met	T	0.0067 (0.0023–0.011)	0.0024 (0–0.0070)	0.4 (0.05–3.0)	0.3
4	Val	C	0.0030 (0–0.0059)	0.0024 (0–0.0070)	0.9 (0.1–7.7)	0.9

Table V. OR for MTH1 genotypes by smoking dosage and age

SNP	Stratification	No of controls (%)		No of cases (%)		Crude OR	Adjusted OR[a]	*P* for interaction[a]
		Major homozygote	Minor allele carrier	Major homozygote	Minor allele carrier	(95% CI, *P*)	(95% CI, *P*)	
Val83Met	py = 0	319 (83)	67 (17)	5 (63)	3 (38)	2.8 (0.7–12.2, 0.16)	2.9 (0.7–12.7, 0.16)	
	0 < py < 50	178 (82)	38 (18)	59 (69)	26 (31)	2.1 (1.2–3.7, 0.014)	2.3 (1.2–4.4, 0.011)	0.15
	py ≥ 50	54 (78)	15 (22)	88 (77)	26 (23)	1.1 (0.5–2.2, 0.87)	1.1 (0.5–2.3, 0.85)	
T/C in exon 2	py = 0	316 (82)	68 (18)	5 (63)	3 (38)	2.8 (0.7–12.0, 0.16)	2.8 (0.6–12.3, 0.17)	0.11
	0 < py < 50	181 (84)	35 (16)	59 (69)	26 (31)	2.3 (1.3–4.1, 0.006)	2.6 (1.3–4.9, 0.005)	
	py ≥ 50	54 (78)	15 (22)	88 (77)	26 (23)	1.1 (0.5–2.2, 0.87)	1.1 (0.5–2.3, 0.85)	

[a]Adjusted for gender and age.

in the capacity to prevent mutations of the cancer-related genes caused by incorporation of oxidatively damaged dNTPs during DNA replication (20). In the present study, SNPs in the *MTH1* gene were found to be associated with SCLC risk. To the best of our knowledge, SNPs in the *MTH1* gene were found for the first time as being associated with risks for human cancers by a case–control study. However, the possibility of false positives (type I statistical errors) must be considered. We performed 50 separate tests of significance in the analysis. A consecutive Bonferroni adjustment to yield an experiment-wide type I error rate of 0.05 would demand a test-wise *P*-value of 0.001. Therefore, the association of the MTH1-Val83Met SNP would not be considered significant on an experiment-wide level after Bonferroni adjustment. Thus, the association requires confirmation in other population samples, although the present study proposed *MTH1* as a candidate gene responsible for SCLC susceptibility.

The two *MTH1* SNPs, Val83Met and exon 2-T/C, examined in the present study were suggested to cause functional differences, although their effects on mutation suppression efficiency against oxidative DNA damages are unknown (20–22). These two SNPs were in linkage disequilibrium, and the risky allele of each SNP (i.e. the 83Met and exon 2-C alleles) was on the same haplotype (haplotype #2) in most of the Japanese population. Thus, at present, it is unclear whether both or one of the two SNPs are responsible for the SCLC susceptibility. It is also possible that other SNPs consisting of the haplotype are responsible. Further biological and genetic analyses of the *MTH1* SNPs will elucidate the issue.

Interestingly, ORs for carriers of the 83Met and C alleles were more evidently increased in light smokers than in heavy smokers. Tobacco smoke is known to cause oxidative damages on genomic DNA and nucleoside triphosphates (26). Therefore, individuals carrying the 83Met and C alleles might be more prone to acquiring gene mutations even by a low-dose exposure of carcinogens, and therefore, the effects of *MTH* SNPs might have more prominently appeared under the condition of a low-dose exposure of tobacco smoke. On the other hand, the effects of the SNPs might be masked under the condition of a high-dose exposure of tobacco smoke, since, under such a condition, environmental factors (i.e. carcinogens in tobacco smoke) rather than genetic factors predominantly determine the risk for SCLC. However, the interaction of *MTH1* SNPs with smoking on SCLC risk in the present study was not statistically significant; therefore, further case–control studies are necessary to elucidate how *MTH1* SNPs contribute to SCLC risk of smokers.

We previously examined the same 50 SNP set for associations with lung SQC and ADC risk using the same controls (13). In the study, frequencies of the MTH1-83Met allele in SQC and ADC cases, respectively, were slightly higher than that in controls. However, ORs of the carriers of the allele was not significantly increased (Figure 2). Thus, the MTH1-Val83Met SNP was thought to be associated with SCLC risk but not with NSCLC risk. In the previous study, an SNP, TP53-Arg72Pro, in the *p53* gene was associated with SQC risk, and the association remained significant after Bonferroni adjustment. Association of the SNP with NSCLC and overall lung cancer risks have been observed in several other case–control studies (28–31). The association was also supported by a report that TP53-72Pro protein has a weaker activity than TP53-72Arg protein in inducing apoptosis of human cells suffering from DNA damages (32). Interestingly, the TP53-72Pro allele was marginally significantly over-represented in SCLC cases in the present study. ORs of the homozygotes for the carriers of the TP53-72Pro allele were increased in SCLC cases, although the increase was not statistically significant (Figure 2). Thus, it is possible that the TP53-72Pro allele confers increased susceptibility both to SCLC and NSCLC. In the present study, marginally significant associations with SCLC risk were observed for two other SNPs, BLM-Thr298Met and SNM1-His317Asp. However, such associations were not detected in ADC and

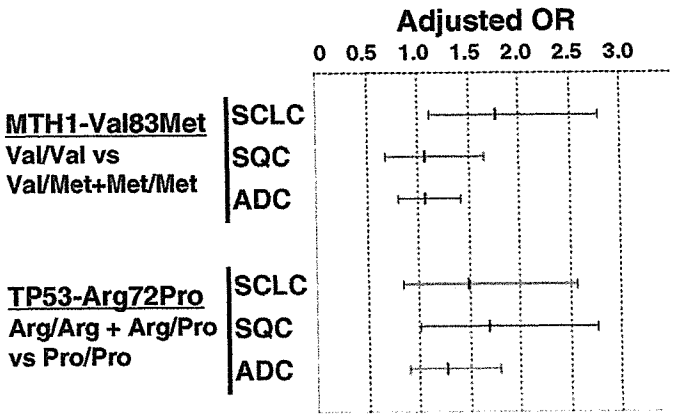

Fig. 2. ORs of the MTH-83Met allele carriers against homozygotes for the MTH-83Val allele and those of homozygotes for the TP53-72Pro allele against others. ORs adjusted for gender, age and smoking dosage are shown. ORs in SQC and ADC cases are from our previous report (13).

