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.

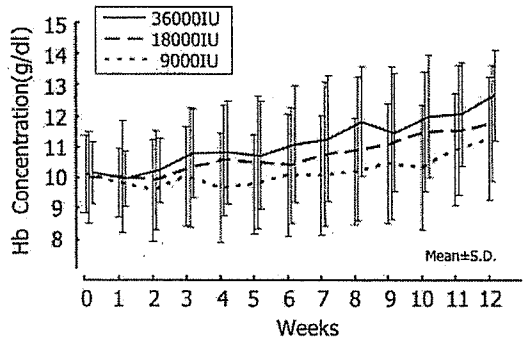

Figure 1. Mean weekly hemoglobin levels for Per Protocol Set population by epoetin-beta dosage Group. Hb, hemoglobin; SD, standard deviation.

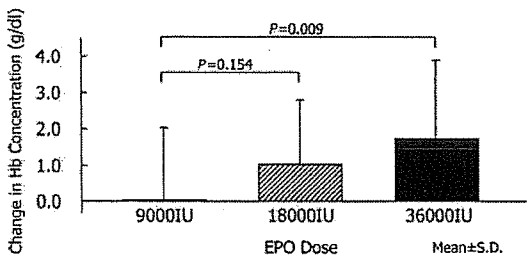

Figure 2. Mean change in hemoglobin level from baseline to last observation (at 12 weeks or 4 weeks after the beginning of a final-course of chemotherapy) by epoetin-beta dosage group (Per Protocol Set population). Hb, hemoglobin; EPO, epoetin-beta; SD, standard deviation.

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,

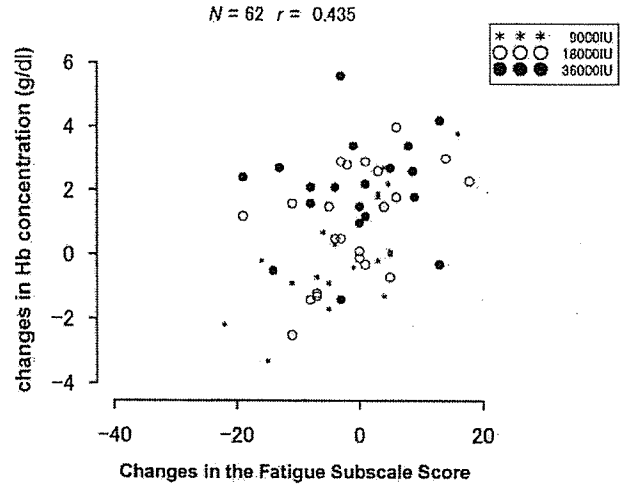

Figure 3. Correlation between change in hemoglobin levels and change in the Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale scores at 7–11 weeks (Per Protocol Set population). Hb, hemoglobin.

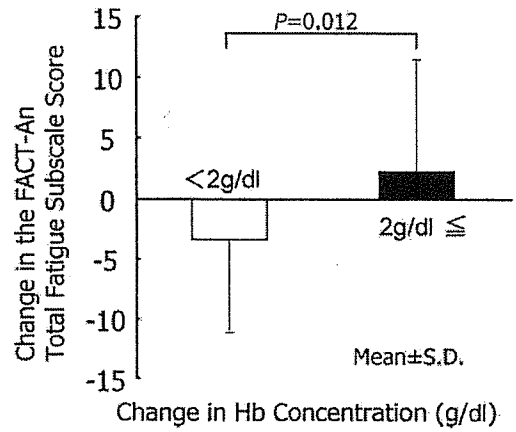

Figure 4. Change in mean Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale score between baseline and 7–11 weeks by change in hemoglobin level (change in hemoglobin of ≥2 g/dl or <2 g/dl from baseline). FACT-An, Functional Assessment of Cancer Therapy–Anemia; Hb, hemoglobin; SD, standard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

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).

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 $\leq$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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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 an 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 estimatio were undertaken using the EM algorithm. The strength of association c *MTH1* (mutT homolog 1) genotypes and haplotypes with SCLC risk wa measured as crude odds ratios (ORs), and ORs were adjusted for gender, ag (49, 50–59, 60–69, 70) and smoking dosage (pack-years: 0, 1–49, 50) wit 95% confidence intervals (CIs) by unconditional logistic regression analysi (17). ORs for carrying a copy of a haplotype were also calculated by th bootstrap method with 5000 resampling. All the statistical analyses wer 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 consiste of patients enrolled in four hospitals in Tokyo and surround ing prefectures. The 685 controls consisted of patients outpatients and healthy volunteers without a history of cance enrolled in two hospitals and a university in the same area Most of the SCLC cases were males and had a smokin habit, as has been reported (18,19). Therefore, the SCLC cases showed a higher fraction of males and smokers than th controls, and the mean smoking dosage of the SCLC case was larger than that of the controls.

All the 685 controls were genotyped for the 50 SNPs wit a success rate of 99.98% in our previous study (13). Th 211 SCLC cases were genotyped for the same 50 SNPs in th present study, and the success rate was 99.94% (Table II) The allele distribution in the SCLC cases was compared wit that in the 685 controls. None of the 50 SNPs deviated fro HWE in cases and controls ($P \geq 0.05$). A significan difference in the allele distribution between the controls an cases was observed in one of the 50 SNPs, MTH1-Val83Me (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. Allel distributions for the other 46 SNPs were not significantly o were marginally significantly different between the control and cases.

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

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

Subject	Institution[a]	No.	Gender (%)		Age	Smoking habit (%)			Pack-years of smoker
			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
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.

