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.

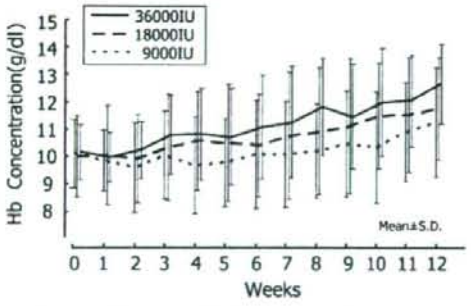

Figure 1. Mean weekly hemoglobin levels for Per Protocol Set population by epoetin-beta dosage Group. Hb, hemoglobin; SD, standard deviation.

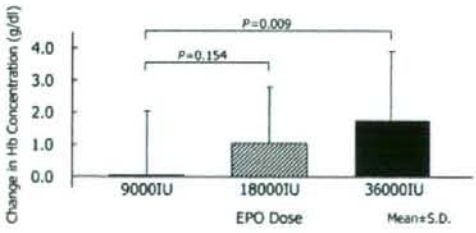

Figure 2. Mean change in hemoglobin level from baseline to last observation (at 12 weeks or 4 weeks after the beginning of a final-course of chemotherapy) by epoetin-beta dosage group (Per Protocol Set population). Hb, hemoglobin; EPO, epoetin-beta; SD, standard deviation.

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).

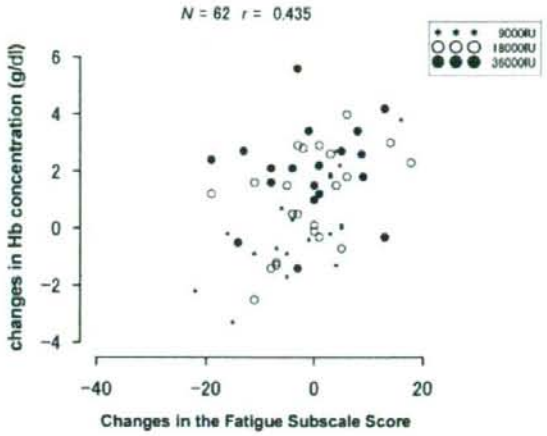

Figure 3. Correlation between change in hemoglobin levels and change in the Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale scores at 7–11 weeks (Per Protocol Set population). Hb, hemoglobin.

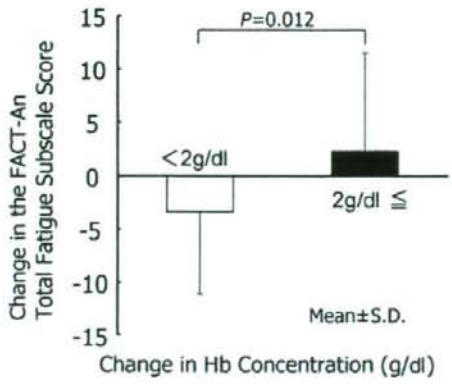

Figure 4. Change in mean Functional Assessment of Cancer Therapy—Anemia total Fatigue subscale score between baseline and 7–11 weeks by change in hemoglobin level (change in hemoglobin of ≥2 g/dl or <2 g/dl from baseline). FACT-An, Functional Assessment of Cancer Therapy–Anemia; Hb, hemoglobin; SD, standard 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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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	rs1847568	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.

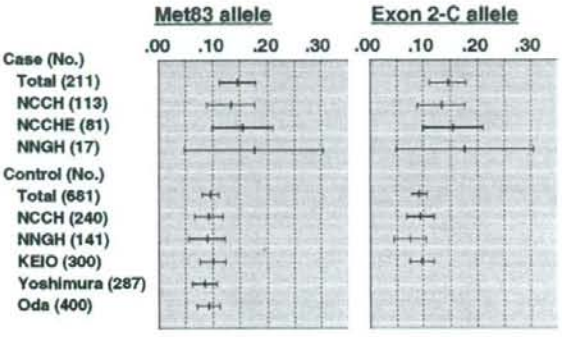

Fig. 1. Frequencies of the MTH-83Met and exon 2-C alleles in cases and controls. Allele frequency is shown with its sampling variations estimated by 95% CI. Frequencies of the MTH-83Met allele in two control populations reported by Yoshimura *et al.* (24) and Oda *et al.* (21) are also shown.

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

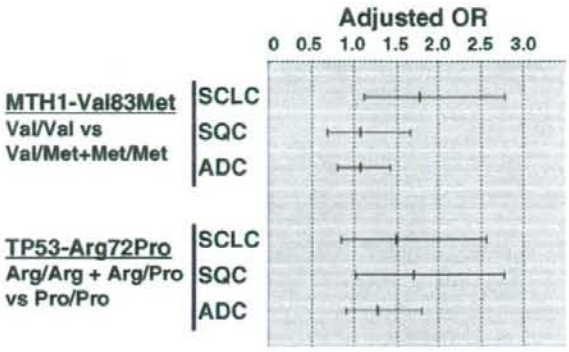

Fig. 2. ORs of the MTH-83Met allele carriers against homozygotes for the MTH-83Val allele and those of homozygotes for the TP53-72Pro allele against others. ORs adjusted for gender, age and smoking dosage are shown. ORs in SQC and ADC cases are from our previous report (13).

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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Docetaxel Consolidation Therapy Following Cisplatin, Vinorelbine, and Concurrent Thoracic Radiotherapy in Patients with Unresectable Stage III Non-small Cell Lung Cancer

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Background: To evaluate the feasibility and efficacy of docetaxel consolidation therapy after concurrent chemoradiotherapy for unresectable stage III non–small cell lung cancer (NSCLC).