SQC (Table II). SNPs that showed association with SQC or ADC risk, such as LIG4-Ile658Val, POLI-Thr706Ala and REV1-Phe257Ser, were not associated with SCLC risk in this study. Thus, genes involved in the susceptibility might be overlapped but different between SCLC and NSCLC.

In the present and previous studies (13), we examined the associations of 50 SNPs in 36 DNA repair genes with SCLC and NSCLC risks. The studies led us to identify several DNA repair genes commonly or specifically involved in the susceptibility to SCLC and NSCLC. The results supported the idea that inherited variations in DNA repair genes are involved in susceptibility to lung cancer of each individual. Further examination of SNPs in DNA repair genes in the present and also in other sets of subjects will help us understand genetic factors responsible for the susceptibility to lung cancer. In addition, studies up to the present suggested that polymorphisms of genes involved in metabolism of carcinogens in cigarette smoke, such as *CYP1A1, CYP2A6* and *GSTM1*, are also responsible for the susceptibility to lung cancer (4–7). It is possible that such polymorphisms modify the effect of SNPs in DNA repair genes on risk for lung cancer. Therefore, combined effects of polymorphisms in DNA repair genes and metabolic genes on risks for SCLC and NSCLC should be also further investigated.

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References

1. Jemal,A., Tiwari,R.C., Murray,T., Ghafoor,A., Samuels,A., Ward,E. Feuer,E.J. and Thun,M.J. (2004) Cancer statistics, 2004. *CA Cancer J Clin.*, **54**, 8–29.
2. Travis,W., Nicholson,S., Hirsch,F.R. *et al.* (2004) Small Cell Carcinoma In Travis,W.D., Brambilla,E., Muller-Hermelink,H.K. and Harris,C.C. (eds) *World Health Organization Classification of Tumors: Pathology and Genetics, Tumours of Lung, Pleura, Thymus and Heart*.pp. 31–34.
3. Jackman,D.M. and Johnson,B.E. (2005) Small-cell lung cancer. *Lancet* **366**, 1385–1396.
4. Sugimura,H., Wakai,K., Genka,K. *et al.* (1998) Association of Ile462Val (exon 7) polymorphism of cytochrome P450 IA1 with lung cancer in the Asian population: further evidence from a case–control study in Okinawa *Cancer Epidemiol. Biomarkers Prev.*, **7**, 413–417.
5. Bartsch,H., Nair,U., Risch,A., Rojas,M., Wikman,H. and Alexandrov,K (2000) Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 3–28.
6. Fujieda,M., Yamazaki,H., Saito,T. *et al.* (2004) Evaluation of CYP2A6 genetic polymorphisms as determinants of smoking behavior and tobacco related lung cancer risk in male Japanese smokers. *Carcinogenesis*, **25** 2451–2458.
7. Stucker,I., Hirvonen,A., de Waziers,I., Cabelguenne,A., Mitrunen,K. Cenee,S., Koum-Besson,E., Hemon,D., Beaune,P. and Loriot,M.A. (2002 Genetic polymorphisms of glutathione *S*-transferases as modulators of lung cancer susceptibility. *Carcinogenesis*, **23**, 1475–1481.
8. Shields,P.G. and Harris,C.C. (2000) Cancer risk and low-penetrance susceptibility genes in gene–environment interactions. *J. Clin. Oncol.*, **18** 2309–2315.
9. Spitz,M.R., Wei,Q., Dong,Q., Amos,C.I. and Wu,X. (2003) Genetic susceptibility to lung cancer: the role of DNA damage and repair. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 689–698.
10. Amos,C.I., Xu,W. and Spitz,M.R. (1999) Is there a genetic basis for lung cancer susceptibility? *Recent Results Cancer Res.*, **151**, 3–12.
11. Mohrenweiser,H.W., Wilson,D.M. III and Jones,I.M. (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat. Res.*, **526**, 93–125.
12. Goode,E.L., Ulrich,C.M. and Potter,J.D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1513–1530.
13. Sakiyama,T., Kohno,T., Mimaki,S. *et al.* (2005) Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. *Int. J. Cancer*, **114**, 730–737.
14. Paz-Elizur,T., Krupsky,M., Blumenstein,S., Elinger,D., Schechtman,E and Livneh,Z. (2003) DNA repair activity for oxidative damage and risk of lung cancer. *J. Natl Cancer Inst.*, **95**, 1312–1319.
15. Sakumi,K., Tominaga,Y., Furuichi,M., Xu,P., Tsuzuki,T., Sekiguchi,M and Nakabeppu,Y. (2003) Ogg1 knockout-associated lung tumorigenesis and its suppression by *Mth1* gene disruption. *Cancer Res.*, **63** 902–905.
16. Brambilla,E., Travis,W.D., Colby,T.V., Corrin,B. and Shimosato,Y (2001) The new World Health Organization classification of lung tumours. *Eur. Respir. J.*, **18**, 1059–1068.
17. Breslow,N.E. and Day,N.E. (1980) Statistical methods in cancer research. Volume I—The analysis of case–control studies. *IARC Sci. Publ.*, 5–338.
18. Yoshimi,I., Ohshima,A., Ajiki,W., Tsukuma,H. and Sobue,T. (2003) A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka Cancer Registry, Japan and in the Surveillance, Epidemiology and End Results Program, USA. *Jpn. J. Clin. Oncol.*, **33**, 98–104.
19. Wynder,E.L. and Hoffmann,D. (1994) Smoking and lung cancer: scientific challenges and opportunities. *Cancer Res.*, **54**, 5284–5295.
20. Nakabeppu,Y. (2001) Molecular genetics and structural biology of human MutT homolog, MTH1. *Mutat. Res.*, **477**, 59–70.
21. Oda,H., Taketomi,A., Maruyama,R., Itoh,R., Nishioka,K., Yakushiji,H., Suzuki,T., Sekiguchi,M. and Nakabeppu,Y. (1999) Multi-forms of human MTH1 polypeptides produced by alternative translation initiation and single nucleotide polymorphism. *Nucleic Acids Res.*, **27**, 4335–4343.
22. Yakushiji,H., Maraboeuf,F., Takahashi,M., Deng,Z.S., Kawabata,S., Nakabeppu,Y. and Sekiguchi,M. (1997) Biochemical and physicochemical characterization of normal and variant forms of human MTH1 protein with antimutagenic activity. *Mutat. Res.*, **384**, 181–194.