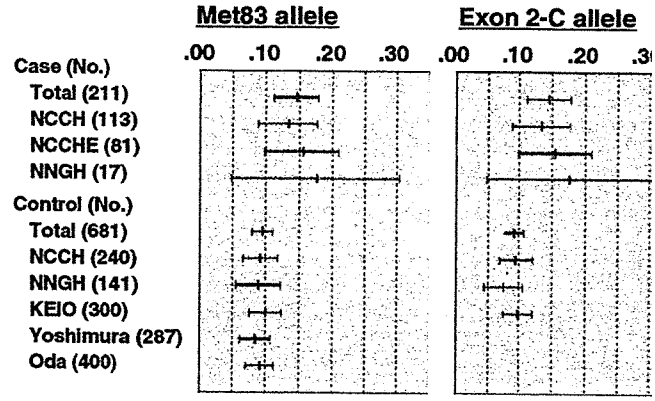

Fig. 1. Frequencies of the MTH-83Met and exon 2-C alleles in cases and controls. Allele frequency is shown with its sampling variations estimated by 95% CI. Frequencies of the MTH-83Met allele in two control populations reported by Yoshimura *et al.* (24) and Oda *et al.* (21 are also shown.

We next assessed the effect of smoking on the contributi of the MTH1-Val83Met and exon 2-T/C SNPs to the SCI risk. ORs in light (PY < 50) smokers and heavy (PY ≥ 5 smokers were compared (Table V). The number of nc smokers in the case subjects was small (i.e. $N < 1$ therefore, they were excluded from the analysis. Increases ORs for the 83Met and exon 2-C alleles were more evident light smokers than in heavy smokers, and the ORs we statistically significant in light smokers but not in hea smokers. *P*-values for interaction of the Val83Met and ex 2-T/C genotypes on the SCLC risk with smoking were 0. and 0.11, respectively. *P*-value for interaction of haploty #2 on the SCLC risk by smoking was calculated as bei 0.095.

Discussion

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

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

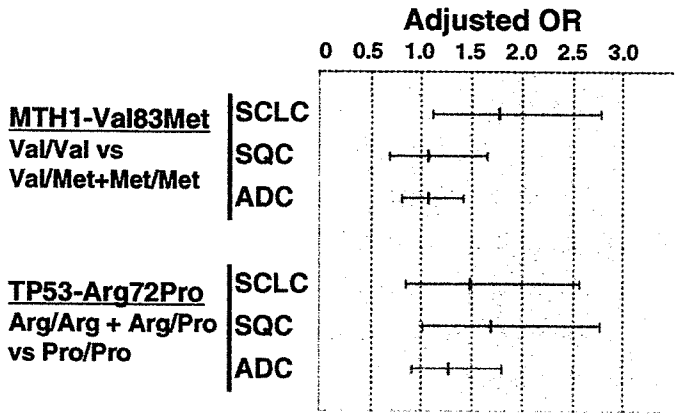

Fig. 2. ORs of the MTH-83Met allele carriers against homozygotes for the MTH-83Val allele and those of homozygotes for the TP53-72Pro allele against others. ORs adjusted for gender, age and smoking dosage are shown. ORs in SQC and ADC cases are from our previous report (13).

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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CLINICAL INVESTIGATION **Lung**

A PHASE II STUDY OF HYPERFRACTIONATED ACCELERATED RADIOTHERAPY (HART) AFTER INDUCTION CISPLATIN (CDDP) AND VINORELBINE (VNR) FOR STAGE III NON–SMALL-CELL LUNG CANCER (NSCLC)

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Purpose: The purpose was to assess the feasibility and efficacy of hyperfractionated accelerated radiotherapy (HART) after induction chemotherapy for Stage III non–small-cell lung cancer.

Methods and Materials: Treatment consisted of 2 cycles of cisplatin 80 mg/m^2 on Day 1 and vinorelbine 25 mg/m^2 on Days 1 and 8 every 3 weeks followed by HART, 3 times a day (1.5, 1.8, 1.5 Gy, 4-h interval) for a total dose of 57.6 Gy.

Results: Thirty patients were eligible. Their median age was 64 years (range, 46–73 years), 24 were male, 6 were female, 8 had performance status (PS) 0, 22 had PS 1, 9 had Stage IIIA, and 21 had Stage IIIB. All but 1 patient completed the treatment. Common grade ≥3 toxicities during the treatment included neutropenia, 25; infection, 5; esophagitis, 5; and radiation pneumonitis, 3. The overall response rate was 83%. The median survival was 24 months (95% confidence interval [CI], 13–34 months), and the 2-year overall survival was 50% (95% CI, 32–68%). The median progression-free survival was 10 months (95% CI, 8–20 months).

Conclusion: Hyperfractionated accelerated radiotherapy after induction of cisplatin and vinorelbine was feasible and promising. Future investigation employing dose-intensified radiotherapy in combination with chemotherapy is needed. © **2005 Elsevier Inc.**

Non–small-cell lung cancer, Hyperfractionated accelerated radiation therapy, Chemoradiotherapy.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death for men and the second for women in Japan. During 2001, approximately 55,000 patients died of lung and bronchus cancer (1). Surgery is the standard of care for patients with Stage I–II non–small-cell lung cancer (NSCLC), but a combination of chemotherapy and thoracic radiotherapy with or without surgery is indicated for the majority of patients with Stage III disease. Cisplatin (CDDP) based chemotherapy with conventional radiotherapy improved survival compared to conventional radiotherapy alone (2–6) and was the standard of care in the 1990s. Recently, concurrent chemoradiotherapy has been revealed to be superior to sequential chemoradiotherapy (7, 8), but it is difficult to give full-dose chemotherapy using newer cytotoxic agents concurrently with radiotherapy, and the optimal combination has not yet been clarified. In the meantime, continuous hyperfractionated accelerated radiotherapy (CHART) with 3 daily fractions to intensify the local effect of radiotherapy has been found to be superior to conventional radiotherapy (9). The survival benefit of CHART was encouraging, but the protocol including treatments on weekends and 6-h intervals between fractions had some difficulties in practicality. Mehta *et al.* introduced hyperfractionated accelerated radiotherapy (HART) (modified CHART) with 3 daily fractions and 4-h interfraction intervals with weekend breaks and also showed promising results similar to those using sequential chemoradiotherapy (10). After these results, we started a Phase II trial to evaluate the feasibility and efficacy of induction chemotherapy with HART for patients with Stage III NSCLC.