Patients and Methods: The eligibility criteria included unresectable stage III NSCLC, no previous treatment, age between 20 and 74 years, and performance status 0 or 1. Treatment consisted of cisplatin (80 mg/m^2 on days 1, 29, and 57), vinorelbine (20 mg/m^2 on days 1, 8, 29, 36, 57, and 64), and thoracic radiotherapy (TRT) (60 Gy/30 fractions over 6 weeks starting on day 2), followed by consolidation docetaxel (60 mg/m^2 every 3 to 4 weeks for three cycles).

Results: Of 97 patients who were enrolled in this study between 2001 and 2003, 93 (76 males and 17 females with a median age of 60) could be evaluated. Chemoradiotherapy was well tolerated; three cycles of chemotherapy and 60 Gy of TRT were administered in 80 (86%) and 87 (94%) patients, respectively. Grade 3 or 4 neutropenia, esophagitis, and pneumonitis developed in 62, 11, and 3 patients, respectively. Docetaxel consolidation was administered in 59 (63%) patients, but three cycles were completed in only 34 (37%) patients. The most common reason for discontinuation was pneumonitis, which developed in 14 (24%) of the 59 patients. During consolidation therapy, grade 3 or 4 neutropenia, esophagitis, and pneumonitis developed in 51, 2, and 4 patients, respectively. A total of four patients died of pneumonitis. We calculated a V_{20} (the percent volume of the normal lung receiving 20 Gy or more) on a dose–volume histogram in 25 patients. Of these, five patients developed grade 3 or more severe radiation pneumonitis. A median V_{20} for these five patients was 35% (range, 26–40%), whereas the median V_{20} for the remaining 20 patients was 30% (range, 17–35%) (p = 0.035 by a Mann–Whitney test). The response rate was 81.7% (95% confidence interval [CI], 72.7–88.0%), with 5 complete and 71 partial responses. The median progression-free survival was 12.8 (CI, 10.2–15.4) months, and median survival was 30.4 (CI, 24.5–36.3) months. The 1-, 2-, and 3-year survival rates were 80.7, 60.2, and 42.6%, respectively.

Conclusion: This regimen produced promising overall survival in patients with stage III NSCLC, but the vast majority of patients could not continue with the docetaxel consolidation because of toxicity.

Key Words: Non-small cell lung cancer, Chemoradiotherapy, Consolidation, Docetaxel.

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Locally advanced unresectable non–small cell lung cancer (NSCLC), stage IIIA with bulky N2 and stage IIIB disease without pleural effusion, is characterized by large primary lesions and/or involvement of the mediastinal or supraclavicular lymph nodes and occult systemic micrometastases. A combination of thoracic radiotherapy and chemotherapy is the standard medical treatment for this disease, but the optimal combination has not been established.[1] Although the available data are insufficient to accurately define the size of a potential benefit,[2] concurrent chemoradiotherapy using a platinum doublet has been shown to be superior to the sequential approach in phase III trials of this disease.[3–5] However, third-generation cytotoxic agents, which have provided better patient survival with extrathoracic spread than the old-generation agents, must be reduced when administered concurrently with thoracic radiotherapy.[6] Thus, it has been hypothesized that the addition of systemic dose chemotherapy with a new cytotoxic agent to concurrent chemoradiotherapy, either as induction or as consolidation chemotherapy, might further improve patient survival.[1]

The consolidation chemotherapy with docetaxel was based on the observation that this drug was highly active in the primary treatment of metastatic NSCLC, producing a response rate (RR) as high as 20% after platinum-based chemotherapy failed.[7–9] Highly encouraging results of a me-

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dian survival time (MST) of more than 2 years and a 3-year survival rate of nearly 40% were obtained in a phase II trial of docetaxel consolidation after chemoradiotherapy with cisplatin and etoposide in patients with stage IIIB NSCLC (SWOG study S9504).[10]

We have developed a combination chemotherapy schedule with cisplatin and vinorelbine concurrently administered with thoracic radiotherapy at a total dose of 60 Gy in 30 fractions in patients with unresectable stage III NSCLC. The results of a phase I study in 18 patients were very promising, with a RR of 83%, a MST of 30 months, and a 3-year survival rate of 50%.[6] Thus, addition of docetaxel consolidation to this regimen is a particularly interesting therapeutic strategy. The objectives of the current study were to evaluate the feasibility of docetaxel consolidation therapy after concurrent chemoradiotherapy with cisplatin and vinorelbine and to evaluate the efficacy and safety of the whole treatment regimen including both the chemoradiotherapy and consolidation therapy in patients with unresectable stage IIIA and IIIB NSCLC.

PATIENTS AND METHODS

Patient Selection

The eligibility criteria were histologically or cytologically proven NSCLC; unresectable stage IIIA or IIIB disease; no previous treatment; measurable disease; tumor within an estimated irradiation field no larger than half the hemithorax; age between 20 and 74 years; Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; adequate bone marrow function (12.0×10^9/liter ≥ white blood cell [WBC] count ≥4.0×10^9/liter, neutrophil count ≥2.0×10^9/liter, hemoglobin ≥10.0 g/dl, and platelet count ≥100×10^9/liter), liver function (total bilirubin ≤1.5 mg/dl and transaminase no more than twice the upper limit of the normal value), and renal function (serum creatinine ≤1.5 mg/dl and creatinine clearance ≥60 ml per minute); and a PaO_2 of 70 torr or more under room air conditions. Patients were excluded if they had malignant pleural or pericardial effusion, active double cancer, a concomitant serious illness such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonia or lung fibrosis identified by a chest x-ray, chronic obstructive lung disease, infection or other diseases contraindicating chemotherapy or radiotherapy, pregnancy, or if they were breast feeding. All patients gave their written informed consent.

Pretreatment Evaluation

The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest x-rays, chest computed tomographic (CT) scan, brain CT scan or magnetic resonance imaging, abdominal CT scan or ultrasonography, and radionuclide bone scan.