23. Sakai,Y., Oda,H., Yoshimura,D., Furuichi,M., Kang,D., Iwai,S., Hara,T. and Nakabeppu,Y. (2006) The GT to GC single nucleotide polymorphism at the beginning of an alternative exon 2C of human MTH1 gene confers an amino terminal extension that functions as a mitochondrial targeting signal. *J. Mol. Med.*, in press.
24. Yoshimura,K., Hanaoka,T., Ohnami,S., Kohno,T., Liu,Y., Yoshida,T., Sakamoto,H. and Tsugane,S. (2003) Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene–environment studies on cancer: data from population-based Japanese random samples. *J. Hum. Genet.*, **48**, 654–658.
25. Tsuzuki,T., Egashira,A., Igarashi,H. *et al.* (2001) Spontaneous tumorigenesis in mice defective in the MTH1 gene encoding 8-oxo-dGTPase. *Proc. Natl Acad. Sci. USA*, **98**, 11456–11461.
26. Loft,S. and Poulsen,H.E. (1996) Cancer risk and oxidative DNA damage in man. *J. Mol. Med.*, **74**, 297–312.
27. Hou,S.M., Falt,S., Yang,K., Nyberg,F., Pershagen,G., Hemminki,K. and Lambert,B. (2001) Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency in lung cancer patients and population controls. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 133–140.
28. Kiyohara,C., Yoshimasu,K., Shirakawa,T. and Hopkin,J.M. (2004) Genetic polymorphisms and environmental risk of lung cancer: a review. *Rev. Environ. Health*, **19**, 15–38.
29. Wu,X., Zhao,H., Amos,C.I., Shete,S., Makan,N., Hong,W.K., Kadlubar,F.F. and Spitz,M.R. (2002) p53 genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. *J. Natl Cancer Inst.*, **94**, 681–690.
30. Mechanic,L.E., Marrogi,A.J., Welsh,J.A., Bowman,E.D., Khan,M.A., Enewold,L., Zheng,Y.L., Chanock,S., Shields,P.G. and Harris,C.C. (2005) Polymorphisms in XPD and TP53 and mutation in human lung cancer. *Carcinogenesis*, **26**, 597–604.
31. Fan,R., Wu,M.T., Miller,D., Wain,J.C., Kelsey,K.T., Wiencke,J.K. and Christiani,D.C. (2000) The p53 codon 72 polymorphism and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1037–1042.
32. Dumont,P., Leu,J.I., Della Pietra,A.C. III, George,D.L. and Murphy,M. (2003) The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat. Genet.*, **33**, 357–365.

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ORIGINAL ARTICLE

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Pilot phase II study of weekly chemotherapy with paclitaxel and carboplatin for refractory or relapsed small-cell lung cancer

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Abstract *Purpose*: The safety and efficacy of weekly chemotherapy with paclitaxel and carboplatin for the treatment of patients with refractory or relapsed small-cell lung cancer (SCLC) were evaluated. *Patients and methods*: Paclitaxel (100 mg/m^2) and carboplatin (with a target area under the concentration versus time curve of 2 mg min/ml using the Calvert formula) were administered to patients with previously- treated SCLC on days 1 and 8 at every 3–4 weeks. *Results*: A total of 29 patients (pts) [male/female, 26/3 pts; median age 62.7 years (43–74); performance status 0/1/2, 9/10/10 pts] were enrolled between March 2000 and June 2002. The mean number of cycles administered per pt was 3 (1–7). The overall response rate was 69% (95% confidence interval 52–86%), and 83% (15/18) in sensitive pts and 45% (5/11) in refractory pts ($P<0.01$). The overall median survival time was 29.6 weeks with a 1-year survival rate of 37% [34.1 weeks in sensitive pts and 23.1 weeks in refractory pts ($P=0.085$), 46.9 weeks in PS 0–1 and 16.3 weeks in PS 2 ($P<0.001$)]. The median time to progressive disease was 16.4 weeks [21.7 weeks in sensitive pts and 15.3 weeks in refractory pts ($P=0.32$)]. Hematologic toxicities observed included grade ≥3 neutropenia in 55%, grade ≥3 anemia in 36%, and grade ≥3 thrombocytopenia in 3%. Non-hematologic toxicities were mild except for grade 3 diarrhea in three pts and grade 3 pneumonitis in one pt. *Conclusion*: Weekly chemotherapy with paclitaxel and carboplatin was well-tolerated and gave a high-response rate in pts with refractory or relapsed small-cell lung cancer.

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Keywords Small-cell lung cancer · Second line chemotherapy · Weekly chemotherapy · Carboplatin · Paclitaxel

Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of the total number of lung cancer patients. It grows more rapidly and shows a higher incidence of remote metastasis than non-small-cell lung cancer (NSCLC). It is apparently more sensitive to chemotherapy and radiotherapy than NSCLC, but is cured only in a small number of patients and recurs in a great majority of them. Recurrent SCLC is less responsive to chemotherapy, and the median survival time from recurrence to death is 2–3 months [3]. Chemotherapy has been reported to contribute to the improvement of symptoms and prolongation of the survival time in patients with recurrent SCLC [2, 6]. In general, first-line chemotherapy is conducted for sensitive disease (relapse ≥90 days after completion of first-line chemotherapy). For refractory disease (relapse during first-line chemotherapy or less than 90 days after completion of initial chemotherapy), however, salvage chemotherapy is undertaken due to the lack of a standard chemotherapy regimen. However, no standard chemotherapy has been established for recurrent SCLC [17].