METHODS AND MATERIALS

Eligibility criteria

Eligibility criteria included previously untreated patients with pathologically proven NSCLC with clinical tumor-node-metastasis system Stage III, and pathologic N2 was also required for Stage

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IIIA; age, 20 to 74 years; performance status (PS) (based on Eastern Cooperative Oncology Group [ECOG] scale) 0 to 1; measurable disease; adequate hematologic (WBC count ≥4,000/mm^3, platelet count ≥100,000/mm^3, and hemoglobin ≥9.5 g/dL), hepatic (AST and ALT level ≤2 times the upper limit of normal and total bilirubin level ≤ the upper limit of normal), and renal (creatinine ≤1.2 mg/dL and creatinine clearance ≥60 mL/min) functions; PaO_2 ≥70 torr; no pleural and pericardial effusion; radiation field encompassed one-half or less of the ipsilateral lung; and no serious comorbidity. All patients signed written informed consent in accordance with our institutional review board.

Pretreatment evaluation included history and physical examination; serum chemistries (lactate dehydrogenase, alkaline phosphatase, AST, ALT, bilirubin, albumin, creatinine, and calcium); chest radiograph; CT scan of the chest; ultrasound of the abdomen; MRI or CT scan of the brain; and bone scintigraphy.

Treatment details

The treatment consisted of 2 cycles of CDDP 80 mg/m^2 on Day 1 and vinorelbine (VNR) 25 mg/m^2 on Days 1 and 8 every 3 weeks followed by HART; 3 times a day with minimal interval of 4 hours for a total dose of 57.6 Gy in 36 fractions over 2.5 weeks.

Radiation therapy was started after the patient recovered from the toxicity of chemotherapy and was delivered with megavoltage equipment. Lung heterogeneity corrections were not used. The first and third fraction of each day consisted of anterior-posterior opposed fields that encompassed the primary tumor, the metastatic lymph nodes, and the regional lymph nodes with a 1.5 to 2-cm margin. The fraction size was 1.5 Gy. Regional nodes excluding the contralateral hilar and supraclavicular nodes were included in these fractions. However, lower mediastinal nodes were included only if the primary tumor was located in the lower lobe of the lung. The second fraction of each day consisted of bilateral oblique fields that encompassed the primary tumor and the metastatic lymph nodes with a 1.5 to 2-cm margin; the fraction size was 1.8 Gy. Attempts were made to design the field of the second fraction to minimize the irradiated volume of the esophagus without compromising the margin around the tumor or spinal cord.

Toxicity assessment

Patients were observed weekly during treatment to monitor toxicity. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Late toxicity was graded according to the Radiation Therapy Oncology Group (RTOG)/European Organization for Research and Treatment of Cancer late radiation morbidity scoring scheme. Late toxicity was defined as that occurring more than 90 days after treatment initiation.

Follow-up evaluation

The following evaluations were performed until disease progression every 2 months for the first year, every 3 months for the second year, and every 6 months thereafter: physical examination, toxicity assessment, and chest radiograph. CT scan of the chest was performed at 1, 3, 6, 9, 12, 18, and 24 months after the treatment and when indicated thereafter. Restaging at 6 months after the treatment was also performed with ultrasound of the abdomen, MRI or CT scan of the brain, and bone scintigraphy.

Response assessment

Complete response (CR) was defined as complete disappearance of all measurable and assessable lesions for ≥4 weeks, partial response (PR) was defined as a decrease of 50% or more from baseline in the sum of products of perpendicular diameters of all measurable lesions for ≥4 weeks, and progressive disease (PD) was defined as an increase of 25% or more from baseline in the sum of products of perpendicular diameters of all measurable lesions or the appearance of any new lesion. Stable disease was defined as the remainder of evaluable patients without CR, PR, or PD.

Pattern of failure

Patterns of failure were defined as first site of failure. Local/regional failure included the primary tumor and regional lymph nodes. Distant failure included any site beyond the primary tumor and regional lymph nodes.

Statistics

A Simon's two-stage optimal design was used for this study with the assumption that a protocol compliance rate of less than 60% would not be feasible, and protocol compliance rate of 80% or greater with α error of 0.10 and β error of 0.10 would warrant further investigation of this regimen. In the first stage, 11 assessable patients were entered. If fewer than 7 patients completed the treatment, accrual would be stopped with the conclusion that the regimen was not feasible for further investigation. If 7 or more patients completed the treatment, an additional 27 patients would be accrued in the second study. According to this design, this study would be determined to be feasible and be proceeded to a multicenter Phase II study if 27 patients completed the treatment. The actuarial median survival time and 2-year survival were estimated by the Kaplan–Meier method (11).

RESULTS

Patient population

Between July 1999 and March 2001, 30 patients were enrolled in the study. The accrual was stopped, because 29 of 30 patients completed the treatment, and conclusions could be drawn at that time. The patients' median age was 64 years (range, 46–73 years), 24 were male, and 6 were female. The patient and tumor characteristics are summarized in Table 1.

Treatment compliance and toxicity

All patients completed 2 cycles of induction chemotherapy. Six of 30 patients required dose modification, and 13 patients had treatment delay. The median time to start of HART from start of chemotherapy was 49 days (range, 41–62 days). Twenty-nine of 30 patients completed HART, and the median overall treatment time of HART was 17 days (range, 16–22 days). In total, 29 of 30 patients (97%; 95% confidence interval [CI], 83–100%) completed this combined treatment.

The toxicity profile of the treatment is shown in Tables 2 and 3. Common Grade 3 or greater acute toxicities were neutropenia, 25 (83%); infection, 5 (17%); esophagitis, 5 (17%); and radiation pneumonitis, 3 (19%). There were 2 cases of treatment-related death due to radiation pneumonitis. As of the date of this analysis, 2 cases with Grade

Table 1. Patient and tumor characteristics

Number of patients	30
Age	
Median	64
Range	46–73
Gender	
Male	24
Female	6
Performance status	
0	8
1	22
Weight loss	
<5%	25
≥5%	5
Tumor and lymph nodes	
T1N2	3
T1N3	1
T2N2	5
T2N3	5
T3N2	1
T4N0	1
T4N1	4
T4N2	9
T4N3	1
Stage	
IIIA	9
IIIB	21
Histology	
Squamous	13
Nonsquamous	17

3 s.c. tissue fibrosis and 1 case with spontaneous rib fracture were observed as late toxicities.