Treatment Schedule

Treatment consisted of a chemoradiotherapy phase with three cycles of cisplatin and vinorelbine followed by a consolidation phase with three cycles of docetaxel (Figure 1). Cisplatin 80 mg/m^2 was administered on days 1, 29, and 57 by intravenous infusion for 60 minutes with 2500 to 3000 ml of fluid for hydration. Vinorelbine diluted in 50 ml of normal saline was administered intravenously on days 1, 8, 29, 36, 57, and 64. All patients received prophylactic antiemetic therapy consisting of a $5HT_3$-antagonist and a steroid.

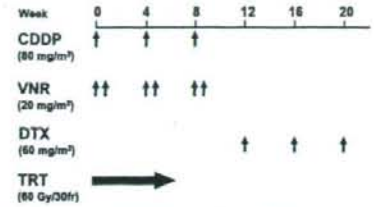

FIGURE 1. Treatment schema. CDDP, cisplatin; DTX, docetaxel; TRT, thoracic radiotherapy; VNR, vinorelbine.

Radiation therapy was delivered with megavoltage equipment (≥6 MV) using anterior/posterior opposed fields up to 40 Gy in 20 fractions including the primary tumor, the metastatic lymph nodes, and the regional nodes. A booster dose of 20 Gy in 10 fractions was given to the primary tumor and the metastatic lymph nodes for a total dose of 60 Gy using bilateral oblique fields. A CT scan–based treatment planning was used in all patients. The clinical target volume (CTV) for the primary tumor was defined as the gross tumor volume (GTV) plus 1 cm taking account of subclinical extension. CTV and GTV for the metastatic nodes (>1 cm in shortest dimension) were the same. Regional nodes, excluding the contralateral hilar and supraclavicular nodes, were included in the CTV, but the lower mediastinal nodes were included only if the primary tumor was located in the lower lobe of the lung. The planning target volumes for the primary tumor, the metastatic lymph nodes, and regional nodes were determined as CTVs plus 0.5- to 1.0-cm margins laterally and 1.0- to 2.0-cm margins craniocaudally, taking account of setup variations and internal organ motion. Lung heterogeneity corrections were not used.

The criteria for starting consolidation chemotherapy were completion of three cycles of cisplatin and vinorelbine and a full dose of thoracic radiotherapy, the absence of progressive disease, adequate general condition within 6 weeks of the start of the third cycle of cisplatin and vinorelbine (PS 0 or 1, WBC count ≥3.0×10^9/liter, neutrophil count ≥1.5×10^9/liter, hemoglobin ≥9.0 g/dl and platelet count ≥100×10^9/liter, total bilirubin ≤1.5 mg/dl and transaminase no more than twice the upper limit of the normal value, and a PaO_2 of 70 torr or more at room air). Docetaxel (60 mg/m^2) was administered intravenously for 1 hour every 3 to 4 weeks for three cycles.

Toxicity Assessment and Treatment Modification

Complete blood cell counts and differential counts, routine chemistry determinations, and a chest x-ray were performed once a week during the course of treatment. Acute toxicity was graded according to the NCI Common Toxicity Criteria, and late toxicity associated with thoracic radiother-

apy was graded according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer late radiation morbidity scoring scheme. Vinorelbine administration on day 8 was omitted if any of the following were noted: WBC count $<3.0 \times 10^9$/liter, neutrophil count $<1.5 \times 10^9$/liter, platelet count $<100 \times 10^9$/liter, elevated hepatic transaminase level or total serum bilirubin of at least grade 2, fever ≥38°C, or PS ≥2. Subsequent cycles of cisplatin and vinorelbine chemotherapy were delayed if any of the following toxicities were noted on day 1: WBC count $<3.0 \times 10^9$/liter, neutrophil count $<1.5 \times 10^9$/liter, platelet count $<100 \times 10^9$/liter, serum creatinine level ≥1.6 mg/dl, elevated hepatic transaminase level or total serum bilirubin of at least grade 2, fever ≥38°C, or PS ≥2. The dose of cisplatin was reduced by 25% in all subsequent cycles if the serum creatinine level rose to 2.0 mg/dl or higher. The dose of vinorelbine or docetaxel was reduced by 25% in all subsequent cycles if any of the following toxicities were noted: WBC count $<1.0 \times 10^9$/liter, platelet count $<10 \times 10^9$/liter, or grade 3 or 4 infection or liver dysfunction. Thoracic radiotherapy was suspended if any of the following were noted: fever ≥38°C, grade 3 esophagitis, PS of 3, or PaO_2 <70 torr. Thoracic radiotherapy was terminated if any of the following were noted: grade 4 esophagitis, grade 3 or 4 pneumonitis, PS of 4, or duration of radiotherapy of over 60 days. The use of granulocyte colony-stimulating factor during radiotherapy was not permitted unless radiotherapy was on hold. The criteria for termination of docetaxel consolidation were not defined in the protocol.

Response Evaluation

Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumor.[11] Local recurrence was defined as tumor progression in the primary site and in the hilar, mediastinal, and supraclavicular lymph nodes after a partial or complete response; regional recurrence as the development of malignant pleural and pericardial effusions; and distant recurrence as the appearance of a distant metastasis.

Study Design, Data Management, and Statistical Considerations

This study was conducted at three institutions: the National Cancer Center Hospital, National Cancer Center Hospital East, and Tochigi Cancer Center. The protocol and consent form were approved by the institutional review board of each institution. Registration was conducted at the registration center. Data management, periodic monitoring, and the final analysis were performed by the study coordinator.

