Figure 2b

No. of Risk allele	Genotype DQA1	Genotype TERT	Freq[a]	Odds ratio (95%CI) 0 1 2 3 4 5	OR	*P* value
0	other/other	-	0.40		1.0	
1	other/*03	-	0.47		1.49	2.5×10^{-5}
2	*03/*03	-	0.13		1.90	1.8×10^{-6}
0	-	T/T	0.38		1.0	
1	-	T/G	0.48		1.32	3.5×10^{-3}
2	-	G/G	0.14		2.21	4.8×10^{-10}
0	other/other	T/T	0.15		1.0	
1	other/other	T/G	0.19		1.27	0.13
1	*03/other	T/T	0.06		1.44	0.027
2	*03/*03	T/T	0.18		1.97	3.1×10^{-3}
2	*03/other	T/G	0.22		1.99	6.3×10^{-6}
2	other/other	G/G	0.07		2.23	2.3×10^{-4}
3	*03/*03	T/G	0.05		2.42	1.9×10^{-5}
3	*03/ other	G/G	0.06		3.06	2.2×10^{-9}
4	*03/*03	G/G	0.02		4.76	4.2×10^{-7}
Per risk-allele				◊	1.43	7.8×10^{-16}
P value for interaction						0.88

[a]Frequency in controls.

Supplementary Table I. Inflation factors for the 1st and 2nd stages of GWAS

Repeat unit	1st stage		2nd stage	
	No. of markers	Inflation factor[a]	No. of markers	Inflation factor[a]
All	23,010	0.639	1,328	-
2-bp	16,545	0.520	897	-
3-bp	1,295	0.919	86	0.812
4-bp	4,502	0.958	303	1.026
5-bp	648	1.022	41	1.265
6-bp	20	0.947	1	-
3~6-bp	6,465	0.955	431	1.010

[a]The mean of the lower 90% of the test statistics (-log$P$ values by Fisher's exact test) divided by the mean of the lower 90% of the expected values.

Supplementary Table II. Linkage disequilibrium between the D6S0067i polymorphisms and SNPs/alleles in the 6p21.31 locus

SNP/Allele	Controls		ADC cases	
	D'	R^2	D'	R^2
rs17426593	0.516	0.225	0.603	0.349
rs34843907	0.531	0.152	0.688	0.280
DQA1_2_145	0.514	0.223	0.607	0.352
DQA1_2_150	0.531	0.152	0.689	0.283
DQA1*01	0.531	0.152	0.689	0.283
DQA1*03	0.516	0.225	0.607	0.352

Supplementary Table III. Linkage disequilibrium between exonic and intronic *HLA-DQA1* SNPs among case and control populations

Population	DQA1_2_145 and rs17426593		DQA1_2_150 and rs34843907	
	D'	R^2	D'	R^2
Controls	1.000	0.983	1.000	0.996
ADC cases	1.000	0.996	0.996	0.992

Supplementary Table IV. Correlation coefficients of HLA-*DQA1* alleles determined by exonic SNPs and intronic SNPs

Population	R^2	
	DQA1*01	DQA1*03
Controls	1.000	0.983
ADC cases	0.996	0.996

Supplementary Table V. ORs of the DQA1*03 and DQA1*01 alleles for lung cancer risk

Allele	Category	Histological type	Subgroup	No.		Crude			Adjusted		
				Case	Control[a]	OR	(95% CI)	*P* value	OR	(95% CI)	*P* value
DQA1*03	NCCH	ADC		1,656	1,173	1.35	(1.21 - 1.51)	5.6×10^{-8}	1.36 [b]	(1.20 - 1.54)	5.3×10^{-7}
			Smoker	896	363	1.46	(1.22 - 1.75)	2.9×10^{-5}	1.48 [c]	(1.23 - 1.77)	2.7×10^{-5}
			Non-smoker	760	610	1.27	(1.09 - 1.48)	0.0022	1.27 [c]	(1.08 - 1.50)	0.0045
			Male	924	675	1.35	(1.16 - 1.57)	1.4×10^{-4}	1.39 [d]	(1.18 - 1.65)	9.7×10^{-5}
			Female	732	498	1.27	(1.09 - 1.49)	0.0021	1.35 [d]	(1.12 - 1.61)	0.0012
		SQC		390	1,173	1.27	(1.08 - 1.50)	0.0047	1.40 [b]	(1.13 - 1.74)	0.0024
		SCC		297	1,173	1.17	(0.98 - 1.41)	0.089	1.22 [b]	(0.97 - 1.54)	0.095
	NNGH	ADC		84	145	1.57	(1.07 - 2.30)	0.022	1.70 [b]	(1.14 - 2.53)	0.0087
		SQC		52	145	2.07	(1.32 - 3.27)	0.0016	2.13 [b]	(1.31 - 3.49)	0.0023
DQA1*01	NCCH	ADC		1,656	1,173	0.78	(0.70 - 0.87)	6.0×10^{-6}	0.77 [b]	(0.68 - 0.87)	1.4×10^{-5}
			Smoker	896	363	0.78	(0.65 - 0.92)	0.0040	0.78 [c]	(0.65 - 0.93)	0.0051
			Non-smoker	760	610	0.78	(0.67 - 0.91)	0.0016	0.77 [c]	(0.65 - 0.90)	0.0014
			Male	924	675	0.83	(0.72 - 0.96)	0.0150	0.82 [d]	(0.69 - 0.96)	0.0150
			Female	732	498	0.74	(0.62 - 0.87)	4.6×10^{-4}	0.72 [d]	(0.60 - 0.86)	2.8×10^{-4}
	NNGH	ADC		84	145	0.77	(0.52 - 1.13)	0.18	0.73 [b]	(0.49 - 1.09)	0.12

[a]Information on smoking was not available for 200 subjects.

[b]Adjusted for age, sex and smoking.

[c]Adjusted for age and sex.

[d]Adjusted for sex and smoking.

Supplementary Table VI. Differences in the allele distribution of SNPs in lung cancer susceptibility loci identified by GWASs

Allele	Chromosomal location	Position	Gene	Category	Minor allele frequency		OR (95% CI, *P*)
					Control	Case	
rs2736100-G	5p15.33	1339516	*TERT* (intron 2)	All	0.377	0.444	1.38 (1.23 - 1.56, 6.3×10^{-8})[a]
				ADC		0.465	1.46 (1.30 - 1.65, 6.6×10^{-10})[a]
				SQC		0.382	0.95 (0.77 - 1.19, 0.68)[a]
				SCC		0.407	1.07 (0.85 - 1.35, 0.58)[a]
				Non-smoker	0.380	0.470	1.47 (1.25 - 1.73, 4.1×10^{-6})[b]
				Smoker	0.372	0.431	1.29 (1.08 - 1.53, 4.2×10^{-3})[b]
rs401681-T	5p15.33	1375087	*CLPTM1L* (intron 13)	All	0.334	0.312	0.88 (0.78 - 0.99, 0.044)[a]
				ADC		0.314	0.89 (0.79 - 1.01, 0.077)[a]
				SQC		0.300	0.88 (0.70 - 1.10, 0.27)[a]
				SCC		0.315	0.87 (0.68 - 1.11, 0.27)[a]
				Non-smoker	0.333	0.297	0.81 (0.68 - 0.96, 0.014)[b]
				Smoker	0.336	0.319	0.96 (0.80 - 1.15, 0.65)[b]
rs1051730-T	15q25.1	76681394	*CHRNA3* (Y215Y)	All	0.015	0.032	1.79 (1.19 - 2.78, 9.5×10^{-3})[a]
				ADC		0.030	1.72 (1.14 - 2.69, 9.5×10^{-3})[a]
				SQC		0.033	2.29 (1.14 - 4.72, 0.020)[a]
				SCC		0.037	2.22 (1.09 - 4.58, 0.027)[a]
				Non-smoker	0.015	0.030	1.65 (0.94 - 3.02, 0.083)[b]
				Smoker	0.017	0.033	1.94 (1.09 - 3.79, 0.023)[b]

[a]Adjusted for sex, age and smoking.