In recent years, a number of institutions have undertaken weekly chemotherapy for lung cancer and reported the outcome [11, 14]. Weekly chemotherapy is being reported to be useful for recurrent SCLC as well [1, 4, 7, 10]. It is considered to be more suitable than the standard chemotherapy conducted every 3–4 weeks for recurrent cases with impaired bone marrow due to initial chemotherapy because it uses smaller doses of anticancer drugs in each administration cycle and it is possible to titrate their doses after starting the treatment depending on hemotoxicity and the patients' physical condition.

When used alone, paclitaxel was reported to produce good therapeutic results in patients with refractory SCLC with a response rate of 29% and a median survival time of 100 days [15]. When coadministered with carboplatin, paclitaxel showed even better results with a response rate of 73.5% and a median survival time of 31 weeks [5]. This report prompted us to conduct the present study to evaluate the efficacy and safety of weekly chemotherapy using carboplatin and paclitaxel in recurrent SCLC patients.

Patients and methods

Patient selection

All patients with histologically or cytologically confirmed SCLC with documented progression after chemotherapy were eligible for this phase II trial. Patients with either limited- or extensive-stage disease were allowed. The trial was initiated after a rest period of at least 4 weeks following previous chemotherapy (2 weeks in the case of radiotherapy). Patients were required to have recovered completely from prior therapy, with no ongoing toxicity greater than grade 1.

Other eligibility criteria included expected survival of 12 weeks, age $\leq$ 75 years, Eastern cooperative oncology group performance score of 0–2, measurable lesions, and adequate hematological function. Primary refractory disease was defined as relapse during first-line chemotherapy or less than 90 days after completing initial chemotherapy, and sensitive disease was defined as relapse $\geq$90 days after completion of first-line chemotherapy.

The ethical committee of the Tochigi cancer center approved the protocols. Written informed consent stating that the patient was aware of the investigational nature of this treatment regimen was obtained in every case.

Treatment

Paclitaxel was administered at a dose of 100 mg/m^2 intravenously during a 1-h infusion on days 1and 8 of the treatment cycle. Carboplatin was given at a dose designed to give an area under the curve (AUC) of 2 on days 1 and 8 with the use of the Calvert formula: 2 $\times$ (creatinine clearance + 25). Prior to each treatment, patients were given 50 mg diphenhydramine orally, and an H2 blocker intravenously along with 16 mg dexamethasone . Intrvenously administered antiemetics, 3 mg graniston, were used. The length of each chemotherapy cycle was 21 days. Patients who experienced grade 4 leukopenia or neutropenia that lasted for three days or more , or who experienced grade 4 thrombocytopenia, reversible grade 2 neurotoxicity, or liver dysfunction, received reduced doses of both paclitaxel and carboplatin (paclitaxel 80 mg/m^2, carboplatin AUC1.5) for the next cycle. If non-hematologic toxicities of grade 3 or more occurred, treatment was stopped. Subsequent courses of chemotherapy were started after 3–4 weeks when the leukocyte count was 3,000/mm^3 or more, the neutrophil count 1,500/mm^3 or more, the platelet count 75,000/mm^3 or more, serum creatinine less than 1.5 mg/dl, GOT and GPT less than twice the upper limit of the normal range, and neurotoxicity was grade 1 or less. If these variables did not return to adequate levels by the first day of the next course of chemotherapy, treatment was withheld until full recovery. If more than 6 weeks passed from the time of the last treatment before these criteria were satisfied, or if more than dose reduction were indicated, the patient was taken off the study at that time, but still included in the analysis.

Evaluation of response and toxicity

Pretreatment evaluation included medical history, physical examination, complete blood count, bone marrow examination, serum biochemical analyses, chest roentgenogram, electrocardiogram, and urinalysis. All patients underwent radionuclide bone scan, bone marrow aspiration or biopsy, magnetic resonance or computerized tomography (CT) of the brain, and CT of thorax and abdomen. Complete blood count, biochemical tests, serum electrolytes, urinalysis, and chest roentgenograms were obtained weekly during this phase II trial.

Response and toxicity were evaluated on the basis of tumor images obtained by CT and other techniques, laboratory data and subjective/objective symptoms before, during, and after administration of the study drugs and during the period from completion of treatment to final analysis. Measurable disease parameters were determined every 4 weeks by various means such as CT. Evaluation was made in compliance with response evaluation criteria in solid tumors (RECIST) guidelines [16] for anti-tumor activity, and with NCI common toxicity criteria Version 2 for safety. Patients were withdrawn from the study if evidence of tumor progression was observed. The Institutional Ethical Review Committee approved the study.

Statistical analyses

Time to progression was measured as a period from the start of this treatment to the identifiable time for progression. Survival time was measured from the start of the present treatment until death or last follow-up. The Kaplan–Meier method was used to calculate survival curves. Survival differences between subgroups were compared using the log-rank test. The chi-square test was used to compare the percentage of patients in each group.

Primary endpoints were response rate and toxicity; secondary endpoints were survival and time to pro-

gression. We chose a 50% response rate as a desirable target level and a 25% response rate as an undesirable target. Our design had a power in excess of 95% and less than 20% type I error, requiring 26 patients. Considering the percentage of probable dropout cases, 29 patients were required.

Results

Patient characteristics

Twenty-nine patients were enrolled in this study from March 2000 to June 2002. All patients were assessed for toxicity, response and survival. Characteristics of the 29 patients are listed in Table 1. There were 11 refractory cases and 18 sensitive cases against the first-line chemotherapy.

Efficacy of treatment

The mean number of cycles administered per patient was three, and ranged from one to seven. There were no cycles of dose reduction. One patient achieved a complete response (CR) and 19 patients showed partial response (PR). Overall response rate was 69% (20/29) [95% confidence interval (CI) 52–86%]. The response rate was 83% (15/18, 95% CI: 66–100%) in sensitive cases and 45% (5/11, 95% CI: 16–75%) in refractory cases, with significant differences between the two groups ($P < 0.01$). The median time to progressive disease was 16.4 weeks [21.7 weeks in sensitive pts and 15.3 weeks in refractory pts ($P = 0.32$)]. The overall median survival time was 29.6 weeks (Fig. 1) with no significant differences between sensitive cases (34.1 weeks) and refractory cases (23.1 weeks) ($P = 0.085$). The median survival time differed significantly between PS 0 or 1 patients (46.9 weeks) and PS 2 patients (16.3 weeks) ($P < 0.001$). The 1-year survival rate was 38% (11/29).