The primary objective of the current study was to evaluate the feasibility of docetaxel consolidation therapy. The secondary endpoints were toxicity observed during chemoradiotherapy and consolidation therapy, the best response, and overall survival in all patients eligible to participate in this study. Because no standard method to evaluate consolidation chemotherapy after chemoradiotherapy has been established, we arbitrarily defined the primary endpoint of this study as a ratio (R) of the number of patients receiving docetaxel without grade 4 nonhematological toxicity or treatment-related death to the total number of patients receiving docetaxel. The sample size was initially estimated to be 34 patients with a power of 0.80 at a significance level of 0.05, under the assumption that a R of 0.95 would indicate potential usefulness, whereas a R of 0.8 would be the lower limit of interest, and that 85% of patients would move into the consolidation phase. An analysis of the first 13 patients, however, showed that only 8 (61%) patients advanced into the consolidation phase. The reasons for not receiving docetaxel were disease progression in one, delay in completion of chemoradiotherapy in two, grade 3 esophagitis in one, and death due to hemoptysis in one patient. Considering that the SWOG trial S9504 included 83 patients, we decided to revise the number of patients in the current study. According to Simon's two-stage minimax design, the required number of patients was calculated to be 59 with a power of 0.80 at a significance level of 0.05, under the assumption that a R of 0.85 would indicate potential usefulness, whereas a R of 0.7 would be the lower limit of interest.[12] Assuming that 61% of registered patients would move into the consolidation phase, the sample size was determined to be 97 patients.

Overall survival time and progression-free survival time were estimated by the Kaplan–Meier method, and confidence intervals (CI) were based on Greenwood's formula.[13] Overall survival time was measured from the date of registration to the date of death (from any cause) or to the last follow-up. Progression-free survival time was measured from the date of registration to the date of disease progression, death (from any cause), or the last follow-up. Patients who were lost to follow-up without event were censored at the date of their last known follow-up. A CI for RR was calculated using methods for exact binomial CIs. The Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses.

RESULTS

Registration and Characteristics of the Patients

A total of 97 patients were enrolled in this study between April 2001 and June 2003. Four patients were excluded from this study before the treatment was started because the radiation treatment planning disclosed that their tumors were too advanced for curative thoracic radiotherapy. Thus, 93 patients who received the protocol-defined treatment were the subjects of this analysis (Figure 2). There were 76 males and 17 females, with a median age of 60 (range 31–74). Body weight loss was less than 5% in 77 patients; adenocarcinoma histology was noted in 57 patients, and stage IIIA disease was noted in 41 patients (Table 1).

Treatment Delivery

Treatment delivery was generally well maintained in the chemoradiotherapy phase (Table 2). Full cycles of cisplatin and vinorelbine and the full dose of thoracic radiotherapy were administered in 80 (86%) and 87 (94%) patients, respectively. Delay in radiotherapy was less than 5 days in 61 (66%) patients. In contrast, the delivery of docetaxel was poor (Table 2). A total of 59 (63%) patients could enter the consolidation phase, and only 34 (37%) patients completed three cycles of docetaxel chemotherapy. The reasons for not

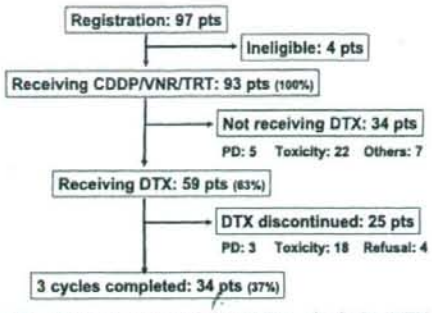

FIGURE 2. Patient registration. CDDP, cisplatin; DTX, docetaxel; TRT, thoracic radiotherapy; VNR, vinorelbine.

receiving consolidation were toxicity in 22 (65%) patients including pneumonitis in seven patients, myelosuppression in five patients, esophagitis in four patients, liver dysfunction in two patients, infection in two patients, other toxicity in two patients, progressive disease in five (15%) patients, patient refusal in three (9%) patients, early death due to hemoptysis in one (3%) patient, and other reasons in three (9%) patients. Of the 59 patients, 18 (31%) discontinued docetaxel consolidation because of toxicity, including pneumonitis (n = 14) and esophagitis, infection, gastric ulcer, and allergic reaction (n = 1 each), four (7%) because of patient refusal, and three (5%) because of progressive disease.

Toxicity

Acute severe toxicity in the chemoradiotherapy phase was mainly leukopenia and neutropenia, whereas grade 3 or 4 thrombocytopenia was not noted (Table 3). Severe nonhematological toxicity was sporadic, and grade 3 esophagitis and pneumonitis were observed in only 11 (12%) and 3 (3%) patients, respectively. Acute severe toxicity in the consolidation phase also consisted of neutropenia and associated infection (Table 4). In addition, grade 3 or 4 pneumonitis developed in 4 (7%) patients. The R observed in this study was 0.05 (3 out of 57 patients), which was much lower than the hypothetical value. Grade 3 or 4 late toxicities were included lung toxicity in four patients, esophageal toxicity in two patients, renal toxicity in one patient, and a second esophageal cancer that developed 35.4 months after the start of the chemoradiotherapy in one patient. Treatment-related

TABLE 1. Patient Characteristics

Characteristics	n	%
Gender		
Male	76	82
Female	17	18
Age median (range)	60	31–74
Weight loss		
<5%	76	81
5–9%	12	13
≥10%	3	3
Unknown	2	2
Histology		
Adenocarcinoma	57	61
Squamous cell carcinoma	23	25
Large cell carcinoma	12	13
Others	1	1
Stage		
IIIA	41	44
IIIB	52	56

TABLE 2. Treatment Delivery

Variables	n	%
Cisplatin and vinorelbine chemotherapy		
Total number of cycles		
3	80	86
2	10	11
1	3	3
Number of vinorelbine skips		
0	63	68
1	25	27
2–3	5	5
Thoracic radiotherapy		
Total dose (Gy)		
60	87	94
50–59	4	4
<50	2	2
Delay (days)		
<5	61	66
5–9	20	22
10–16	6	6
Not evaluable (<60 Gy)	6	6
Docetaxel consolidation		
Number of cycles		
3	34	37
2	12	13
1	13	14
0	34	34

TABLE 3. Acute Toxicity in Chemoradiotherapy (n = 93)