[b]Adjusted for sex and age.

Supplementary Table VII. ORs for genotypes of the *HLA-DQA1, TERT* and *CHRNA3* loci

Locus	Number of risk allele	Genotype	Control (%)	Case (%)	OR* (95% CI)	*P*
HLA-DQA1	0	other/other	389 (40.2)	509 (30.7)	Reference	
	1	*03/other	455 (47.0)	859 (51.9)	1.49 (1.24 - 1.79)	2.5×10^{-5}
	2	*03/*03	124 (12.8)	288 (17.4)	1.90 (1.45 - 2.48)	1.8×10^{-6}
rs2736100 (TERT)	0	T/T	373 (38.5)	488 (29.5)	Reference	
	1	T/G	460 (47.5)	796 (48.0)	1.32 (1.10 - 1.60)	3.5×10^{-3}
	2	G/G	135 (14.0)	372 (22.5)	2.21 (1.72 - 2.86)	4.8×10^{-10}
rs1051730 (CHRNA3)	0	G/G	939 (97.0)	1,558 (94.1)	Reference	
	1	G/A	28 (2.9)	95 (5.7)	1.82 (1.18 - 2.89)	6.4×10^{-3}
	2	A/A	1 (0.1)	3 (0.2)	0.94 (0.11 - 19.0)	0.960

[a]Adjusted for sex, age and smoking.

Supplementary Table VIII. Risk of combined *HLA-DQA1, TERT* and *CHRNA3* genotypes for lung adenocarcinoma

Number		Genotype		Control (%)		Case (%)		OR* (95% CI)			*P*
Gene	Risk allele										
2		rs2736100 (*TERT*)	*03 (HLA-DQA1)								
	0	T/T	other/other	148	(15.3)	149	(9.0)	Reference			
	1	T/G	other/other	188	(19.4)	249	(15.0)	1.27	(0.93	- 1.74)	0.13
	1	T/T	*03/other	176	(18.2)	243	(14.7)	1.44	(1.04	- 1.99)	0.027
	2	T/T	*03/*03	49	(5.1)	96	(5.8)	1.97	(1.25	- 3.13)	3.1×10^{-3}
	2	T/G	*03/other	212	(21.9)	415	(25.1)	1.99	(1.47	- 2.70)	6.3×10^{-6}
	2	G/G	other/other	53	(5.5)	111	(6.7)	2.23	(1.45	- 3.45)	2.3×10^{-4}
	3	T/G	*03/*03	60	(6.2)	132	(8.0)	2.42	(1.61	- 3.68)	1.9×10^{-5}
	3	G/G	*03/other	67	(6.9)	201	(12.1)	3.06	(2.11	- 4.48)	2.2×10^{-9}
	4	G/G	*03/*03	15	(1.5)	60	(3.6)	4.76	(2.53	- 9.47)	4.2×10^{-7}
Per risk-allele								1.43	(1.31	- 1.56)	7.8×10^{-16}
P value for interaction											0.88
2		rs2736100 (*TERT*)	rs1051730 (*CHRNA3*)								
	0	T/T	G/G	362	(37.4)	457	(39.7)	Reference			
	1 or 2		G/A + A/A	11	(1.1)	31	(2.7)	1.73	(0.84	- 3.80)	0.14
	1	T/G	G/G	445	(46.0)	749	(65.0)	1.32	(1.09	- 1.60)	4.5×10^{-3}
	2 or 3		G/A + A/A	15	(1.5)	47	(4.1)	2.40	(1.30	- 4.69)	4.9×10^{-3}
	2	G/G	G/G	132	(13.6)	352	(30.6)	2.22	(1.71	- 2.88)	9.3×10^{-10}
	3 or 4		G/A + A/A	3	(0.3)	20	(1.7)	4.27	(1.38	- 18.8)	9.9×10^{-3}
Per risk-allele								1.48	(1.33	- 1.66)	3.9×10^{-11}
P value for interaction											0.73
2		*03 (HLA-DQA1)	rs1051730 (*CHRNA3*)								
	0	other/other	G/G	380	(39.3)	470	(40.8)	Reference			
	1	*03/other	G/G	440	(45.5)	809	(70.2)	1.53	(1.26	- 1.84)	1.1×10^{-5}
	2 or 3		G/A + A/A	15	(1.5)	50	(4.3)	2.47	(1.36	- 4.74)	2.5×10^{-3}
	2	*03/*03	G/G	119	(12.3)	279	(24.2)	2.01	(1.54	- 2.65)	2.9×10^{-7}
	3 or 4		G/A + A/A	5	(0.5)	9	(0.8)	1.37	(0.44	- 4.77)	0.59
Per risk-allele								1.35	(1.21	- 1.50)	5.1×10^{-7}
P value for interaction											0.083
3											
	0			144	(14.9)	132	(8.0)	Reference			
	1			356	(36.8)	479	(28.9)	1.45	(1.08	- 1.94)	0.013
	2			319	(33.0)	619	(37.4)	2.15	(1.60	- 2.88)	3.0×10^{-7}
	3			127	(13.1)	349	(21.1)	3.11	(2.24	- 4.35)	9.3×10^{-12}
	4			22	(2.3)	73	(4.4)	4.16	(2.39	- 7.50)	2.0×10^{-7}
	5			0	(0)	4	(0.2)	-	(-	- -)	-
	6			0	(0)	0	(0)	-	(-	- -)	-
Per risk-allele								1.45	(1.40	- 1.50)	2.5×10^{-17}

[a]Adjusted for sex, age and smoking.

Supplementary Table IX. Association of 10 SNPs commonly analyzed in the present and other GWASs with lung cancer risk

SNP	Genome location	Gene	Position	Allele	Minor allele frequency		Allele OR		*P* value	
					Japanese*	Others	Japanese[a]	Others	Japanese*	Others
rs7192	32,519,624	*HLA-DRA*	exon 4	G/T	0.444	0.370	0.76	0.98 [b]	0.0016	0.65
rs3129763	32,698,903			G/A	0.064	0.270	1.10	1.14 [b]	0.60	0.0048
								1.11 [e]		3.6×10^{-7}
rs2187668	32,713,862	*HLA-DQA1*	intron 1	G/A	0.033	0.100	1.21	1.27 [b]	0.42	5.0×10^{-4}
								1.22 [c]		2.3×10^{-6}
								1.18 [e]		6.9×10^{-10}
rs2647012	32,772,436			G/A	0.214	0.330	0.61	1.00 [b]	2.3×10^{-5}	0.95
rs1794282	32,774,504			G/A	0.000	0.080	-	1.29 [b]	-	8.0×10^{-4}
								1.20 [c]		7.2×10^{-5}
								1.26 [d]		6.0×10^{-5}
								1.20 [e]		6.7×10^{-10}
rs2239800	32,821,245	*HLA-DQA2*	intron 2	T/C	0.289	0.130	1.01	0.95 [b]	0.88	0.47
rs1573649	32,839,236			T/C	0.426	0.420	0.81	1.01 [b]	0.021	0.82
rs2071469	32,892,761	*HLA-DOB*	5'UTR	G/A	0.424	0.430	1.20	1.00 [b]	0.037	0.95
rs1057373	32,921,257	*TAP1*	3'UTR	G/T	0.105	0.080	1.18	0.98 [b]	0.23	0.78
rs17587	32,933,068	*PSMB9*	exon 3	G/T	0.243	0.280	0.90	1.05 [b]	0.33	0.33