Response and survival

Of 30 patients, 2 achieved CR, and 23 achieved PR with a response rate of 83% (95% CI, 65–94%). Five patients remained in a stable disease state, and there were no PD patients. With a median follow-up period of 40 months for surviving patients, the median survival and the 2-year and 3-year survivals (Fig. 1) were 24 months (95% CI, 13–34 months), 50% (95% CI, 32–68%), and 32% (95% CI, 15–49%), respectively. The median progression-free survival and the 1-year progression-free survival (Fig. 2) were 10 months (95% CI, 8–20 months) and 47% (95% CI, 29–65%), respectively.

Pattern of failure

At the time of this analysis, 22 of 30 patients (73%) showed tumor progression, 2 patients (7%) had died as a result of treatment, and 6 patients (20%) were alive without disease progression. The patterns of first failure were as follows: local/regional only, 13 (43%); local/regional and distant, 4 (13%); distant only, 5 (17%).

DISCUSSION

In the 1970s, treatment of locally advanced NSCLC was by conventional radiotherapy alone. In the 1980s, sequential chemotherapy and conventional radiotherapy were revealed to be superior to conventional radiotherapy alone. In the 1990s, optimal sequences of chemoradiotherapy and radiation fractionation were investigated. The West Japan Lung Cancer Group compared sequential vs. concurrent radiotherapy with induction CDDP, vindesine, and mitomycin (7). In an RTOG 9410 trial, induction CDDP and vinblastine plus sequential standard radiotherapy, CDDP and vinblastine plus concurrent standard radiotherapy, and CDDP and etoposide plus concurrent twice-daily hyperfractionated radiotherapy were compared (8). Both trials showed similar results; concurrent chemoradiotherapy was superior to the sequential approach and achieved 5-year survivals for concurrent and sequential approach of approximately 20% and 10%, respectively. However, twice-daily hyperfractionated radiotherapy, which seemed to be promising in a preceding RTOG 9015 trial (12), failed to show a survival advantage over standard once-daily radiotherapy, and concurrent chemotherapy and once-daily radiotherapy is the standard of care today. Recently, a Czech randomized Phase II trial (13) suggested a similar advantage of the concurrent approach using CDDP and VNR, a newer cytotoxic agent. However, there remains some argument that newer cytotoxic agents cannot be delivered as full-dose chemotherapy with concurrent radiotherapy, and the survival advantage of newer cytotoxic agents over old ones has not yet been demonstrated in Stage III NSCLC patients. The optimal schedule and fractionation of thoracic radiotherapy in combination with chemotherapy also remains to be determined.

Another promising regimen was altered fractionation of radiotherapy such as CHART or HART, 3 times a day with a fraction interval of 4 to 6 hours over 2.5 weeks or less. CHART was developed at Mount Vernon Hospital, United Kingdom, in the 1980s. It was designed to combine both a shortening of the overall treatment time of radiotherapy, which is analogous to the concept of dose intensification of cytotoxic chemotherapy, and a reduction in dose per fraction. The rationale was to overcome accelerated repopulation of the tumor during the course of radiotherapy, which may lead to local failure, and to reduce normal tissue toxicities that depend on the dose per fraction. After the results of a randomized trial that showed survival benefits of CHART over conventional

Table 2. Hematologic toxicities (n = 30)*

	Grade					
	0	1	2	3	4	≥Grade 3 (%)
Leukopenia	1	3	8	16	2	18 (60)
Neutropenia	3	0	2	6	19	25 (83)
Thrombocytopenia	20	7	1	2	0	2 (7)
Anemia	1	10	16	3	0	3 (10)

* National Cancer Institute–Common Toxicity Criteria version 2.

Table 3. Nonhematologic toxicities (n = 30)*

	Grade						
	0	1	2	3	4	5	≥Grade 3 (%)
Acute toxicity							
Nausea	7	16	4	3	0	0	3 (10)
Vomiting	23	3	4	0	0	0	0
Infection	20	3	2	5	0	0	5 (17)
Esophagitis	1	11	13	4	1	0	5 (17)
Pneumonitis	18	4	5	1	0	2	3 (10)
Late radiation morbidity†							
Esophagus	26	1	0	0	0	0	0
Heart	26	0	1	0	0	0	0
Lung	9	13	5	0	0	0	0
Subcutaneous tissue	17	6	2	2	0	0	2 (7)
Bone	26	0	0	0	1	0	1 (3)

* National Cancer Institute–Common Toxicity Criteria version 2.
† Three patients died within 90 days of the beginning of radiotherapy.

radiotherapy (9), the Department of Health in the United Kingdom recommended CHART as the radiotherapy schedule of choice in inoperable NSCLC, and a CHART implementation group was formed to facilitate its introduction throughout the United Kingdom (14). There were difficulties in changing departmental working hours and a lack of sufficient financial support in UK hospitals to introduce CHART into routine practice (15), although it was suggested that CHART gave more benefit than any sequential combination of conventional radiotherapy and chemotherapy with minimally increased toxicity. To make the accelerated regimen more widely applicable, Continuous Hyperfractionated Accelerated Radiotherapy Week-End Less (CHARTWEL) and HART were introduced and were found to be as effective as CHART. Both CHARTWEL and HART showed improved survival over conventional radiotherapy, but the local tumor control was still unsatisfactory. Radiation dose escalation and use of chemotherapy combined with CHARTWEL/HART were also investigated to improve the local control and survival. Saunders *et al.* (16) reported on CHARTWEL combined with induction chemotherapy (17). In that study, 113 patients were enrolled, and dose escalation from 54 Gy to 60 Gy with or without chemotherapy was successfully achieved. Locoregional control at 2 years was 37% and 55% for CHARTWEL 54 Gy and 60 Gy alone, respectively, compared with 72% in those treated with 60 Gy and induction chemotherapy. These results suggested that chemotherapy improved locoregional control, but unfortunately they failed to show a statistically significant survival advantage, because of the relatively small number of patients and imbalanced tumor characteristics enrolled in each arm. The advantage of dose-escalated CHARTWEL against conventional radiotherapy is currently being investigated in a German Phase