	Grade			
Toxicity	3	4	3 + 4	%
Leukopenia	54	18	72	77
Neutropenia	33	29	62	67
Anemia	21	0	21	23
Infection	15	1	16	17
Esophagitis	11	0	11	12
Hyponatremia	11	0	11	12
Anorexia	9	1	10	11
Nausea	5	—	5	5
Pneumonitis	3	0	3	3
Syncope	2	0	2	2
Hyperkalemia	2	0	2	2
Ileus	0	1	1	1
Cardiac ischemia	1	0	1	1

TABLE 4. Acute Toxicity in Consolidation Therapy (n = 57)

Toxicity	Grade 3	Grade 4	Grade 3 + 4	%
Leukopenia	33	11	44	77
Neutropenia	24	26	50	88
Anemia	5	0	5	9
Infection	5	1	6	11
Esophagitis	2	0	2	3
Anorexia	1	0	1	2
Pneumonitis	2	2	4	7

death was observed in four (4%) patients. Of these, three received docetaxel, and one did not. The reason for death was pneumonitis in all patients. We calculated a V_{20} (the percent volume of the normal lung receiving 20 Gy or more) on a dose–volume histogram in 25 patients. Of these, five patients developed grade 3 or severer radiation pneumonitis. A median V_{20} for these five patients was 35% (range, 26–40%), whereas that for the remaining 20 patients was 30% (range, 17–35%) (p = 0.035 by a Mann–Whitney test).

Objective Responses, Relapse Pattern, and Survival

All 93 patients were included in the analyses of tumor response and survival. Complete and partial responses were obtained in 5 (5%) and 71 patients (76%), respectively, for an overall RR of 81.7% (95% CI, 72.7–88.0%). Stable and progressive diseases occurred in 12 (13%) and 5 (5%) patients, respectively. With a median follow-up period of 29.7 months, 38 patients developed locoregional recurrence, 32 developed distant recurrence, 4 developed both locoregional and distant recurrences, and 19 did not. The median progression-free survival time was 12.8 (95% CI, 10.2–15.4) months (Figure 3). Two patients underwent salvage surgery for a recurrent primary tumors. Conventional chemotherapy and gefitinib monotherapy were administered after recurrence in 20 and 25 patients, respectively. The median overall survival time was 30.4 (95% CI, 24.5–36.3) months. The 1-, 2-, and 3-year survival rates were 80.7, 60.2, and 42.6%, respectively. (Figure 4).

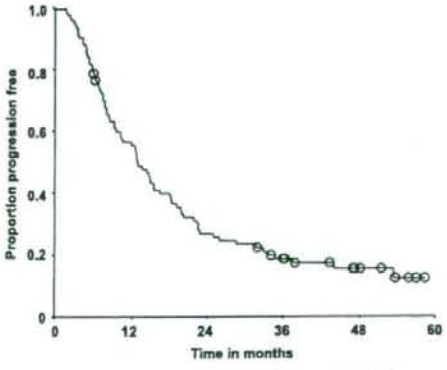

FIGURE 3. Progression-free survival (n = 93). The median progression-free survival time was 12.8 (95% CI, 10.2–15.4) months.

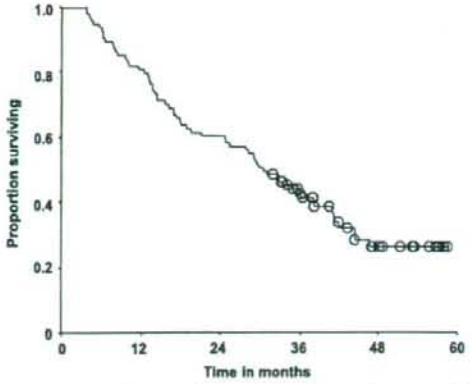

FIGURE 4. Overall survival (n = 93). The median overall survival time was 30.4 (95% CI, 25.4–35.4) months. The 1-, 2-, and 3-year survival rates were 80, 60, and 40%, respectively.

DISCUSSION

This study showed that concurrent chemoradiotherapy with cisplatin, vinorelbine, and standard thoracic radiotherapy was well tolerated, with a high completion rate exceeding 80%. The incidence of acute toxicity, including 67% (62/93) of grade 3 or 4 neutropenia, 12% (11/93) of grade 3 esophagitis, and 3% (3/93) of grade 3 pneumonitis, were comparable with other reports of concurrent chemoradiotherapy.[3,4,10] In contrast, consolidation docetaxel could be administered in only 59 of 93 (63%) patients eligible to participate in this study. Of the remaining 34 patients, 22 (65%) patients did not receive consolidation chemotherapy because of toxicities affecting various organs. Other studies also showed that not all patients proceeded to the consolidation phase after completion of concurrent chemoradiotherapy: 61 to 78% of patients after two cycles of cisplatin and etoposide with radiotherapy,[3,10] and 54 to 75% of patients after weekly carboplatin and paclitaxel with radiotherapy.[14,15] Thus, for 20 to 40% of the patients, concurrent chemoradiotherapy was as much as they could undergo, and the additional chemotherapy was not practical.

Furthermore, the number of patients who fulfilled the three cycles of consolidation docetaxel was only 34 (58%) of the 59 patients, which corresponded to only 37% of those eligible in this study. The reason for the termination of docetaxel in the 25 patients was toxicity in 18 (72%) patients, especially pneumonitis in 14 (56%) patients. The grade of pneumonitis during the consolidation phase was within grade 2 in most cases, and this was probably because docetaxel was discontinued early. Considering that pneumonitis associated with cancer treatment is more common in Japan, docetaxel consolidation is not thought to be feasible in the Japanese population. The MST and the 3-year survival rate in all eligible patients were 33 months and 44% in this study, but docetaxel consolidation was unlikely to contribute to these promising results because only 37% of patients received full cycles of docetaxel. This contrasts clearly with the result of

the SWOG study S9504, a phase II trial of two cycles of cisplatin and etoposide with thoracic radiation followed by three cycles of docetaxel. In this trial, 75% of patients starting consolidation and 59% of those entering the trial received full cycles. In addition, docetaxel consolidation seemed to prolong survival, although this was drawn from a retrospective comparison of the results between the two SWOG studies S9504 and S9019.[10]