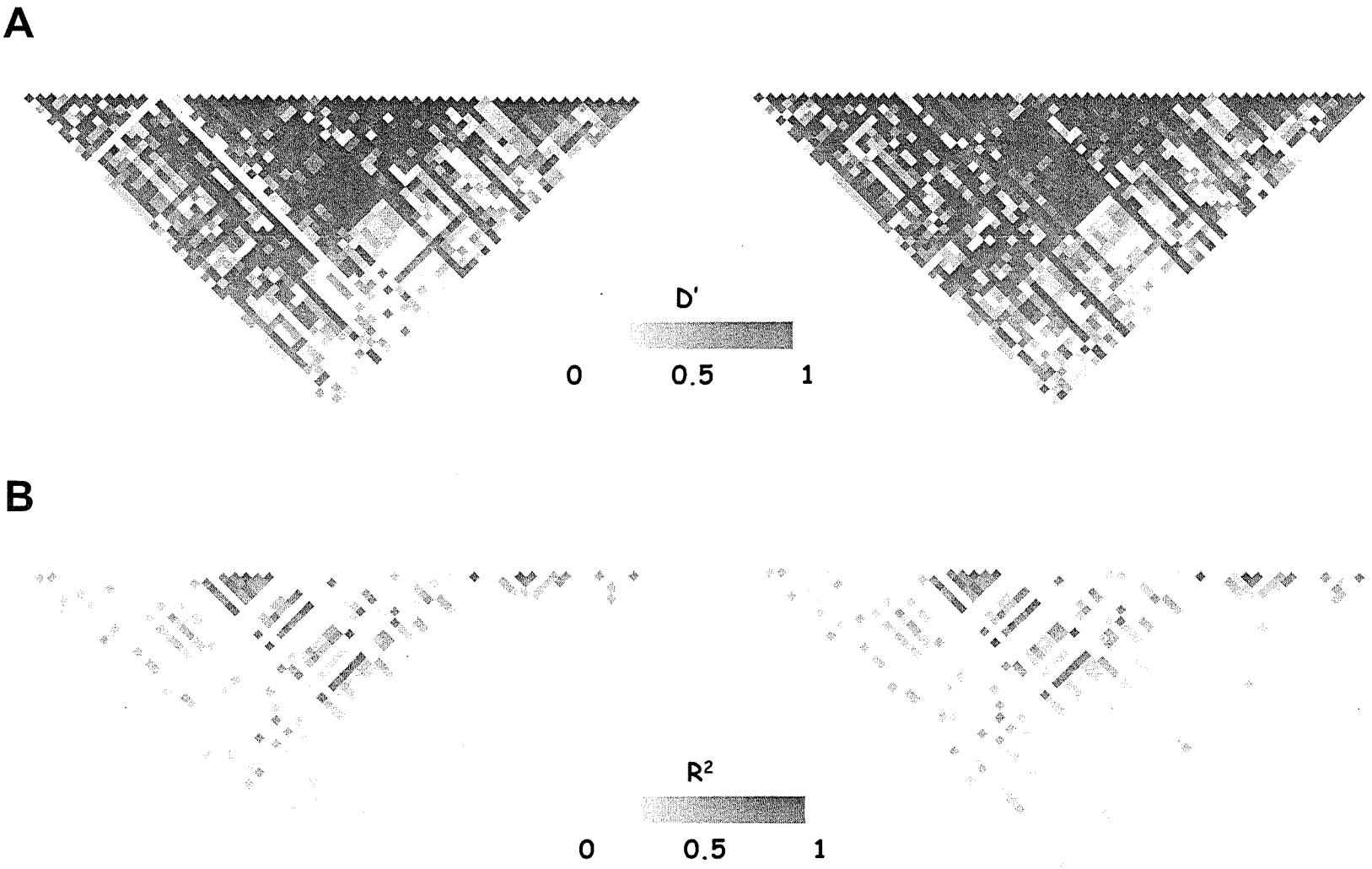
[a]Association with lung adenocarcinoma risk on 525 cases and 525 controls.

[b]Association with lung cancer risk on 1,989 cases and 2,625 controls in European countries[8].

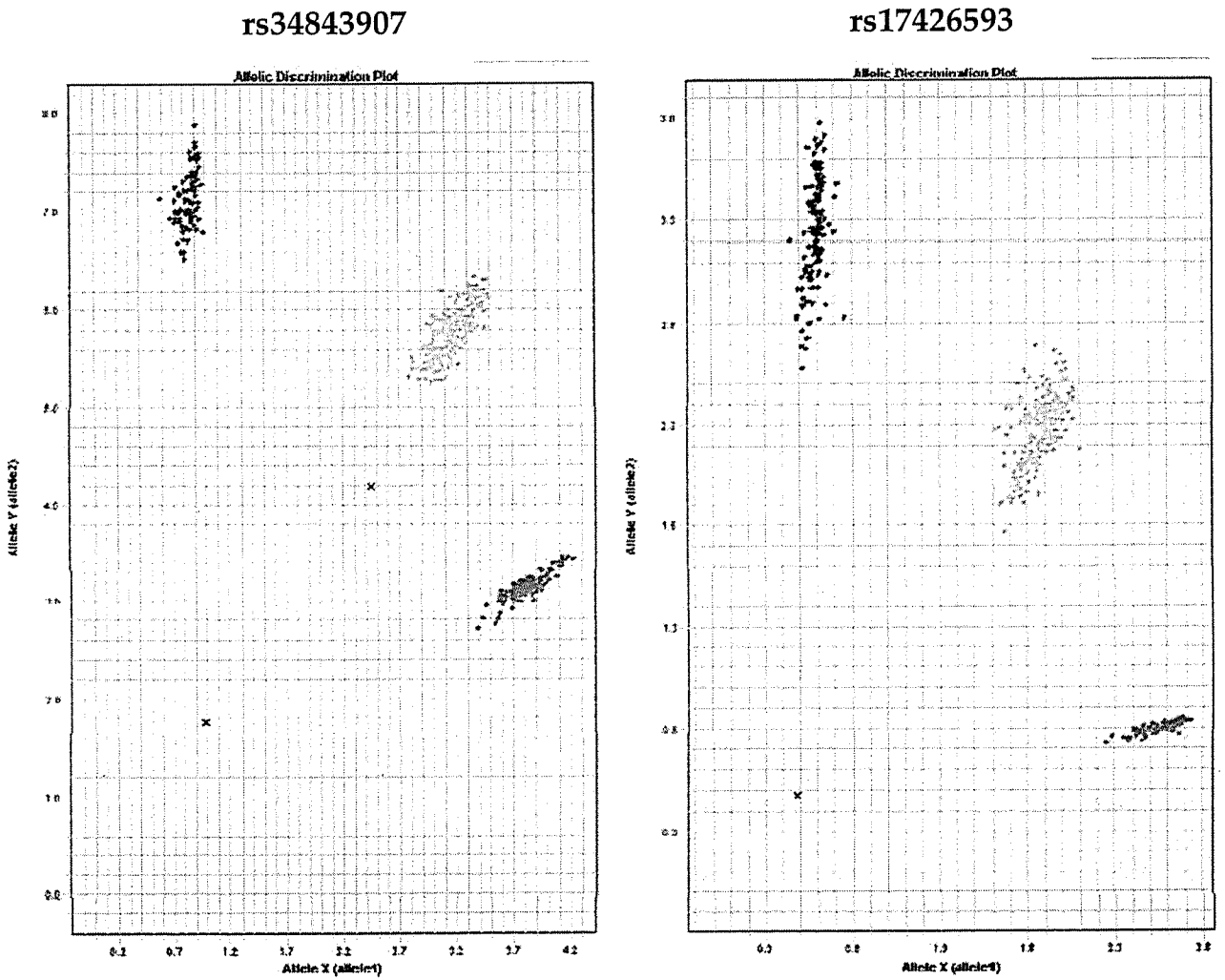
[c]Association with lung cancer risk on 5,095 cases and 5,200 controls in European countries and USA[4].