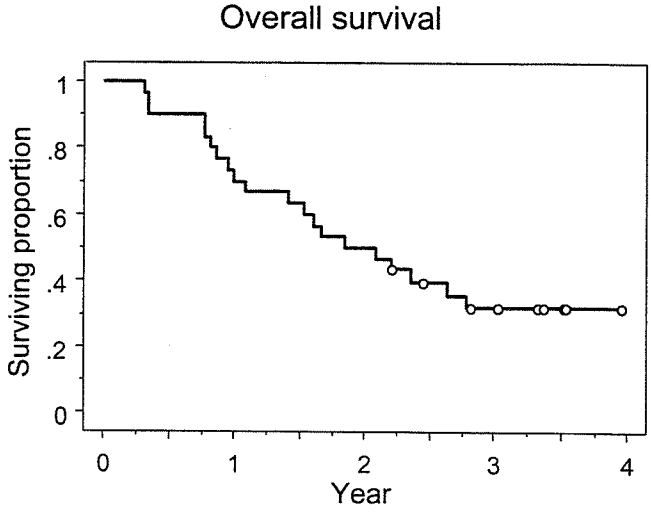

Fig. 1. Overall survival for all patients enrolled in this study.

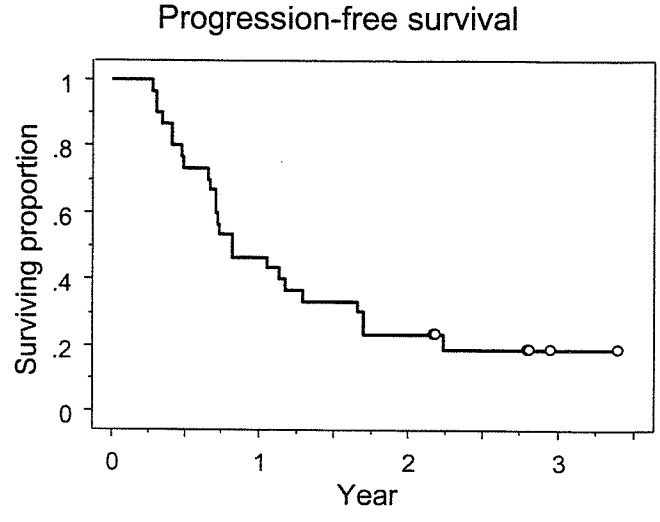

Fig. 2. Progression-free survival for all patients enrolled in this study.

III trial (18). Belani *et al.* reported the results of a randomized Phase III trial (19) that compared conventional radiotherapy with HART after induction chemotherapy (ECOG 2597). This study randomized 119 patients and unfortunately was closed because of slow accrual, but the results were provocative: The median survival time and the 2-year survivals for conventional radiotherapy and HART were 13.7 months and 33% vs. 22.2 months and 48%, respectively. These results seemed to be reliable despite the modest number of patients, because the median survival time of 13.7 months for the conventional radiotherapy arm was similar to that of a sequential chemoradiotherapy trial (2). The optimum chemotherapy regimen in combination with radiotherapy has not yet been determined, and we used a CDDP/VNR regimen instead of the carboplatin/paclitaxel regimen used in the ECOG 2597 trial. Both regimens are standards for advanced-stage NSCLC (20, 21). The compliance and toxicity profiles of chemotherapy in our study were acceptable, the incidence of esophagitis after HART was less than we expected, and the survival figure was nearly identical to that of the ECOG 2597 trial. This suggests that HART after induction CDDP/VNR or carboplatin/paclitaxel can achieve reproducible and promising results.

The pattern of failure in our study showed that local failure was still high (17 of 30, 57%) compared with distant metastasis (9 of 30, 30%), and further improvement of local control is needed. Future directions may include further dose intensification of radiotherapy and introduction of molecular-targeted agents. Recent innovation of information technology has made it possible to use sophisticated three-dimensional conformal radiotherapy (3DCRT). This can deliver intensified radiation doses to the tumor while minimizing the doses to the normal tissues that prevented further dose escalation using conventional two-dimensional radiotherapy. There have been several reports evaluating dose-intensified 3DCRT (22–25), and the technique is now under investigation in combination with cytotoxic chemotherapy in the Radiation Therapy Oncology Group trial (RTOG L-0117). Currently, molecular-targeted agents are being investigated most enthusiastically in Phase II and Phase III trials (26–29). It will be determined in the near future whether or not the combination of these agents has a survival impact. However, the optimal combination of these agents, newer cytotoxic agents, radiation fractionation, and 3DCRT will still need to be determined. Further investigation employing dose-intensified radiotherapy will be necessary to make a great leap in the treatment of locally advanced NSCLC.

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Pilot Study of Concurrent Etoposide and Cisplatin Plus Accelerated Hyperfractionated Thoracic Radiotherapy Followed by Irinotecan and Cisplatin for Limited-Stage Small Cell Lung Cancer: Japan Clinical Oncology Group 9903

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Abstract

Purpose: Irinotecan and cisplatin (IP) significantly improved survival compared with etoposide and cisplatin (EP), in patients with extensive-stage small cell lung cancer (SCLC) in a previous Japan Clinical Oncology Group (JCOG) randomized trial. JCOG9903 was conducted to evaluate the safety of sequentially given IP following concurrent EP plus twice-daily thoracic irradiation (TRT) for the treatment of limited-stage SCLC (LSCLC).

Experimental Design: Between October 1999 and July 2000, 31 patients were accrued from 10 institutions. Thirty patients were assessable for toxicity, response, and survival. Treatment consisted of etoposide 100 mg/m^2 on days 1 to 3, cisplatin 80 mg/m^2 on day 1, and concurrent twice-dailyTRTof 45 Gy beginning on day 2. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 60 mg/m^2 on day 1, with three 28-day cycles.