There is no widely used definition of consolidation therapy following chemoradiotherapy. Given that consolidation therapy is arbitrarily defined as chemotherapy with three cycles or more after the completion of concurrent chemoradiotherapy, only one randomized trial is available in the literature. The randomized phase III trial of standard chemoradiotherapy with carboplatin and paclitaxel followed by either weekly paclitaxel or observation in patients with stage III NSCLC showed that only 54% of patients proceeded to randomization, and overall survival was worse in the consolidation arm (MST, 16 versus 27 months).[15] Thus, there have been no data supporting the use of consolidation therapy, especially when a third-generation cytotoxic agent such as paclitaxel and vinorelbine is incorporated into concurrent chemoradiation therapy.

The low complete-response rate of 5% in this study may be explained partly by an inability to distinguish between inactive scarring or necrotic tumor and active tumor after radiotherapy. Positron emission tomography (PET) using 18F-fluorodeoxyglucose showed a much higher rate of complete response than conventional CT scanning and provided a better correlation of the response assessment using PET with patterns of failure and patient survival.[16] In addition, the high locoregional relapse rate in this study clearly showed that the conventional total dose of 60 Gy was insufficient. Three-dimensional treatment planning, omission of elective nodal irradiation, and precise evaluation of the gross tumor volume by PET may facilitate the escalation of the total radiation dose without enhanced toxicity.

In conclusion, cisplatin and vinorelbine chemotherapy concurrently combined with standard thoracic radiotherapy and followed by docetaxel consolidation produced promising overall survival in patients with stage III NSCLC, but the vast majority of patients could not continue with the docetaxel consolidation because of toxicity.

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A Literature Review of Molecular Markers Predictive of Clinical Response to Cytotoxic Chemotherapy in Patients with Lung Cancer

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Background: To find candidate genes for a predictive chemosensitivity test in patients with lung cancer by using a literature review.
Methods: Using MEDLINE searches, "in vitro chemosensitivity associated genes" and articles on association of the gene alteration with clinical chemosensitivity in lung cancer patients were selected. We calculated odds ratios (ORs) and their 95% confidence intervals (95% CIs) of response rates for patients who had tumors with or without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.
Results: Of the 80 in vitro chemosensitivity-associated genes identified, 13 genes were evaluated for association with clinical chemosensitivity in 27 studies. The median (range) number of patients in each study was 50 (range, 28-108). The response rates of lung cancer with high and low P-glycoprotein expression were 0% and 73% to 85%, respectively ($p < 0.001$). Glutathione S-transferase pi expression (OR 0.22, 95% CI 0.06-0.79), excision repair cross-complementing 1 alterations (combined OR 0.53, 95% CI 0.28-1.01; $p = 0.055$), and tumor suppressor p53 mutation (combined OR 0.25, 95% CI 0.12-0.52) were associated with clinical chemosensitivity.
Conclusion: In total, 80 in vitro chemosensitivity-associated genes were identified in the literature, and high and low P-glycoprotein, glutathione S-transferase pi expression, excision repair cross-complementing 1 alterations, and tumor suppressor p53 mutation were candidates for future clinical trials of chemosensitivity tests in lung cancer patients.

Key Words: chemotherapy, drug response, molecular markers, prediction, lung cancer

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Lung cancer is the leading cause of death in many countries despite extensive basic research and clinical trials. Approximately 80% of patients with lung cancer have developed distant metastases either by the time of initial diagnosis or during recurrence after surgery for local disease. Systemic chemotherapy against lung cancer, however, has limitations in efficacy such that patients with distant metastases rarely live long.[1]

Tumor response to chemotherapy varies among patients, and objective tumor response rates to standard chemotherapy regimens are approximately 20 to 40% in patients with non—small-cell lung cancer and 60 to 90% in patients with small-cell lung cancer. Thus, it would be extremely useful to know in advance whether patients have tumors that respond to chemotherapy agents and whether the tumors would be resistant to such therapy. For this purpose, cell culture-based chemosensitivity tests have been investigated for more than 20 years, but they are not widely accepted because of technical problems such as the large amount of material required, a low success rate for the primary culture, length of time required, and poor correlation with the clinical response.[2–5]

To overcome these obstacles, DNA-, RNA-, and protein-based chemosensitivity tests have been created, but gene alterations that are predictive of the clinical drug response are not established. Recently, as many as 400 genes whose expression was associated with drug response were identified by cDNA microarray studies, but their functions do not seem to be related to drug sensitivity or resistance.[6–10] In addition, the genes identified by microarray studies were highly unstable and depended on the selection of patients used for gene identification.[11,12] The purpose of this study was to provide an overview of gene alterations in lung cancer that are associated with chemotherapy drug response to identify candidate genes for predictive chemosensitivity tests in patients with lung cancer.

MATERIALS AND METHODS

Because one set of genes associated with chemosensitivity is those directly involved in drug resistance mechanisms, we conducted a MEDLINE search for articles on tumor drug resistance published in the years 2001–2003. This search yielded 112 studies, including several review articles. By searching manually through these articles, we identified 134 genes or gene families that may be involved in drug resistance based on their function. We conducted the second MEDLINE searches for papers of in vitro studies on the 134 genes or gene families by using their names as a keyword.