[d]Association with lung cancer risk on 2,971 cases and 3,746 controls in European countries, Canada and USA[5].

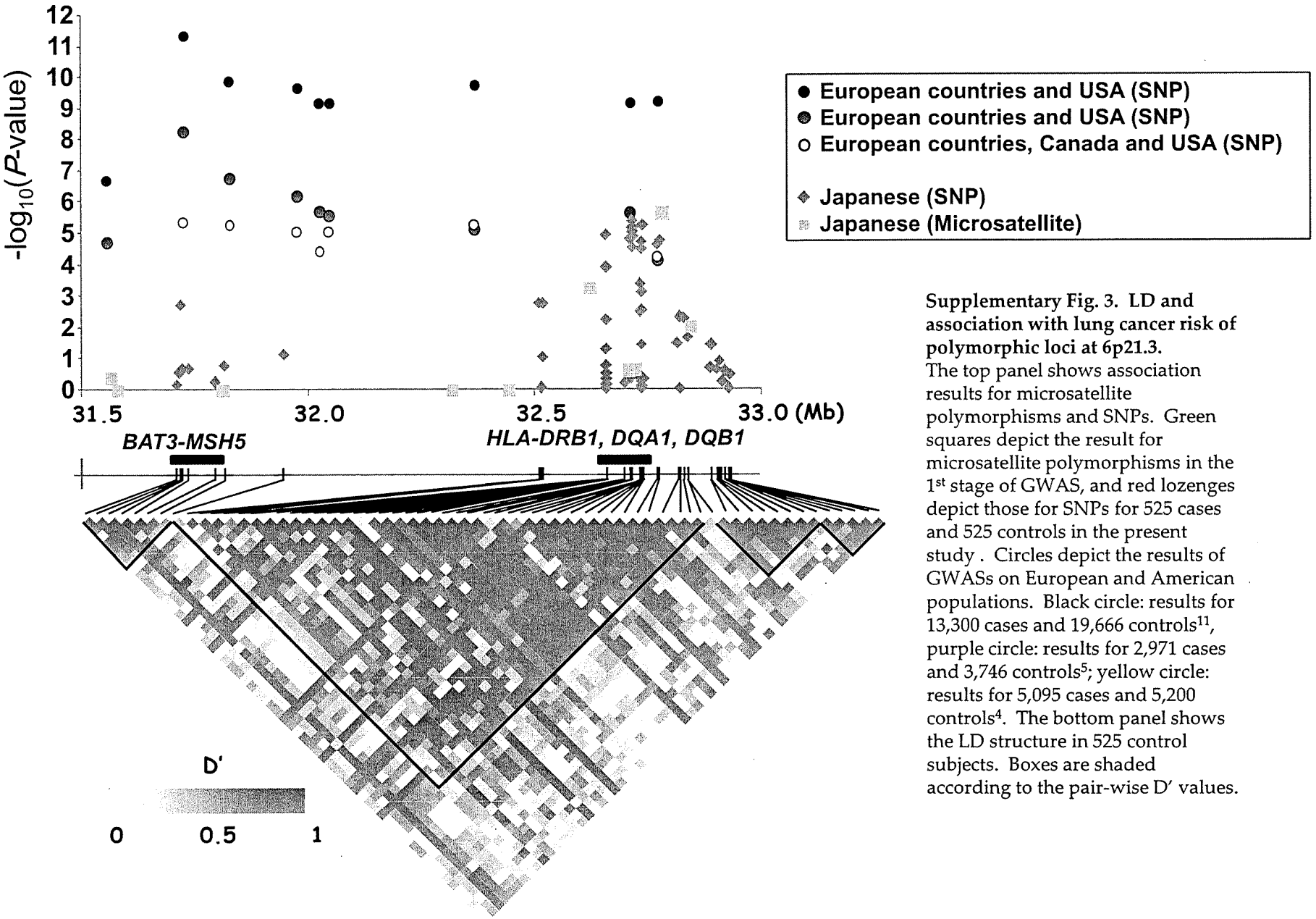
[e]Association with lung cancer risk on 13,300 cases and 19,666 controls in European countries and USA[11].



Supplementary Fig. 1. Linkage disequilibrium among 55 SNPs in the 6p21.31 locus.
(A) D′ value. (B) R^2 value. Results in 525 cases (left) and 525 controls (right) are shown. Boxes are shaded according to the pair-wise D′ or R^2 values. A SNP, DRB1_2_61, was monomorphic in the cases, therefore, D′ and R^2 values were not plotted in the cases.



Supplementary Fig. 2. The Taqman cluster plots for the rs34843907 and rs17426593 SNPs.
Left: rs34843907. Red: homozygotes for the A allele; Green: heterozygotes; Blue: homozygotes for the C allele.
Right: rs17426593. Red: homozygotes for the C allele; Green: heterozygotes; Blue: homozygotes for the T allele.
Undetermined genotypes are labeled X.



Supplementary Fig. 3. LD and association with lung cancer risk of polymorphic loci at 6p21.3.
The top panel shows association results for microsatellite polymorphisms and SNPs. Green squares depict the result for microsatellite polymorphisms in the 1st stage of GWAS, and red lozenges depict those for SNPs for 525 cases and 525 controls in the present study . Circles depict the results of GWASs on European and American populations. Black circle: results for 13,300 cases and 19,666 controls[11], purple circle: results for 2,971 cases and 3,746 controls[5]; yellow circle: results for 5,095 cases and 5,200 controls[4]. The bottom panel shows the LD structure in 525 control subjects. Boxes are shaded according to the pair-wise D′ values.

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Re-challenge chemotherapy for relapsed non-small-cell lung cancer

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ABSTRACT

There has been no report about re-challenge chemotherapy (RC) consisting of the same regimen as first-line chemotherapy in non-small-cell lung cancer (NSCLC). The aim of this study was to evaluate the efficacy of RC as second-line chemotherapy in patients with relapsed NSCLC. We conducted a retrospective review of 28 consecutive NSCLC patients who were treated with RC and compared their clinical outcomes with those of 38 consecutive NSCLC patients who were treated with docetaxel (DOC) at our hospital between July 1992 and December 2003. The RC group consisted of 21 men and 7 women, with a median age of 62 years (range, 42–76 years). Most first-line regimens were platinum-based and the median administered course was 3 (range, 2–7). All patients had responded to the first-line chemotherapy and had performance status (PS) 1 at relapse. The median interval from the end of first-line chemotherapy to relapse was 5.0 months (range, 1.6–36.1 months). The overall response rate of RC was 29%. The median survival time from the beginning of RC was 17.0 months and the 1-year survival rate was 60%. RC led to a significantly better overall survival rate than DOC ($p = 0.0342$). RC could be an active second-line regimen in patients with relapsed NSCLC who responded to first-line chemotherapy.