Toxicities

Table 2 lists the toxicities observed during this study. Hematological and blood biochemical reactions included a high incidence of leukopenia and neutropenia, leukopenia, and neutropenia of grade 3 or higher occurred in 55 and 55%, respectively. All neutropenia patients recovered upon treatment with G-CSF. Anemia and thrombocytopenia of grade 3 or higher occurred in 27 and 3%, respectively. Subjective and objective symptoms observed included grade 3 diarrhea in three patients who all showed improvement after administration of anti-cholinergic drugs, and grade 3 pneumonitis in one, who showed rapid recovery following administration of steroids. Other subjective and objective symptoms observed were of grade 2 or less and included nausea in 34%, vomiting in 10%, alopecia in 59%, neuropathy in 28%, and flushing in 17%. All of these toxicities disappeared or improved by symptomatic treatment. There were no toxic deaths.

Discussion

No standard chemotherapy for recurrent SCLC has been established since only two Phase III clinical studies have been reported to date on chemotherapy for this disease [13, 17]. In contrast, many studies have been undertaken on salvage chemotherapy for recurrent SCLC, with monotherapy with new third-generation anti-cancer agents and platinum-based multi-drug chemotherapy being the mainstay in recent years [1, 4, 5, 8–10, 14, 15]. Some institutions administer anti-cancer drugs on a weekly basis (weekly chemotherapy) [1, 4, 7, 10]. This treatment regimen makes it possible to titrate the dose of anti-cancer drugs depending on adverse reactions and the patients' physical condition after starting the treatment by dividing the dose into some installments.

The results reported with weekly chemotherapy are summarized in Table 3 [1, 4, 7, 10]. While the study by Goto et al. [4] included only sensitive cases, all other studies included 35–64% of refractory cases. The overall response rate ranged between 31% and 88%: 37–91% in sensitive cases and 23–83% in refractory cases. No study, apart from ours, reported any significant difference between sensitive and refractory cases. The overall median survival time was 6.1–11.8 months with no significant differences between sensitive and refractory cases [10]. In our study, the median survival time was 46.9 weeks in PS 0 or 1 patients and 16.3 weeks in PS 2 patients ($P < 0.001$). Naka et al. [10] reported significant differences between PS 0 or 1 patients (6.9 months) and PS 2 patients (3.8 months) [10]. Hemotoxicity was the main adverse reaction in all studies. Thrombocytopenia was milder in our study than in other studies. Diarrhea also showed a high incidence in regimens including CPT-11.

Groen et al. [5] reported therapeutic results similar to ours with carboplatin and paclitaxel therapy: overall response rate of 73.5% and overall median survival time of 31 weeks. They administered carboplatin and paclitaxel at AUC 7 and 175 mg/m^2, respectively at an interval of 3 weeks. These doses were 1.7 and 0.88 times that obtained by us. . The main adverse reaction was hemotoxicity in both studies, but thrombocytopenia was milder in our study. In the study by Groen et al., 22 and 4 of 34 patients received RBC transfusions and platelet transfusions, respectively [5].

In a phase III trial, which compared topotecan versus cyclophosphamide, doxorubicin and vincristine (CAV) in patients with recurrent SCLC [17], the response rate was 24.3 and 18.3%, respectively; time to progression 13.3 and 12.3 weeks; median survival time 25.0 and 24.7 weeks; 1-year survival rate 14.2 and 14.4%. In our study, the response rate was 69%, time to progression 16.4 weeks,

Table 1 Patient characteristics

Eligible patients	29
Gender	
Male	26
Female	3
Age (years)	
Median	63
Range	43–74
Performance status	
0	9
1	10
2	10
Disease extent at relapse	
Limited disease	7
Extensive disease	22
Relapse type	
Refractory case	11
Sensitive relapse case	18
Prior therapy	
Chemotherapy alone	21
Chemotherapy and irradiation	8
Prior chemotherapy regime	
CBDCA + ETOP	3
CDDP + ETOP(PE)	11
CODE + PE	1
CDDP + CPT-11(PI)	9
CDDP + ETOP + CPT-11	3
PE + PI	2
Response to prior chemotherapy	
Complete response	4
Partial response	21
Stable disease	3
Progressive disease	1

CBDCA carboplatin, *ETOP* etoposide, *CDDP* cisplatin, *CODE* cisplatin/vincristine/doxorubicin/etoposide, *CPT-11* irinotecan

median survival time 29.6 weeks, and 1-year survival rate 37%, and our study showed better therapeutic performance in terms of all four parameters although ours was a pilot study and direct comparisons cannot be made.

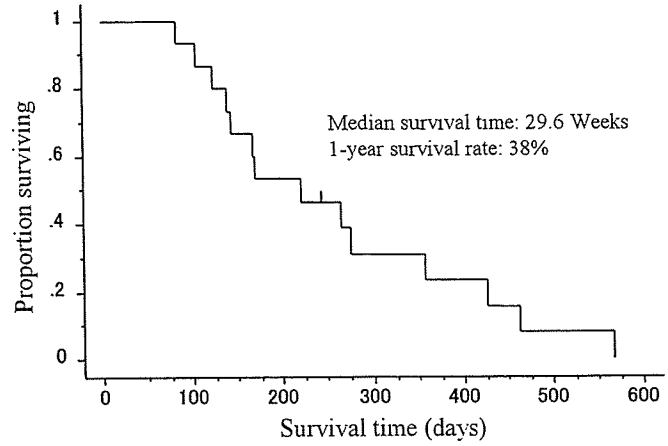

Fig. 1 Kaplan–Meier estimated overall survival curves. Median survival time, 29.6 weeks; 1-year survival rate, 38%

In Japan, cisplatin and irinotecan chemotherapy is the standard therapy for untreated patients in extensive SCLC. Only 8 of 40 patients in the study by Goto et al. [4] and 14 of 29 in our study received irinotecan-based regimens in initial therapy, and no other weekly chemotherapy studies included in Table 3 used such regimens. Carboplatin and paclitaxel combination chemotherapy appears rational in patients with recurrence following initial therapy with cisplatin and irinotecan because the two regimens are not cross resistant.