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From the 134 genes, we selected genes that met the following definition of "in vitro chemosensitivity associated genes": 1) alteration of the gene was identified in a human drug-induced resistant, solid tumor cell line; 2) transfection of the gene induced drug resistance; or 3) down-regulation of the gene or its encode protein increased drug sensitivity. In this latter category, we included studies in which the gene expression or function was suppressed by antisense RNA, hammerhead ribozyme, or an antibody against the gene product. We excluded studies in which drugs were used to inhibit function because the specificity of the drug against the target may not have been complete. We performed a third MEDLINE search for articles on the association between the gene alteration and chemosensitivity of lung cancer cell lines by using the name of the gene as a keyword. Articles in which the association was evaluated in 20 or more cell lines were included in this study. Finally, we searched MEDLINE for studies on the association between the gene alteration and clinical drug response in patients with lung cancer by using the name of the gene as a keyword. Articles in which the association was evaluated in 25 or more patients with advanced lung cancer were included in this study. Studies in which gene expression was evaluated with microarray were excluded because result analysis and interpretation of this technique have not been established, as indicated by the fact that the list of genes identified by microarray studies was highly variable without overlap between these gene sets.[11,12] Clinical studies on concurrent chemoradiotherapy were excluded. We constructed 2 × 2 tables from the response data and calculated odds ratios (ORs), their variances, and 95% confidence intervals (95% CIs) for the patients who had tumors with gene alteration relative to those who had tumors without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.[13] When a response rate was 0, association with gene alteration was evaluated using the χ^2 test because 95% CIs for ORs cannot be calculated. The name of each gene was standardized according to Human Gene Nomenclature Database of National Center for Biotechnology Information.

RESULTS

Of the 134 genes or gene families found, a gene alteration in drug-induced resistant cells, an increased or decreased resistance in transfected cells, and an altered sensitivity in gene down-regulated cells were reported for 45, 57, and 32 genes, respectively. In total, 80 genes met the definition of "in vitro chemosensitivity associated gene" (Table 1).

Gene alteration was associated with in vitro chemosensitivity in 15 (50%) of 30 studies on 15 (56%) of 27 gene alterations (Table 2). Clinical drug response was evaluated in 27 studies on 13 gene alterations. The methods used to identify gene alteration included immunohistochemical protein expression analysis (n = 18), polymerase chain reaction (PCR)-based mRNA expression analysis (n = 3), and PCR-based mutation analysis (n = 6). All but one clinical study was retrospective, and the median (range) number of patients in each study was 50 (28-108). Gene alteration was associated with clinical response in 8 of the 27 (30%) studies (Table 2).

TABLE 1. In Vitro Chemosensitivity-Associated Genes

Transporters: ABCA2, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCG2, MVP, ATP7A, ATP7B, SLC29A1, SLC28A1, SLC19A1
Drug targets: TUBB, TUBB4, TUBA, TYMS, TOP1, TOP2A, TOP2B, DHFR,
Target-associated proteins : MAP4, MAP7, STMN1, KIF5B, HSPA5, PSMD14, FPGS
Intracellular detoxifiers: GSTP1, GPX, GCLC, GGT2, MT, RRM2, AKR1B1
DNA damage recognition and repair proteins: HMGB1, HMGB2, ERCC1, XPA, XPD, MSH2, MLH1, PMS2, APEX1, MGMT, BRCA1, GLO1
Cell cycle regulators: RB1, GML, CDKN1A, CCND1, CDKN2A, CDKN1B
Mitogenic signal regulators: ERBB2, EGFR, KRAS2, HRAS, RAF1
Survival signal regulators: AKT1, AKT2
Integrin: ITGB1
Transcription factors: JUN, FOS, MYC, NFKB1
Apoptosis regulators: TP53, MDM2, TP73, BCL2, BCL2L1, MCL1, BAX, BIRC4, BIRC5, TNFRSF6, CASP3, CASP8, HSPB1

We evaluated the association between transporter P-glycoprotein/multidrug resistance 1 (ABCB1) expression and clinical chemosensitivity in four studies. The response rate of lung cancer with high ABCB1 expression was consistently 0%, whereas that for lung cancer with low ABCB1 expression was 73 to 85% (Table 3). Among drug targets, only topoisomerase II-beta (TOP2B) expression was associated with clinical drug response in patients with small-cell lung cancer (OR 0.29, 95% CI 0.09-0.95). The intracellular detoxifier glutathione s-transferase pi (GSTP1) was associated with both in vitro and clinical drug response (OR 0.22, 95% CI 0.06-0.79) (Table 4). DNA repair gene excision repair cross-complementing 1 (ERCC1) alterations were associated with drug response among patients with non—small-cell lung cancer with marginal statistical significance; the combined OR (95% CI) for ERCC1 alteration was 0.53 (0.28-1.01; p = 0.055) (Table 5). Tumor suppressor p53 (TP53) mutation was the only alteration associated with drug response among patients with non—small-cell lung cancer among genes involved in cell cycle and apoptosis. A combined OR (95% CI) for TP53 among patients with non—small-cell lung cancer was 0.25 (0.12-0.52) (Table 6). B-cell CLL/lymphoma 2 (BCL2) and its family protein expression was not associated with clinical drug response (Table 7).

DISCUSSION

We identified 80 in vitro chemosensitivity-associated genes in our literature search. Of these, 13 were evaluated clinically in 27 studies; ABCB1, TOP2B, GSTP1, and ERCC1 expression and TP53 mutation were associated with changes to drug responses among patients with lung cancer.