1. Introduction

Lung cancer is one of the most common causes of death from cancer worldwide. Non-small-cell lung cancer (NSCLC) accounts for at least 80% of all lung cancer cases and about 65–80% of NSCLC patients present with locally advanced or metastatic disease [1]. Today, standard first-line chemotherapy for advanced NSCLC patients with a good performance status (PS) is considered to be the platinum-based doublet regimen [2,3]. In a Japanese phase III study comparing four different platinum doublet regimens, response rates of 30–33%, and median survival time (MST) of 11–14 months were reported [2].

The prognosis of patients who have relapsed or have refractory NSCLC after first-line chemotherapy and did not receive additional therapy is abysmal: MST after relapse was reported to be only 3 months [4]. Some cytotoxic agents, such as docetaxel (DOC) [5,6] and pemetrexed [7], or molecular target agents, such as gefitinib [8] and erlotinib [9], are active in the second-line or third-line setting; however, further progress is needed in the treatment of relapsed NSCLC.

In general, relapsed tumors are thought to have acquired resistance to previously administered drugs [10,11]; however, we consider that some relapsed tumors of NSCLC might still be sensitive to the prior chemotherapy, as in sensitive relapse in small-cell lung cancer (SCLC). In this study, we conducted a retrospective review of 28 consecutive NSCLC patients who received re-challenge chemotherapy (RC) as second-line chemotherapy and compared their clinical outcomes with those of 38 consecutive NSCLC patients who were treated with DOC, which has been used as standard second-line chemotherapy in Japan, at our hospital to evaluate the efficacy of RC for relapsed NSCLC.

2. Materials and methods

2.1. Patients

Between July 1992 and December 2003, 3934 consecutive NSCLC patients were treated at the National Cancer Center Hospital East, Japan, including 579 patients who had received second-line chemotherapy. In this study, we conducted a retrospective review of the 28 consecutive patients who underwent RC and the 38 consecutive patients who had responded to first-line therapy and received DOC as the second-line therapy, and compared the clinical outcomes between the two treatment groups. In both the groups, patients received second-line chemotherapy after they experienced disease progression. All patients in the RC group had Eastern Cooperative Oncology Group (ECOG) PS 1; therefore, DOC group patients were restricted to PS ≤1 to balance patient backgrounds.

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Table 1
Patient characteristics.

Characteristics	RC (n=28)	DOC (n=38)	p value
Age (years)			
Median	62	67	0.388
Range	42–76	47–77	
Gender (%)			
Male	21(75)	33(87)	0.333
Female	7(25)	5(13)	
Smoking status (%)			
Non-smoker	4(14)	4(11)	0.714
Smoker	24(86)	34(89)	
PS (ECOG) (%)			
0	0(0)	4(11)	0.131
1	28(100)	34(89)	
Clinical stage (%)			
IIB	0(0)	2(5)	0.029
IIIA	1(4)	7(18)	
IIIB	14(50)	22(58)	
IV	13(46)	7(18)	
Histology (%)			
Ad	18(64)	16(42)	0.087
Sq	7(25)	17(45)	
Others	3(11)	5(13)	

RC, re-challenge chemotherapy; DOC, docetaxel; PS, performance status; ECOG, Eastern Cooperative Oncology Group; Ad, adenocarcinoma; Sq, squamous cell carcinoma.

2.2. Treatment and response assessment

RC was defined as the same chemotherapy regimen as the first-line chemotherapy. The Response Evaluation Criteria in Solid Tumors (RECIST) [12] was used to evaluate the response of patients and objective tumor response was assessed as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). This response assessment was based on the disease progressive state after first-line therapy in both the groups.

2.3. Statistical analysis

All statistical analyses were performed with SPSS 11.0 statistical software (Dr. SPSS II for Windows, Standard version 11.0; SPSS Inc., Chicago, IL). Differences in patient characteristics between groups were tested for significance using the χ^2 test or Fisher's exact test, as appropriate, and the Mann–Whitney U-test was used to compare the number of courses and intervals. Overall survival (OS) was measured from the start of second-line chemotherapy to the date of death from any cause or the date that patients were last known to be alive. Survival rates were calculated by the Kaplan–Meier method, and the statistical significance of any difference in OS was evaluated by the log-rank test. p values <0.05 were considered significant.

3. Results

3.1. Patient characteristics

Patient characteristics are shown in Table 1. All clinical data were retrieved from medical records. The two treatment groups were well balanced for age, gender, smoking status, and PS, with the exception of histology and clinical stage: the RC group had more advanced stages (p=0.029) and tended to have more adenocarcinoma than the DOC group (p=0.087).

The majority of patients were treated with a platinum-based regimen as first-line chemotherapy and only two patients in each group had received non-platinum chemotherapy (Table 2). All patients in both groups had responded (PR/CR) to first-line chemotherapy. The median number of cycles of first-line chemotherapy was 3 in each group and the median interval from the end of first-line chemotherapy to relapse was 5.0 months (range, 1.6–36.1 months) in the RC group and 7.6 months (range, 0.7–41.1 months) in the DOC group (p=0.165).

Table 2
Details of first-line chemotherapy.

	RC (n=28)	DOC (n=38)	p value
Course			
Median	3	3	0.098
Range	2–7	1–6	
Regimen, cases	10	4	
CDDP+MMC+VDS			
CDDP+VDS	7	2	
CDDP+VNR	3	28	
CDDP+DOC	3	0	
CDDP+GEM	1	0	
CDDP+CPT	1	2	
CBDCA+PTX	1	0	
GEM+VNR	2	1	
VNR	0	1	
Response, cases			
CR	1	1	1.000
PR	27	37	
Interval from the end of first-line chemotherapy to relapse, months			
Median	5.0	7.6	0.165
Range	1.6–36.1	0.7–41.1	

RC, re-challenge chemotherapy; DOC, docetaxel; CDDP, cisplatin; MMC, mitomycin C; VDS, vindesine; VNR, vinorelbine; GEM, gemcitabine; CPT, camptothecin; CBDCA, carboplatin; PTX, paclitaxel; CR, complete response; PR, partial response.

Patients in the RC group received a median of 1.5 cycles (range, 1–3) of RC, whereas 2.0 cycles (range, 1–10) of DOC in the DOC group. One patient in the RC group discontinued RC with cisplatin and docetaxel at 1 cycle because of severe allergy and no patients died of toxicity from RC. The proportion of patients who received third-line and fourth-line chemotherapy was well balanced between the two treatment groups (Table 3).

3.2. Response and survival

Response and survival in each group are shown in Table 4, and the Kaplan–Meier curve for overall survival is shown in Fig. 1. The median follow-up time was 20.4 months. In the RC group, 20 patients died during the follow-up period. One patient died of

Table 3
Additional chemotherapy after re-challenge chemotherapy and docetaxel.

	RC (n=28)	DOC (n=38)
Third-line chemotherapy		
CDDP+VNR	2(8)	0(0)
DOC	3(11)	0(0)
Gefitinib	3(11)	8(21)
Erlotinib	0(0)	1(3)
GEM	0(0)	4(11)
GEM+VNR	1(3)	2(5)
Total (%)	9(32)	15(40)
Fourth-line chemotherapy		
DOC	1(3)	0(0)
Gefitinib	2(8)	1(3)
GEM+VNR	0(0)	1(3)
GEM+DOC	1(3)	0(0)
CBDCA+PTX	0(0)	1(3)
Total (%)	4(14)	3(8)

RC, re-challenge chemotherapy; DOC, docetaxel; CDDP, cisplatin; VNR, vinorelbine; GEM, gemcitabine; CBDCA, carboplatin; PTX, paclitaxel.