Conclusion

Weekly chemotherapy with paclitaxel and carboplatin is tolerable and an active regimen for patients with refractory or relapsed SCLC. It is to be recommended as a candidate regimen in planning a phase III clinical study in refractory or relapsed SCLC, and this regimen will ultimately be evaluated in a phase III clinical study.

Table 2 Toxicities ($n = 29$)

	Grade (common toxicity criteria)				Grade ≤ 3 (%)
	1	2	3	4	
Leukopenia	1	7	14	2	16 (55%)
Neutropenia	1	5	9	7	16 (55%)
Anemia	5	8	6	2	8 (27%)
Thrombocytopenia	8	3	1	0	1 (3%)
Diarrhea	7	0	3	0	3 (10%)
Pneumonitis	0	0	1	0	1 (3%)
Nausea	9	1	0	–	
Vomiting	3	0	0	–	
Fatigue	3	3	0	0	
Alopecia	17	0	–	–	
Neuropathy	8	0	0	0	
Flushing	5	–	–	–	
Edema	4	0	0	0	
Arthralgia	3	0	0	0	
Rash	3	0	0	0	
Arrythmia	2	0	0	0	

Table 3 Weekly chemotherapy studies for relapsed small-cell lung cancer

References	Regimen	No. of pts	% of ref pts (%)	RR	RR in sen pts (%)	RR in ref pts (%)	MST (months)
7	CODE	17	35	88	91	83	8.2
10	CPT-11/CBDCA	28	46	31	37	23	6.1
1	CPT-11/CDDP	25	64	80	78	81	7.9
4	CPT-11/CDDP/ETOP	40	0	78	78	–	11.8
Present study	CBDCA/PTX	29	38	69	83	45	7.4

pts patients, *ref* refractory, *sen* sensitive, *RR* response rate, *MST* median survival time, *CODE* cisplatin/vincristine/doxorubicin/etoposide, *CPT-11* irinotecan, *ETOP* etoposide, *CDDP* cisplatin, *PTX* paclitaxel, *CBDCA* carboplatin

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References

1. Ando M, Kobayashi K, Yoshimura A, Kurimoto F, Seike M, Nara M, et al (2004) Weekly administration of irinotecan (CPT-11) plus cisplatin for refractory or relapsed small cell lung cancer. Lung Cancer 44:121–127
2. Einhorn LH, Pennington K, McClean J (1990) Phase II trial of daily oral VP-16 in refractory small cell lung cancer: a Hoosier Oncology Group study. Semin Oncol 17:32
3. Glisson BS (2003) Recurrent small cell lung cancer. Update. Semin Oncol 30:72
4. Goto K, Sekine I, Nishiwaki Y, Kakinuma R, Kubota K, Matsumoto T, et al (2004) Multi-institutional phase II trial of irinotecan, cisplatin, and etoposide for sensitive relapsed small-cell lung cancer. Br J Cancer 91:659–665
5. Groen HJ, Fokkema E, Biesma B, Kwa B, van Putten JW, Postmus PE, et al (1999) Paclitaxel and carboplatin in the treatment of small-cell lung cancer patients resistant to cyclophosphamide, doxorubicin, and etoposide: a non-cross-resistant schedule. J Clin Oncol 17:927–932
6. Johnson DH, Greco FA, Strupp J, Hande KR, Hainsworth JD (1990) Prolonged administration of oral etoposide in patients with relapsed or refractory small-cell lung cancer: a phase II trial. J Clin Oncol 8:1613
7. Kubota K, Nishiwaki Y, Kakinuma R, Hojo F, Matsumoto T, Ohmatsu H, et al (1997) Dose-intensive weekly chemotherapy for treatment of relapsed small-cell lung cancer. J Clin Oncol 15:292–296
8. Masters GA, Declerck L, Blanke C, Sandler A, DeVore R, Miller K, et al (2003) Phase II trial of gemcitabine in refractory or relapsed small-cell lung cancer: eastern cooperative oncology group trial 1597. J Clin Oncol 21:1550–1555
9. Masuda N, Fukuoka M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, et al (1992) CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10:1225–1229
10. Naka N, Kawahara M, Okishio S, Hosoe S, Ogawara M, Atagi S, et al (2002) Phase II study of weekly irinotecan and carboplatin for refractory or relapsed small-cell lung cancer. Lung Cancer 37:319–323
11. Neubauer M, Schwartz J, Caracandas J, Conkling P, Ilegbodu D, Tuttle T, et al (2004) Results of a phase II study of weekly paclitaxel plus carboplatin in patients with extensive small-cell lung cancer with Eastern cooperative oncology group performance status of 2, or age? 70 years. J Clin Oncol 22:1872–1877
12. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346:85–91
13. Schiller JH, Adak S, Cella D, DeVore RF III, Johnson DH (2001) Topotecan versus observation after cisplatin plus etoposide in extensive-stage small-cell lung cancer: E7593-A phase III trial of the Eastern cooperative oncology group. J Clin Oncol 19:2114–2122
14. Sekine I, Nishiwaki Y, Kakinuma R, Kubota K, Hojo F, Matsumoto T, et al (2003) Phase I/II trial of weekly cisplatin, etoposide, and irinotecan chemotherapy for metastatic lung cancer. JCOG 9507. Br J Cancer 88:808–813
15. Smit EF, Fokkema E, Biesma B, Groen HJ, Snoek W, Postmus PE (1998) A phase II study of paclitaxel in heavily pretreated patients with small-cell lung cancer. Br J Cancer 77:347–351
16. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
17. von Pawel J, Schiller JH, Shepherd FA, Fields SZ, Kleisbauer JP, Chrysson NG, et al (1999) Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. J Clin Oncol 17:658

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CT-guided needle biopsy of lung lesions: A survey of severe complication based on 9783 biopsies in Japan

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Abstract

Purpose: The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

Materials and methods: Postal questionnaires regarding CT-guided needle biopsy were sent out to multiple hospitals in Japan. The questions regarded: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates and numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax and other rare complications. Each severe complication was followed with additional questions.