Classical drug resistance is believed to be the result of molecular changes inhibiting the drug-target interaction. ABCB1, an ATP-binding cassette protein, acts as an energy-dependent transmembrane efflux pump and decreases the intracellular accumulation of anticancer drugs, including anthracyclines, vinca alkaloids, taxanes, and epipodophyllotox-

TABLE 2. Chemosensitivity-Associated Genes and Association with Chemosensitivity

		Association with chemosensitivity					
		In vitro studies (*n*)			Clinical studies (*n*)		
Category	No of Genes	Total	Yes	%	Total	Yes	%
Transporter	15	9	5	55	4	4	100
Drug target	8	2	1	50	5	1	20
Target-associated protein	7	0	0		0	0	
Intracellular detoxifier	7	3	3	100	1	1	100
DNA repair	10	1	1	100	6	0	0
Damage recognition protein	2	0	0		0	0	
Cell cycle	6	4	2	50	2	0	0
Mitogenic signal	5	3	1	33	1	0	0
Survival signal	2	0	0		0	0	
Transcription factor	4	3	0	0	0	0	
Cell adhesion-mediated drug-resistance protein	1	0	0		0	0	
Apoptosis	13	5	2	40	8	2	25
Total	80	30	15	50	27	8	30

TABLE 3. ABCB1 (P-Glycoprotein) and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio
Yeh et al.[30]	Non-small cell	Paclitaxel	IHC	Low	35	80	0
				High	15	0	$p < 0.001$*
Kawasaki et al.[31]	Small cell	CAV or EP	IHC	Low	26	85	0
				High	4	0	$p < 0.001$*
Hsia et al.[32]	Small cell	EP	IHC	Low	37	73	0
				High	13	0	$p < 0.001$*
Savaraj et al.[33]	Small cell	CAV, CEV, or EP	RT-PCR	Low	24	75	0
				High	7	0	$p < 0.001$*
Combined odds ratio for ABCB expression in patients with SCLC: 0							

IHC, Immunohistochemical analysis; RR, response rate; RT-PCR, reverse transcriptase-polymerase chain reaction.
*Calculated using the χ^2 test because the confidence interval cannot be calculated.

ins. Overexpression of this protein gives tumor cells a multidrug resistance phenotype in vitro, which is thought to be associated with clinical chemoresistance.[14] Our review showed that the response rate of tumors with ABCB1 overexpression was 0 in all studies of lung cancer, whereas that for lung cancer tumors with low ABCB1 expression was 73 to 85% (Table 3).

There is a close relationship between drug sensitivity and quantitative and qualitative alterations of the drug's target, including tamoxifen sensitivity and estrogen receptor expression and trastuzumab response and Her-2/neu overexpression in breast cancer,[15] imatinib resistance and BCR-ABL gene amplification and mutations in Philadelphia chromosome-positive leukemias,[16] and imatinib response and KIT gene mutations in gastrointestinal stromal tumors.[17] In all of these cases, the target molecule is a receptor or a mutated tyrosine kinase located at the entry of growth-stimulating signal transduction pathways. Recently, gefitinib, a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), has been developed, and two large phase II trials showed a response rate of 18% and 12% in patients with non—small-cell lung cancer who were previously treated with conventional chemotherapy.[18,19] Responses to the drug have been unpredictable, but mutations of the EGFR gene were identified in patients with gefitinib-responsive lung cancer.[20,21] Furthermore, all mutations in these tumors were restricted to the activation loop of the kinase domain of EGFR, which are in distinct contrast to mutations in extracelluar and regulatory domains of EGFR in glioblastoma, which are unresponsive to gefitinib.[22] Thus, molecular developments of structure and function of the targets hold the promise of targeted cancer therapy. The target molecules of many anticancer cytotoxic agents have not been clearly defined; therefore, the relationship between the target molecule status and sensitivity to the agent has not been established. TOP2B expression was associated with drug response in patients with small-cell lung cancer, with a response rate of 71% for high TOP2B expression tumors versus 90% for low TOP2B expression tumors (OR 0.29, 95% CI 0.09-0.95).[23] This result, however, is in contrast with the idea that a higher

TABLE 4. Drug Targets, Intracellular Detoxifier, and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio (95% CI)
Beta-tubulin class III							
Rosell et al.[34]	Non-small cell	Paclitaxel, Vinorelbine	Real-time PCR	Low	13	46	0.39
				High	24	25	(0.09-1.62)
Topoisomerase II-alpha							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	65	85	0.65
				High	23	80	(0.20-2.17)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	30	47	0.67
				High	8	38	(0.14-3.40)
Topoisomerase II-beta							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	48	90	0.29
				High	35	71	(0.09-0.95)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	18	50	0.86
				High	13	46	(0.21-3.58)
Glutathione s-transferase pi							
Nakanishi et al.[36]	Non-small cell	Cisplatin-based	IHC	Low	17	47	0.22
				High	37	16	(0.06-0.79)

CI, confidence interval; IHC, immunohistochemical analysis; PCR, polymerase chain reaction; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.

TABLE 5. DNA Repair Genes and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Alteration	Patients (*n*)	RR (%)	Odd ratio (95% CI)
Excision repair cross-complementing 1 expression							
Lord et al. [37]	Non-small cell	Cisplatin, gemcitabine	Real-time PCR	Low	23	52	0.38
				High	24	36	(0.11-1.26)
Excision repair cross-complementing 1 (ERCC1) polymorphism at codon 118							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	C/C	54	54	0.61
			Hybridization	C/T or T/T	53	42	(0.28-1.31)
Combined odds ratio (95% C.I.) for ERCC1 alteration in patients with NSCLC0.53 (0.28-1.01, $p = 0.055$)							
Xeroderma pigmentosum group D polymorphism							
At codon 231							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	G/G	100	48	1.08
			Hybridization	G/A or A/A	8	50	(0.26-4.57)
At codon 312							
Camps et al.[39]	Non-small cell	Cisplatin, gemcitabine	PCR	G/G	18	17	3.33
			Sequencing	G/A or A/A	15	40	(0.66-16.7)
At codon 751							
Camps et al.[39]	Non-small cell	Cisplatin, gemcitabine	PCR	A/A	22	23	2.04
			Sequencing	A/C or C/C	16	38	(0.49-8.45)
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	A/A	96	49	0.74
			Hybridization	A/C	12	42	(0.22-2.51)
Combined odds ratio (95% CI) for XPD polymorphism in patients with NSCLC: 1.38 (0.68-2.78).							

CI, confidence interval; PCR, polymerase chain reaction; RR, response rate; NSCLC, non—small-cell lung cancer; XPD, xeroderma pigmentosum group D.