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Table 4
Treatment efficacy of re-challenge chemotherapy and docetaxel.

	RC (n=28)	DOC (n=38)	p value
Response (%)			
Overall response	8(29)	3(7.9)	0.043
CR	0	0	
PR	8	3	
SD	13	16	
PD	7	19	
Survival			
Median, months	17.0	9.0	0.0342
Range	0.4–43.0	1.3–31.4	
1-Year survival	60	29	

RC, re-challenge chemotherapy; DOC, docetaxel; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

unknown cause after receiving chemotherapy with cisplatin, mitomycin C, and vindesine (CMV), and 19 patients died of lung cancer. The overall response rate was 29% in the RC group and 8% in the DOC group, respectively (p = 0.043). The median survival time (MST) and 1-year survival rate from the beginning of second-line chemotherapy were 17.0 months and 60% in the RC group, and 9.0 months and 29% in the DOC group, respectively. The OS of the RC group was significantly better than that of the DOC group (p = 0.0342).

4. Discussion

To our knowledge, this is the first report to evaluate the efficacy of RC in relapsed NSCLC patients, demonstrating an excellent response rate and survival.

DOC is a standard second-line chemotherapy regimen, and has been most widely used in Japan. A randomized phase III study comparing DOC with best supportive care showed better OS for DOC patients (7.5 months vs. 4.6 months, p = 0.047) [6]. Another phase III study showed that the 1-year survival rate of patients who received DOC 75 mg/m^2 was significantly better than that of patients who received vinorelbine or ifosfamide (32% vs. 19%; p = 0.025) [5]; however, these studies included patients who had not responded to first-line chemotherapy and patients who had PS 2.

In this study, we also conducted a retrospective review of patients who had received DOC as the second-line chemotherapy under the same conditions, namely, patients with PS 0–1 and complete or partial response to prior therapy in the same period. The MST and 1-year survival rate in the DOC group were 9.0 months and 29%, respectively. These survival data were comparable to those demonstrated in the phase III studies above. By contrast, the RC group showed MST of 17.0 months and a 60% 1-year survival rate. These were significantly better than in the DOC group, although more stage IV patients were included in the RC group. The RC group tended to include more adenocarcinoma patients; however, subsequent treatments were similar between groups and the proportion of patients administered gefitinib was almost the same. These results suggested that RC had a sufficient anti-tumor effect and could be an effective second-line regimen for certain types of relapsed NSCLC.

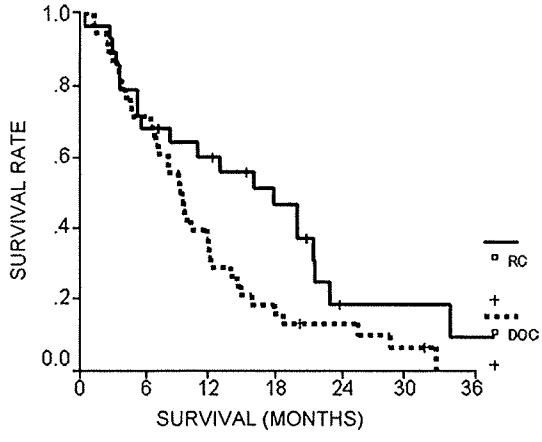

Fig. 1. Kaplan–Meier curve for overall survival. Overall survival in the re-challenge chemotherapy (RC) group was significantly better than in the docetaxel (DOC) group (log-rank test, p = 0.0342).

The interval from first-line to second-line chemotherapy was quite variable in both the groups. Therefore, limiting the patients whose treatment-free interval of more than 6 months, MST was 21.4 months in the RC group (n = 11), and 9.5 months in the DOC group (n = 23), respectively (p = 0.0110).

Drug resistance is considered a major limitation of chemotherapy. Resistance to anticancer drugs is most often ascribed to gene mutations, gene amplification, or epigenetic changes that influence the uptake, metabolism, or export of drugs from a single agent [13]. Although a detailed explanation for re-induction has not been presented, the observation of SCLC cell line resistance during exposure to doxorubicin that disappeared after drug withdrawal provides some suggestions [14]. In a group of SCLC patients, 37 responded to first-line treatment, resulting in 6 CR and 17 PR by RC treatment [15]. In a study by Giaccone et al. of a group of 13 patients with a response duration of 30 weeks or longer, RC at relapse resulted in 6 patients having a second response [16]. These results illustrate that RC was effective in sensitive SCLC patients and encouraged us to evaluate the same phenomenon; that is, some tumors had less drug resistance and were sensitive to their previous anticancer drugs during chemotherapy for NSCLC.

The results should be interpreted with caution because there might be a potential imbalance of prognostic factors between the groups. Nevertheless, this study may suggest that RC has potential to become a treatment option for relapsed NSCLC patients if the previous chemotherapy had been effective and relapsed patients maintained good PS.

Conflict of interest statement

We have no conflict of interest and financial support to declare.

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Antitumor Activity of NK012 Combined with Cisplatin against Small Cell Lung Cancer and Intestinal Mucosal Changes in Tumor-Bearing Mouse after Treatment

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Abstract

Purpose: To investigate the advantages of treatment with the SN-38–incorporating polymeric micelles NK012 over CPT-11 in combination with cisplatin [*cis*-dichlorodiammineplatinum (II) (CDDP)] in mice bearing a small cell lung cancer xenograft in terms of antitumor activity and toxicity, particularly intestinal toxicity.

Experimental Design: Cytotoxic effects were evaluated in human small cell lung cancer cell lines [H69, H82, and vascular endothelial growth factor (VEGF)–secreting cells (SBC-3/VEGF and its mock transfectant SBC-3/Neo)]. *In vivo* antitumor effects were evaluated in SBC-3/Neo–bearing and SBC-3/VEGF–bearing mice after NK012/CDDP or CPT-11/CDDP administration on days 0, 7, and 14. Drug distribution was analyzed by high-performance liquid chromatography or fluorescence microscopy, and the small intestine was pathologically examined.

Results: The *in vitro* growth-inhibitory effects of NK012 were 198- to 532-fold more potent than those of CPT-11. A significant difference in the relative tumor volume on day 30 was found between NK012/CDDP and CPT-11/CDDP treatments ($P = 0.0058$). Inflammatory changes in the small intestinal mucosa were rare in all NK012-treated mice but were commonly observed in CPT-11–treated mice. Moreover, a large amount of CPT-11 was excreted into the feces and high CPT-11 concentration was detected in the small intestinal epithelium. On the other hand, a small amount of NK012 was found in the feces and NK012 was weakly and uniformly distributed in the mucosal interstitium.

Conclusions: NK012/CDDP combination may be a promising candidate regimen against lung cancer without severe diarrhea toxicity and therefore warrants further clinical evaluation.

SN-38 or 7-ethyl-10-hydroxy-camptothecin is a biologically active metabolite of irinotecan hydrochloride (CPT-11) and is formed through CPT-11 conversion by carboxylesterases. SN-38 is active against various human cancers, such as colorectal, lung, and ovarian cancer (1–4). Although SN-38 shows up to 1,000-fold more potent cytotoxic activity against various cancer cell lines than CPT-11 *in vitro* (5), it has been clinically unavailable because of its water-insoluble nature, and the conversion rate from CPT-11 to SN-38 is <10% of the original CPT-11 volume in the body (6, 7).