Results: There were no treatment-related deaths. The response rate was 97% (complete response, 37%; partial response, 60%). Median overall survival was 20.2 months; 1-, 2-, and 3-year survival rates were 76%, 41%, and 38%, respectively. Of the 24 patients who started the IP regimen, 22 received two or more cycles. Hematologic toxicities of grade 3 or 4 included neutropenia (67%), anemia (50%), and thrombocytopenia (4%). Nonhematologic toxicities of grade 3 or 4 included diarrhea (8%), vomiting (8%), and febrile neutropenia (8%). Of the 20 patients with recurrence, none had local recurrence alone and only two had both local and distant metastasis as the initial sites of disease progression.

Conclusions: IP following concurrent EP plus twice-dailyTRT is safe with acceptable toxicities. A randomized phase III trial comparing EP with IP following EP plus concurrentTRT for LSCLC is ongoing (JCOG0202).

Despite efforts to curb smoking, lung cancer remains the leading cause of cancer deaths in many industrialized countries. Small cell lung cancer (SCLC) accounts for about 15% of all lung cancer histology. Whereas combination chemotherapy is the cornerstone of SCLC treatment, meta-analyses showed that adding thoracic radiotherapy to combination chemotherapy significantly improves the survival of patients with limited-stage SCLC (LSCLC; i.e., disease confined to the hemithorax; refs. 1, 2). Several randomized trials have shown that early use of concurrent thoracic radiotherapy is superior to sequential or late use when etoposide and platinum are employed as combination chemotherapy (3–5). An intergroup phase III study showed accelerated hyperfractionated radiotherapy with etoposide and cisplatin (EP) to be superior to standard fractionation, with 5-year survival rates of 26% and 16%, respectively (6). Although substantial progress has been made during the past two decades, many LSCLC patients experience tumor recurrence and succumb to the disease, indicating the need for improved LSCLC therapy.

The Japan Clinical Oncology Group (JCOG) previously conducted a randomized phase III trial comparing irinotecan and cisplatin (IP) with EP in patients with extensive-stage SCLC. The response rate and overall median survival were significantly better for IP (i.e., 84.4% and 12.8 months with IP versus 67.5% and 9.4 months with EP, respectively). The 2-year

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survival rates were 19.5% for IP and 5.2% for EP (7). These encouraging results prompted us to explore the use of IP in LSCLC. We therefore undertook a pilot study to evaluate the safety of IP following concurrent EP plus twice-daily thoracic irradiation (TRT) for LSCLC.

Experimental design

Eligibility criteria. Patients with histologically or cytologically documented LSCLC, defined as disease confined to one hemithorax including bilateral supraclavicular nodes, were enrolled in this study. Additional eligibility criteria consisted of measurable or assessable disease, age <75 years, Eastern Cooperative Oncology Group performance status of 0 to 2, no previous treatment, leukocyte count $\geq 4{,}000/mm^3$, platelet count $\geq 10^5/mm^3$, hemoglobin ≥ 9.5 g/d, serum creatinine ≤ 1.5 mg/d, creatinine clearance ≥ 60 mL/min, serum bilirubin ≤ 1.5 mg/d, serum transaminase $\leq 2 \times$ ULN, and $PaO_2 \geq 70$ mm Hg. Exclusion criteria included active infection, uncontrolled heart disease or a history of myocardial infarction within the previous 3 months, interstitial pneumonia/active lung fibrosis on chest X-ray, peripheral neuropathy, malignant pleural or pericardial effusion, diarrhea, intestinal obstruction or paralysis, and active concomitant malignancy. The TRT portal should be no more than half of the hemithorax. No prior chemotherapy or radiotherapy was permitted. Pregnant or lactating women were excluded. Before enrollment in the study, each patient provided a complete medical history and underwent physical examination, blood cell count determinations, arterial blood gas, biochemical laboratory examinations, chest X-ray, electrocardiogram, chest computed tomographic scan, and whole-brain computed tomographic or magnetic resonance imaging, abdominal ultrasound and/or computed tomographic, and isotope bone scans. Blood cell counts, differential white counts, and other laboratory data were obtained weekly during each course of chemotherapy. All patients were reassessed at the end of treatment in the same manner as at the time of enrollment.

Treatment plan. Induction chemotherapy consisted of cisplatin 80 mg/m^2 on day 1 and etoposide 100 mg/m^2 on days 1 to 3. TRT was begun on day 2 of the induction chemotherapy and given twice daily (1.5 Gy per fraction, with ≥ 6 hours between fractions) and directed to the primary tumor for a total dose of 45 Gy in 3 weeks. The initial field included the primary disease site with a 1.5-cm margin around the mass, the ipsilateral hilum, the entire width of the mediastinum, and the supraclavicular lymph nodes (only if there was nodal tumor involvement). TRT was done with linear accelerators and the energy was 6 to 10 MV photons. After the administration of 30 to 36 Gy, the radiation field was reduced around the primary tumor and involved lymph nodes using parallel opposed oblique fields to limit the dose to the spinal cord and protect the uninvolved lung field. Following chemoradiotherapy, patients were treated with three cycles of IP. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 60 mg/m^2 on day 1, with three 28-day cycles. If the leukocyte count decreased to $<3{,}000/mm^3$ or the platelet count fell below $100{,}000/mm^3$ on the first day of IP, chemotherapy was withhold until the counts recovered to $\geq 3{,}000/mm^3$ and $\geq 100{,}000/mm^3$, respectively. Administration of irinotecan was skipped on day 8 and/or 15 if the leukocyte count was $\leq 2{,}000/mm^3$, the platelet count was $\leq 50{,}000/mm^3$, or there was any diarrhea regardless of grade, or a fever of $\geq 37.5°C$. The dose of irinotecan in subsequent cycles was reduced by 10 mg/m^2 from the planned dose if grade 4 hematologic toxic effects or grade 2 or 3 diarrhea developed. Administration of granulocyte colony-stimulating factor was prohibited on the days of chemotherapy or radiotherapy. Primary prophylactic granulocyte colony-stimulating factor was not given. For patients who had developed grade 4 neutropenia during the previous cycles of chemotherapy, secondary prophylactic granulocyte colony-stimulating factor administration was allowed. Prophylactic antibiotics were not given.