Results: Data from 9783 biopsies was collected from 124 centers. Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. A total of 39 (35%) hospitals reported 74 (0.75%) cases with severe complications. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, nine cases (0.092%) with hemothorax, and 27 cases (0.26%) with others, including heart arrest, shock, and respiratory arrest. From a total of 62 patients with severe complications, 54 patients (0.55%) recovered without sequela, however one patient (0.01%) recovered with hemiplegia due to cerebral infarction, and the remaining seven patients (0.07%) died.

Conclusions: This is the first national study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.

Keywords: CT-guided needle biopsy; Complication; Lung nodule

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1. Introduction

Transthoracic needle biopsy is a common procedure used mainly to elucidate the nature of pulmonary nodules [1,2]. CT has rapidly become the guidance modality of choice for performing transthoracic needle biopsy due to technical advances in CT and its better detection of pulmonary lesions, which sometimes cannot be identified on chest radiograph [3].

CT-guided needle biopsy is generally regarded as a safe procedure, although pneumothorax and other rare complications can sometimes occur [4]. There have been occasional reports of deaths due to severe complications, such as, air embolism following lung biopsy [5]. Fortunately, these complications are generally very rare; previously published data shows wide variations in complication rates, making them difficult to generalize [5–8].

The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

2. Materials and methods

Postal questionnaires regarding CT-guided needle biopsy were sent out to named radiologists at 101 university hospitals and cancer centers in Japan in August 2001. The radiologists at these hospitals were asked to pass duplications of the questions to other associate hospitals. The questions required information regarding: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates, numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax, severe pulmonary hemorrhage or hemoptysis which was treated with drugs for hemostasis and other rare complications, and mortalities and morbidities after that.

We defined a case as having a severe complication when one of the following criteria was met: (1) the duration of hospital stay was prolonged due to the biopsy, (2) a special technique or treatment was required to treat the complication, (3) a special procedure was required for resuscitation, and (4) shock or pre-shock developed. Each severe complication was followed with additional questions, including diagnosis of the complication, the position of the pulmonary lesion, the distance of the pulmonary lesion from the peripheral pleura, whether the lesion was located near the hilum or large pulmonary vessel, whether there was any reasonable factor causing the complication such as cough during biopsy, biopsy technique (CT-fluoroscopy or Co-axial method), the number of biopsies for each case, type and size of the needle, and presence of significant sequela from the complication.

Furthermore, the questionnaire included the following enquiries: whether emergency medication was prepared for resuscitation in the operating room, whether the patient was treated by the intravenous route and monitors, such as automatic sphygmomanometer, pulse oximetry, and electrocardiography. Finally, availability of access to other departments in case of emergency was questioned. Postal replies of questionnaire had been received for a year, and these answers were analyzed.

3. Results

A total of 9783 biopsy data were collected from 124 centers. The average number of biopsies performed per center was 79 cases, and that per center per year was 21 cases. The number of institutions in which hyperbaric oxygen recompression can be performed was 41 of 114 (37%) hospitals. Patients were kept on peripheral intravenous drip infusion in 86 of 92 (93%) hospitals, automatic sphygmomanometer in 38 of 92 (41%) hospitals, pulse oximetry in 32 of 92 (35%) hospitals, and electrocardiography in 8 of 92 (9%) hospitals.

Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. The number of centers that reported severe complications was 39 (35%) of 114 centers. The total number of overall severe complications was 74 (0.75%) cases. Of these, details of the complications in 64 cases are described in Table 1. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, 10 cases (0.10%) with hemothorax, and 26 cases (0.26%) with others. The others included 14 cases of pneumothorax requiring temporal drainage of the pneumothorax or chest tube insertion, three cases of heart arrest, and so on. There was no report of coughing during needle placement into the thorax in any of the cases with air embolism. Two of six pulmonary lesions were complicated with air emboli located near the large pulmonary vessel, and one lesion contained a cavity (Table 2). Tumor seeding occurred in two cases following CT-guided biopsy performed

Table 1
Summary of 64 cases of severe complications

Severe complications	No.
Pneumothorax requiring drainage of air	14
Tension pneumothorax	10
Hemothorax	10
Air embolism	6
Tumor seeding	6
Pulmonary hemorrhage of hemoptysis	6
Heart arrest	3
Respiratory arrest	1
Shock	1
Cyanosis	1
Cardiac tamponade	1
Pneumomediastinum	1
Mediastinal hematoma	1
Loss of consciousness	1
Severe pain of biopsied site	1
disseminated intravascular coagulation (DIC)	1
Total	64

Table 2
Summary of cases of air embolism

No.	Age	Sex	Size (mm)	Location (lobe)	Distance from pleura (mm)	Large vessel near the nodule	Cavity	CT-fluoloscopy	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle	Sequela
1	72	F	20	Left lower	40	Yes	No	Yes	No	2	Core biopsy	18G	Death
2	59	M	10	Left lower	20	No	No	NA[a]	Yes	1	Core biopsy	18G	Totally improved
3	57	F	7	Right middle	25	No	No	Yes	No	1	Core biopsy	18G	Totally improved
4	74	M	20	Right upper	25	Yes	No	Yes	No	2	Core biopsy	20G	Partially improved
5	57	M	12	Right lower	3	No	No	No	Yes	1	Core biopsy	20G	Totally improved
6	75	M	25	Right lower	18	No	Yes	No	No	1	Core biopsy	18G	Totally improved

[a] NA, information was not available.

by the Co-axial method (Table 3). In one of these two cases, the tip of the outer cannula was placed within the chest wall, so that seeding obviously occurred by direct contact of the inner needle with the biopsy route.

From a total of 62 cases with severe complications, 54 cases (0.55%) were recovered without sequela, and one case (0.01%) recovered but with hemiplegia due to cerebral infarction. Unfortunately, four (0.04%) of the remaining seven cases died just after the CT-guided biopsy procedure; these consisted of one case of air embolism, one case of DIC, and two cases of heart arrest. Three cases (0.03%) of the remaining seven cases died several years later due to tumor seeding. Four cases complicated with air embolism, three of which were treated with hyperbaric oxygen recompression, were recovered without sequela out of a total of six cases. In 23 (50%) of 46 centers, an emergency team was able to attend when a severe complication occurred.