The SN-38–incorporating polymeric micelles NK012 seem to have the advantage of passive targeting of the drug delivery system. In this passive targeting of drug delivery system, the drug accumulates in tumor tissue by using the enhanced permeability and retention effect (8–11). This enhanced permeability and retention effect is based on several pathologic mechanisms, which include hypervascularity, secretion of tumor vascular permeability factors stimulating extravasation of macromolecules including nanoparticles such as liposomes and micelles, and the absence of an effective lymphatic drainage of macromolecules accumulated in solid tumor tissue. Recent studies showed that NK012 has a significantly more potent antitumor activity than CPT-11 against small cell lung cancer (SCLC; ref. 12), colorectal cancer (13), renal cancer (14), pancreatic cancer (15), stomach cancer (16), and glioma (17).

It was previously reported that the SN-38/*cis*-dichlorodiammineplatinum (II) (CDDP) combination showed synergistic effects (18). The median survival of SCLC patients treated with the CPT-11/cisplatin (CDDP) combination was significantly longer than that of SCLC patients treated with the etoposide/CDDP combination in a randomized phase III study ($P = 0.002$) conducted by the Japanese Cooperative Oncology

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Translational Relevance

The SN-38–incorporating polymeric micelles NK012 has been shown to have significant antitumor activity against several cancer mouse models compared with CPT-11. The phase I study showed that patients treated with NK012 did not develop grade 3/4 diarrhea, one of the major adverse effects of CPT-11. Here, the antitumor activity of NK012/cisplatin combination was compared with that of CPT-11/cisplatin combination, one of the most active regimens against SCLC and NSCLC in the clinic. We also evaluated the pharmacologic and toxic profiles of the drug combinations, particularly in terms of diarrhea. NK012/cisplatin showed a significant potent antitumor activity against an SBC-3 xenograft compared with CPT-11/cisplatin. Moreover, inflammatory pathologic changes were rarely observed in the small intestinal mucosa of the NK012-treated mouse but were commonly observed in the CPT-11–treated mouse. NK012/cisplatin combination chemotherapy is thus a promising regimen against lung cancer without severe diarrhea toxicity and therefore warrants further clinical evaluation.

Group (19). Therefore, CPT-11/CDDP is considered to be one of the most active regimens against SCLC in Japan. A recent randomized phase III study showed that CPT-11/CDDP was equal to other platinum-based regimens, such as carboplatin plus paclitaxel, CDDP plus gemcitabine, and CDDP plus vinorelbine, in terms of response rate and overall survival in non-SCLC (NSCLC) patients (20).

One of the major clinically important toxic effects or dose-limiting factors of CPT-11 is severe late-onset diarrhea (21–23). We previously showed that there was no significant difference in the kinetic character of free SN-38 in the small intestine of mice bearing the SCLC cell line SBC-3 and treated with NK012 and CPT-11 (12). Furthermore, in two independent phase I clinical trials in Japan (24) and the United States (25), nonhematologic toxicities were minimal and grade 3/4 diarrhea was absent.

In this context, we conducted this study to investigate the advantages of NK012/CDDP over CPT-11/CDDP in mice bearing a SCLC xenograft in terms of antitumor activity and toxic effects, particularly intestinal toxicity.

Materials and Methods

Drugs and cells. SN-38 and NK012 were prepared by Nippon Kayaku Co. Ltd. CPT-11 was purchased from Yakult Honsha Co. Ltd. CDDP was obtained from WC Heraeus GmbH & Co. KG.

Among the SCLC cell lines used, SBC-3 was kindly provided by Dr. I. Kimura (Okayama University, Okayama, Japan), and H69 and H82 were purchased from the American Type Culture Collection. SBC-3, H69, and H82 were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Cell Culture Technologies) and penicillin, streptomycin, and amphotericin B (100 units/mL, 100 μg/mL, and 25 μg/mL, respectively; Sigma) in a humidified atmosphere containing 5% CO_2 at 37°C. Vascular endothelial growth factor (VEGF)–secreting cells, SBC-3/VEGF and its mock transfectant SBC-3/Neo, were generated from SBC-3 cells transfected with BMG-Neo-VEGF and BMG-Neo, as described (26).

In vitro *study.* The growth-inhibitory effects of NK012, CPT-11, SN-38, and CDDP were examined by tetrazolium salt–based proliferation assay (WST-8 assay; Wako Chemicals). One hundred microliters of a suspension of exponentially growing cells (1×10^5/mL of SBC-3/Neo and SBC-3/VEGF or 1×10^6/mL of H69 and H82) were placed into the wells of a 96-well plate and incubated for 24 h at 37°C. Then, after medium removal, 100 μL of medium containing various concentrations of each drug were added to the wells and then incubated for 72 h at 37°C. After medium removal, 10 μL of WST-8 solution and 90 μL of medium were added to the wells followed by incubation for 1 h at 37°C. The growth-inhibitory effects of each drug were assessed spectrophotometrically (SpectraMax 190, Molecular Devices Corp.). The IC_{50} value was determined on the dose-response curves. The nature of interaction between NK012 and CDDP against SCLC cell lines, SBC-3/Neo, SBC-3/VEGF, H69, and H82, was evaluated by median-effect plot analyses and the combination index method of Chou and Talalay (27).

Experimental mice model. Female BALB/c nude mice (6 wk old) were purchased from SLC Japan. Mice were inoculated s.c. in the flank with 1×10^7 cells/150 μL cell suspension of SBC-3/Neo and SBC-3/VEGF cell lines.

All animal procedures were done in compliance with the guidelines for the care and use of experimental animals established by the Committee for Animal Experimentation of the National Cancer Center; these guidelines meet the ethical standards required by law and also comply with the guidelines for the use of experimental animals in Japan.

In vivo *growth inhibition assay.* When the tumor volume (TV) reached 1,500 mm^3, mice were randomly divided into test groups consisting of five mice per group (day 0). Drugs were i.v. administered into the tail vein on days 0, 7, and 14. NK012 was given at SN-38 equivalent doses of 10 and 5 mg/kg/d, which are one third and one sixth of the maximum tolerated dose, respectively. The reference drug, CPT-11, was given at 22 and 10 mg/kg/d, which are one third and one sixth of the maximum tolerated dose, respectively. CDDP was simultaneously given on the same day at 2.5 mg/kg/d based on a previous report (28). In preliminary experiment, NK012 (5 mg/kg) plus CDDP (2.5 mg/kg) seemed to be superior to NK012 (5 mg/kg) alone in these tumors. NaCl solution (0.9%) was administered i.v. as normal control. The length (a) and width (b) of the tumor masses and body weight (BW) were measured twice a week, and TV was calculated using $TV = (a \times b^2) / 2$. Relative TV (RTV) on day n was calculated using $RTV = TV_n / TV_0$, where TV_n is the TV on day n and TV_0 is the TV on day 0. Relative BW (RBW) was calculated using $RBW = BW_n / BW_0$. Differences in RTV and RBW between the treatment groups on day 30 were analyzed using the unpaired t test.

Pharmacokinetic analysis by high-performance liquid chromatography. Female BALB/c nude mice ($n = 3$) bearing SBC-3/Neo and SBC-3/VEGF tumors (1,500 mm^3) were used for drug pharmacokinetic analysis. NK012 or CPT-11 was administered at an equimolar dose of 20 or 30 mg/kg on day 0, respectively, as reported (12). CDDP was simultaneously given at 2.5 mg/kg. Mice were sacrificed 1, 6, 24, and 72 h (day 3) after administration. Plasma samples, tumors, upper small intestine, and feces were obtained and stored at -80°C until analysis.