Treatment was discontinued in patients with grade 4 nonhematologic toxicity. Prophylactic cranial irradiation (25 Gy in 10 fractions) was conducted for patients showing a complete response or near complete response defined as a reduction of >90% in the sum of the products of the greatest perpendicular dimensions of bidimensional lesions. Tumor responses were assessed radiographically. Standard WHO response criteria (8) were used, and all responses were confirmed ≥ 28 days after initial documentation of the response. JCOG criteria were used to assess toxicity (9). JCOG criteria are similar to those of the National Cancer Institute Common Toxicity Criteria (10). Esophageal toxicity was graded as follows: grade 3, moderate to severe ulceration and edema, cannot eat, requires narcotic drugs; grade 4, serious ulceration and edema, resulting in complete obstruction or perforation.

Statistical consideration. The primary objective of this study was to evaluate the safety and feasibility of sequential administration of IP following EP plus concurrent twice-daily TRT. Simon's optimal two-stage design was used to determine the sample size and decision criteria (11). The regimen would be considered feasible if two cycles or more of IP were completed without grade 4 nonhematologic toxicity or treatment related death in at least 90% of patients and not feasible if the completion rate was $\leq 70\%$. The required number of patients was estimated to be 27, with $\alpha = 0.05$ and $\beta = 0.80$. We determined the planned sample size for the study to be 30 patients accrued over 12 months, with 36 months of additional follow-up.

Time-to-progression was calculated from the date of entry into study until the date of documented progression or death (in the absence of progression). Survival was calculated from the protocol treatment start date until the date of death. Both intervals were determined by the Kaplan-Meier method.

The protocol was approved by the Clinical Trial Review Committee of JCOG and the Institutional Review Board of the participating institutions. All patients provided written informed consent.

Results

Patient characteristics. Between October 1999 and July 2000, 31 patients were accrued from 10 institutions. Patient characteristics are detailed in Table 1. Although eligible, no patients with a performance status of 2 were actually enrolled in this trial. Thirty-one patients ultimately participated. One patient did not receive the protocol treatment because of a problem with the radiation equipment in the institution providing treatment. Thus, this patient was not evaluable.

Adherence to treatment plan. All patients completed concurrent chemoradiotherapy. Six patients did not receive the IP regimen, because of disease progression, septic shock

Table 1. Patient characteristics

Patient registered	31
Assessable	30
Not assessable (not treated)	1
Median age (range)	64 (43-74)
Gender	
Male	27
Female	4
Performance status 0/1	8/23

during chemoradiotherapy, renal dysfunction, or leukocytopenia, and two refused IP. Of the 24 patients given the IP regimen, 22 received two cycles or more of IP. The reasons for terminating IP before the second treatment cycle were grade 4 diarrhea in one patient and refusal, not significant toxicity, in one patient. Of the 22 patients who received two cycles or more of IP, nine received the original planned dose. In five patients, dose reductions in the second cycle of IP were necessary, 11 patients skipped day 8 and/or 15 irinotecan, and one patient had a minor protocol violation. Fifteen patients required that the second cycle of IP be delayed for 1 to 14 days. Of 17 patients (58%) who received the entire treatment, the median time delay from the planned protocol was 4 days (range, 0-21 days). Six patients were able to start the third cycle of IP without delay, relative to the first cycle of IP.

Toxicity. Toxicities associated with concurrent chemoradiotherapy are summarized in Table 2. The major toxicity was neutropenia. One patient had febrile neutropenia and septic shock. The same patient experienced grade 3 fatigue and anterior chest pain. IP was well tolerated (Table 3), despite diarrhea, vomiting, and hematologic toxicities. One patient, who had grade 2 nausea/vomiting, refused further treatment after the first cycle of IP. Another patient, who refused days 8 and 15 irinotecan during the second cycle, had grade 2 diarrhea and nausea/vomiting. No grade 3 or 4 pulmonary toxicity was observed. There were no treatment-related deaths.

Table 2. Major toxicities concurrent EP/TRT ($n = 30$)

Toxicity	Grade 3, no. patients (%)	Grade 4, no. patients (%)
Hematologic		
Anemia	0	0
Leucopenia	13 (43)	15 (50)
Neutropenia	9 (30)	19 (63)
Thrombocytopenia	2 (7)	1 (3)
Nonhematologic		
Esophagitis	2 (7)	0
Infection	1 (3)	0
Hypotension*	0	1 (3)
Fatigue*	1 (3)	0
Anterior chest pain*	1 (3)	0
Febrile neutropenia	2 (7)	

*These events occurred in the same patient.

Table 3. Major toxicities irinotecan and cisplatin (IP), ($n = 24$)

Toxicity	Grade 2, no. patients (%)	Grade 3, no. patients (%)	Grade 4, no. patients (%)
Hematologic			
Anemia	6 (25)	12 (50)	0
Leucopenia	6 (25)	12 (50)	5 (21)
Neutropenia	5 (21)	12 (50)	5 (21)
Thrombocytopenia	5 (21)	1 (4)	0
Nonhematologic			
Diarrhea	4 (17)	1 (4)	1 (4)
Vomiting	3 (13)	2 (8)	0
Febrile neutropenia	—	2 (8)	0
Fever	2 (8)	0	0
Infection	4 (17)	0	0

Neither grade 2, or more severe, late radiation toxicities nor radiation recall reactions were reported.

Response and survival. The overall response rate was 97% (complete response, 37%; partial response, 60%). Overall and progression-free survivals are depicted in Figs. 1 and 2 The median follow-up time of all patients was 20 months and that for surviving patients 40 months. The median progression-free survival was 9 months, and the median overall survival was 20 months. The 24- and 36-month overall survivals were 41% and 38%, the 24- and 36- month progression-free survivals 30% and 26%, respectively.