4. Discussion

Recently, many small pulmonary lesions, which cannot be detected on chest radiograph, have been easily visualized by CT examination in daily clinical work. These lesions are usually followed with CT, or in some cases these are biopsies using CT-guided technique. CT-guided needle biopsy is a widely accepted technique and is one of the principal methods for evaluating a pulmonary lesion [9]. Although it is not rare to have minor complications due to CT-guided needle biopsy, such as, a small amount of pneumothorax and pulmonary hemorrhage, these complications improve without any treatment [5]. On the other hand, it is well known that potentially life-threatening complications such as air embolism and tumor seeding can occur. Fortunately, the frequency of these complications is considered very rare [5]. However, the number of published reports has shown that the incidence of air embolism has been increasing over the last several years. Only seven cases with air embolism were documented in the 20 years before 1995 [10–16], whereas six cases have already been published in the last 10 years [17–22].

This is the first national research study demonstrating the incidence rate of severe complications with respect to CT-guided needle biopsy based on a large number of biopsy cases using a multi-center survey.

The most common complication of transthoracic percutaneous needle biopsy is pneumothorax, with a frequency rate of 0–61%, whereas the incidence of pneumothorax requiring chest tube drainage ranges from 1.6% to 17% [23]. In the present study, the rate of pneumothorax was 35.1%, which is considered comparable to the previous studies.

Sinner's review of the literature determined that there were two cases suspected of air embolism in 2726 patients [5]. He estimated that the relative risk of air embolism per patient was about 0.07%. In the present study of 9783 biopsies, air embolism occurred in six patients, resulting in an incidence

Table 3
Summary of cases of tumor seeding

No.	Age	Sex	Size (mm)	Location	Distance from pleura (mm)	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle
1	72	M	30	Right upper	0	No	1	Core biopsy	18G
2	73	M	30	Left lower	30	Yes	3	Core biopsy	18G
3	71	M	10	Right upper	20	No	2	Aspiration biopsy	22G
4	30	F	28	Left upper	76	No	2	Core biopsy	18G
5	69	M	15	Right lower	0	No	2	Core biopsy	21G
6	77	M	12	Right upper	30	Yes	2	Core biopsy	20G

rate of 0.06%, which also shows no major difference from the previously reported complication rate. However, in the present study, there were several cases of severe complications including cardiac and respiratory arrest, and shock, which can be secondary to air embolism, although it is very difficult to confirm air embolism in the coronary artery in cases of myocardial infarction when the patient has not been scanned at the level of the heart. It is speculated that concurrent cough during the procedure has a high possibility of an air embolism misplacing the biopsy needle into the large vessel adjacent to the pulmonary lesion. Among the total of six cases with air emboli in the present study, two cases demonstrated biopsied pulmonary lesions located close to the large vessels, however the remaining four cases have no close relation to the large vessels. There were no reports of coughing during the procedure in any of the cases complicated by air embolism. Air embolism even occurred in a case in which the nodule was very near the pleura (case no. 5). In our study, all cases with air emboli had undergone CT-guided biopsy using a core biopsy needle of 18–20 gauge, which is greater in diameter than the usually used fine aspiration needles. Having said that, in the previous reviews, most cases with air emboli were biopsied by fine aspiration needles, and there are two prior reports of air embolism following CT-guided lung needle marking using thin needles without recent biopsy [24–26].

Tumor seeding into the needle tract seems to be a rare possibility in several case reports [27–34]. There were six cases (0.06%) of tumor seeding in our study, which is a relatively high frequency compared to previous studies [5,35]. The true incidence of tumor seeding along the needle may be underestimated as not all cases can be diagnosed, and many patients die before these metastases become clinically apparent. Tumor seeding appears to depend on the size of the needle, therefore large-bore needles carry a relatively greater risk of tumor seeding, however tumor seeding following a fine needle aspiration was reported in one case of our study. It is thought that CT-guided biopsy performed using the Co-axial method has less frequency of tumor seeding as the outer cannula minimizes direct contact of the tumor cells with the biopsy route. Surprisingly, tumor seeding occurred in two cases using the Co-axial method. We speculate that the outer cannula was not appropriately placed.

Unfortunately, there were seven patients (0.07%) who died in our study due to complications in the CT-guided needle biopsy. Greene [6] estimated the mortality rate associated with fine needle aspiration to be 0.02%, however Richardson et al. [8] reported eight deaths (0.15%) in their study due to complications in CT-guided needle biopsy. Most of the deaths in the present study were attributed to fatal air embolism. Three cases of air embolism that were treated with hyperbaric oxygen recompression were recovered without sequela, which may suggest hyperbaric oxygen recompression therapy is effective for treatment of air embolism, and for reducing the mortality rate.

Our study has several limitations, including selection bias, the long period of the study, multi-center analysis with a large variety of techniques and CT scanners, and the possibility of missing or misdiagnosing significant complications such as the number of air emboli and tumor seeding. Moreover, our study is a retrospective questionnaire-based analysis rather than a prospective survey.

In conclusion, this is the first nation-wide study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.

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References

[1] Sinner WN. Pulmonary neoplasms diagnosed with transthoracic needle biopsy. Cancer 1979;43:1533–40.
[2] Klein JS, Zarka MA. Transthoracic needle biopsy. J Thorac Imag 1997;12:232–49.
[3] Hirose T, Mori K, Machida S, et al. Computed tomographic fluoroscopy-guided transthoracic needle biopsy for diagnosis of pulmonary nodules. Jpn J Clin Oncol 2000;30:259–62.
[4] Berquist TH, Bailey PB, Cortese DA, et al. Transthoracic needle biopsy: accuracy and complication in relation to location and type of lesion. Mayo Clin Proc 1980;55:475–81.
[5] Sinner WN. Complications of percutaneous transthoracic needle aspiration biopsy. Acta Radiol Diag 1976;17:813–28.