SN-38 was extracted for each sample and reversed-phase high-performance liquid chromatography was done as reported (12).

Pathologic studies of small intestinal mucosa. CPT-11 and NK012 were injected to female BALB/c nude mice ($n = 3$) at the same dose schedules as those used in the treatment experiment. On day 14 after the last dosing, mice were sacrificed and parts of the small intestine were sampled at 5 cm from the pylolic part for the jejunum and 5 cm from the ileocecal junction for the ileum. Samples were fixed in 10% formalin, paraffin embedded, sectioned, and stained with H&E. Inflammation was scored by using an inflammation scale from - to ++, with - indicating absent inflammation, + showing mild

Table 1. *In vitro* growth-inhibitory activity of SN-38, NK012, CPT-11, and CDDP in human SCLC cells

Cell line	IC_{50} (μmol/L)			
	SN-38	NK012	CPT-11	CDDP
SBC-3/VEGF	0.00330 ± 0.00210	0.00365 ± 0.00005	1.11 ± 0.29	2.21 ± 0.36
SBC-3/Neo	0.00872 ± 0.00063	0.0101 ± 0.0006	5.05 ± 0.08	12.8 ± 1.5
H69	0.0205 ± 0.0195	0.0417 ± 0.0052	22.2 ± 5.9	6.23 ± 0.33
H82	0.00716 ± 0.00079	0.00998 ± 0.00328	1.98 ± 0.55	4.08 ± 3.79

inflammation predominantly infiltrated with lymphocytes, and ++ indicating active inflammation infiltrated with lymphocytes and neutrophils.

Distribution of NK012 or CPT-11 in small intestine by fluorescence microscopy. NK012 or CPT-11 was administered to female BALB/c nude mice at 20 or 30 mg/kg on day 0, respectively. Mice were sacrificed 1, 6, 24, and 72 h after drug injection, and the small intestine was excised at the middle portion and embedded in an OCT compound (Sakura Finetechnochemical Co. Ltd.) and frozen at -80°C. Tissue sections (5 μm thick) were prepared using a cryostatic microtome (Tissue-Tek Cryo3, Sakura Finetechnochemical). Frozen sections were examined under a fluorescence microscope (Biorevo, Keyence) at a 358-nm excitation wavelength and a 461-nm emission wavelength to evaluate NK012 or CPT-11 distribution in the small intestine. Because formulations containing SN-38 bound via ester bonds possess a particular fluorescence, both NK012 and CPT-11 were detected under the same fluorescence conditions.

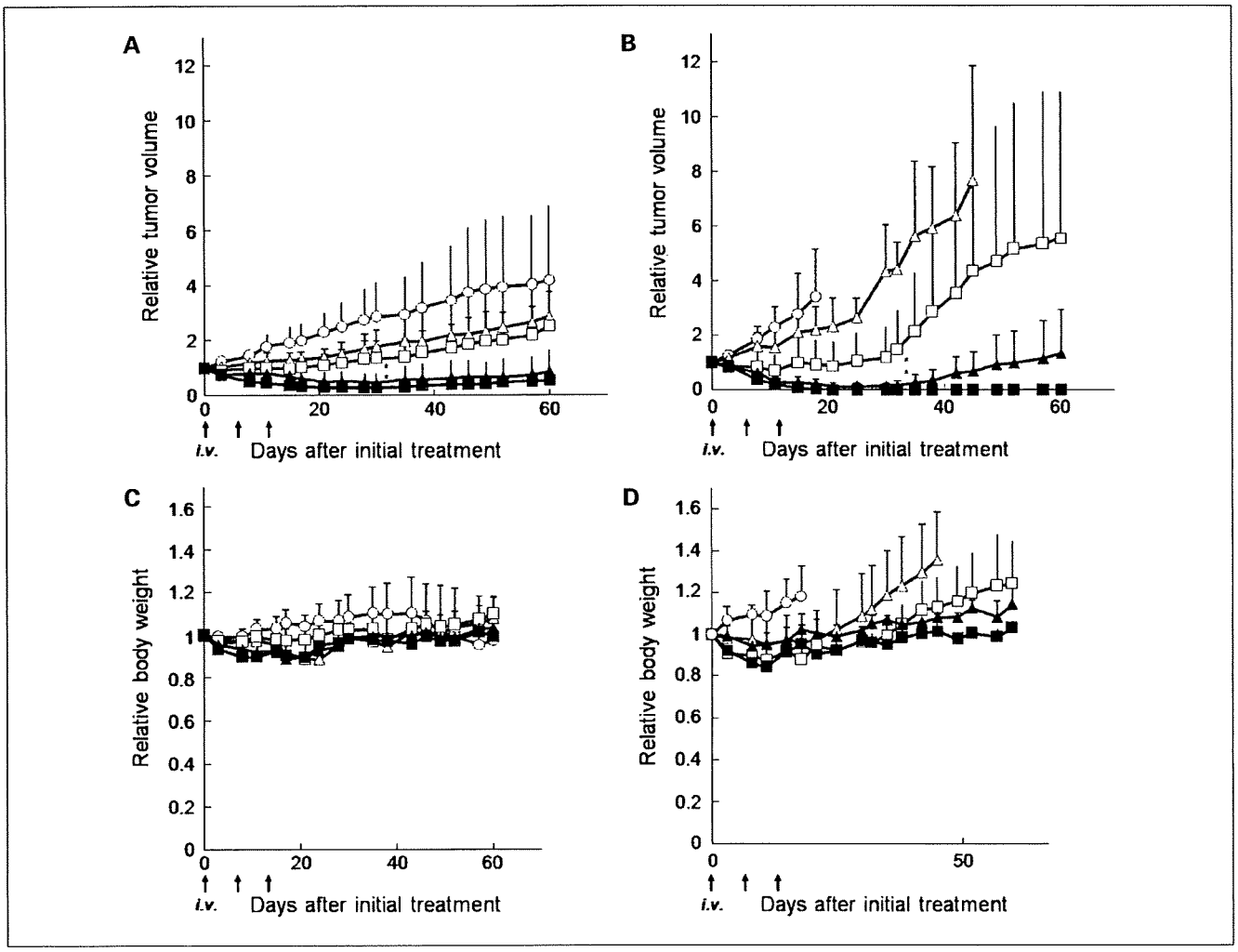

Fig. 1. Growth inhibitory effects of NK012/CDDP and CPT-11/CDDP on SBC-3/Neo and SBC-3/VEGF tumor xenografts. *A* and *B*, RTV in mice treated with NK012/CDDP or CPT-11/CDDP. SBC-3/Neo (*A* and *C*) and SBC-3/VEGF (*B* and *D*) tumors were inoculated s.c. into the flank of mice, as described in Materials and Methods. CPT-11 (10 mg/kg/d; Δ), CPT-11 (22 mg/kg/d; □), NK012 (5 mg/kg/d; ▲), or NK012 (10 mg/kg/d; ■) combined with CDDP (2.5 mg/kg/d) were i.v. administered on days 0, 7, and 14. ○, NaCl solution (0.9%) was i.v. administered as normal control. *Points,* mean; *bars,* SD. *, $P < 0.05$. *C* and *D*, treatment-related BW loss occurred in mice treated with NK012/CDDP and CPT-11/CDDP. *Points,* mean; *bars,* SD.