Pattern of relapse. First sites of disease progression are presented in Table 4. Of the 18 patients who have died to date, all died of progressive disease. Surprisingly, no patient showed relapse solely at the local-regional site (within TRT field). Only two patients had both local and distant involvement. There were 11 patients whose initial site of relapse was the brain. Of these, six had relapses solely in the brain. Whereas two patients had complete response and received prophylactic cranial irradiation, four had partial remission and did not receive prophylactic cranial irradiation.

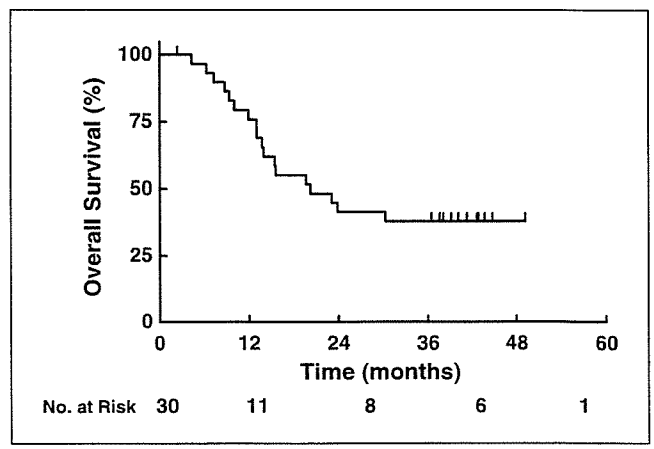

Fig. 1. Overall survival.

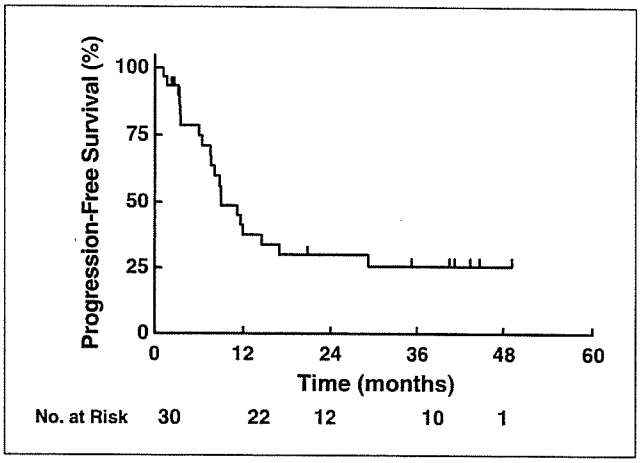

Fig. 2. Progression-free survival.

Other relapse sites included the liver in four patients, bone in three, pleural effusion in three, and supraclavicular lymph nodes in two.

Discussion

Irinotecan is one of the most active agents against SCLC (12). A phase II study of irinotecan and cisplatin yielded a response rate of 86% and median survival of 13.2 months in patients with extensive SCLC (13). A phase III study confirmed excellent results and showed IP to be more effective than etoposide and cisplatin in extensive SCLC (7). Three confirmatory trials, comparing IP with EP for extensive SCLC are ongoing in Europe and the United States. Although dose-finding studies to explore integrating irinotecan into the early concurrent phase of chemoradiation for LSCLC are also currently being conducted by the Radiation Therapy Oncology Group and other U.S. groups. The dose-finding JCOG study of concurrent use of IP with TRT in stage III non-small cell lung cancer showed that the full dose of irinotecan could not be given due to neutropenia, diarrhea, and pulmonary toxicity (14). Thus, we employed IP as a sequential treatment following EP plus concurrent TRT.

The present trial showed IP following concurrent EP plus twice-daily TRT to be safe, with acceptable toxicities. Hematologic toxicities and diarrhea, while on the IP regimen following concurrent chemoradiotherapy, are similar to those of a previous phase III trial conducted by JCOG (JCOG9503; ref. 7). Neither grade 3 or 4 pulmonary toxicity nor treatment related deaths were observed. The West Japan Thoracic Oncology Group conducted a similar phase II study of EP plus twice-daily TRT followed by IP for LSCLC (15). Promising response (88%) and 2-year survival (51%) rates were reported, with acceptable toxicities.

Table 4. Sites of first failure (n = 20)

Site	No. patient (%)
Isolated local-regional failure	0 (0)
Local-regional and distant	2 (10)
Distant	18 (90)
Brain only	6 (30)
Other sites of failure*	12 (60)

*Recurrence at sites other than the primary tumor or brain only.

Local failure is an important problem in the treatment of LSCLC. Turrisi et al. showed the rate of local failure to be reduced in the twice-daily TRT plus EP group as compared with the once-daily TRT plus EP group: the rate was 52% in the group receiving once-daily therapy and 36% in that receiving twice-daily therapy (6). Eighteen percent of patients who received EP plus concurrent twice-daily TRT had first progression within the thorax in the previous JCOG phase III trial (5). It is noteworthy that no patient relapsed solely at the local-regional site and only two patients had both local and distant involvement in the present trial. There may be an interaction between TRT and IP even given sequentially. Another possibility relates to recent improvements in radiotherapeutic techniques with better imaging of the target volume by chest computed tomographic. This possibility should be assessed in a future randomized trial.

It is important to integrate new active anticancer agents to the combined modality treatments for LSCLC. Irinotecan has been clearly shown to have clinical activity in a randomized trial, against extensive-stage SCLC. Several other new agents including targeted therapies have failed to show clinical activity against SCLC. Based on these considerations, we conducted a randomized phase III trial comparing EP with IP following EP plus concurrent TRT for the treatment of LSCLC (JCOG0202). In the JCOG0202, eligible patients were randomized after the completion of induction chemoradiotherapy. Although feasibility may be a limitation of the present study, improvements are anticipated with appropriate use of granulocyte colony-stimulating factor, antibiotics, and patient education.

In summary, irinotecan and cisplatin following EP plus concurrent twice-daily TRT is a safe and active regimen for LSCLC. The observed low rate of local recurrence is encouraging. A randomized phase III trial comparing EP with IP following EP plus concurrent TRT for the treatment of LSCLC is currently under way.

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