Table 2. Prognosis-related genes identified using microarray analysis

P-value	Hazard ratio	Description	Gene	Probe set	Pass	PCR		
0.0002	1.8	Epidermal growth factor receptor	*EGFR*	201984_s_at	2	PCR	1	0.1
0.0005	0.1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54	*DDX54*	219111_s_at			2	0.1
0.0005	0.5	Chimerin (chimaerin) 2	*CHN2*	213385_at			3	0.1
0.0005	6.1	Ubiquitin-like domain containing CTD phosphatase 1	*UBLCP1*	227413_at			4	0.2
0.0006	0.5	PTK2 protein tyrosine kinase 2	*PTK2*	241387_at			5	0.2
0.0008	3.4	Der1-like domain family, member 2	*DERL2*	218333_at			6	0.2
0.0008	0.5	Leucine rich repeat containing 14	*LRRC14*	32062_at			7	0.2
0.0009	4.5	WD repeat domain 33	*WDR33*	222763_s_at		PCR	8	0.2
0.0009	0.1	Rhomboid domain containing 3	*RHBDD3*	217622_at			9	0.2
0.001	0.3	Myosin regulatory light chain interacting protein	*MYLIP*	228098_s_at	3	PCR	10	0.2
0.0013	4.7	Chromosome 14 open reading frame 43	*C14orf43*	225980_at		PCR	11	0.2
0.0013	0.2	BCL6 co-repressor	*BCOR*	223915_at			12	0.2
0.0013	0.5	MAD1 mitotic arrest deficient-like 1 (yeast)	*MAD1L1*	233921_s_at			13	0.2
0.0013	4.9	Chromosome 14 open reading frame 109	*C14orf109*	213246_at			14	0.2
0.0014	4.2	Hypothetical protein LOC124512	*LOC124512*	225808_at			15	0.2
0.0014	5.0	Ring finger protein 167	*RNF167*	212047_s_at			16	0.2
0.0014	0.6	Hypothetical LOC25845	*LOC25845*	225457_s_at			17	0.2
0.0014	4.2	General transcription factor II, i	*GTF2I*	232710_at			18	0.3
0.0014	0.2	Rho guanine nucleotide exchange factor (GEF) 10-like	*ARHGEF10L*	1570511_at			19	0.3
0.0014	0.3	G kinase anchoring protein 1	*GKAP1*	229312_s_at		PCR	20	0.3
0.0015	1.9	Glutathione peroxidase 3 (plasma)	*GPX3*	214091_s_at	2	PCR	21	0.3
0.0016	0.5	Dachshund homolog 1 (*Drosophila*)	*DACH1*	1567101_at	2	PCR	22	0.3
0.0016	0.3	Diacylglycerol kinase, theta 110kDa	*DGKQ*	226605_at			23	0.3
0.0017	0.6	Hepatocellular carcinoma-associated antigen 112	*HCA112*	218345_at			24	0.3
0.0018	3.5	Mediator of RNA polymerase II transcription, subunit 31 homolog	*MED31*	222867_s_at			25	0.3
0.0018	6.9	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	*YWHAE*	210317_s_at		PCR	26	0.3
0.0018	0.1	KH domain containing, RNA binding, signal transduction associated 1	*KHDRBS1*	201488_x_at			27	0.3
0.0019	0.3	Solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	*SLC25A16*	210686_x_at			28	0.3
0.0019	4.9	Hypothetical protein LOC51255	*LOC51255*	223064_at			29	0.3
0.002	0.2	Cyclin L2 /// similar to Aurora kinase A-interacting protein	*CCNL2 /// LOC643556*	222999_s_at			30	0.3
0.002	7.4	Lectin, mannose-binding, 1	*LMAN1*	224629_at			31	0.3
0.002	0.2	Erythrocyte membrane protein band 4.1 like 4A	*EPB41L4A*	228259_s_at			32	0.3
0.0022	0.2	KIAA0999 protein	*KIAA0999*	204155_s_at			33	0.3
0.0022	0.5	ELOVL family member 7	*ELOVL7*	227180_at			34	0.3
0.0023	4.0	Churchill domain containing 1	*CHURC1*	233268_s_at			35	0.4
0.0024	4.0	Yippee-like 2 (*Drosophila*)	*YPEL2*	227020_at			36	0.4
0.0024	5.9	Hermansky–Pudlak syndrome 1	*HPS1*	210112_at			37	0.4
0.0025	0.3	Hypothetical protein LOC285831	*LOC285831*	228857_at			38	0.4
0.0026	3.5	CDC37 cell division cycle 37 homolog (*Saccharomyces cerevisiae*)-like 1	*CDC37L1*	219343_at			39	0.4
0.0026	2.1	Ankyrin repeat and SOCS box-containing 9	*ASB9*	205673_s_at			40	0.4
0.0026	0.2	Hypothetical gene supported by AK125149	*LOC401577*	239247_at			41	0.5
0.0026	0.3	TBC1 domain family, member 23	*TBC1D23*	236755_at			42	0.5
0.0026	0.3	MRNA full length insert cDNA clone EUROIMAGE 2362292		235505_s_at			43	0.5
0.0026	0.4	Dehydrogenase/reductase (SDR family) member 8	*DHRS8*	217989_at			44	0.5
0.0026	0.4	Nuclear receptor coactivator 2	*NCOA2*	242369_x_at			45	0.5
0.0026	0.2	MRNA; cDNA DKFZp667E0114 (from clone DKFZp667E0114)		235660_at			46	0.5
0.0027	0.4	Transforming, acidic coiled-coil containing protein 1	*TACC1*	242290_at			47	0.5
0.0027	0.2	POU domain, class 2, transcription factor 1	*POU2F1*	1562280_at			48	0.5
0.0027	2.9	p21(CDKN1A)-activated kinase 6	*PAK6*	1555310_a_at				0.5
0.0027	0.5	Mannosyl (alpha-1,3-)-glycoprotein β-1,4-N-acetylglucosaminyltransferase, isozyme A	*MGAT4A*	226039_at			50	0.5
0.0027	5.1	Zinc finger CCCH-type containing 14	*ZC3H14*	204216_s_at			51	0.5
0.0028	0.5	Acyl-CoA synthetase short-chain family member 2	*ACSS2*	235805_at			52	0.5
0.0028	0.3	Programmed cell death 6	*PDCD6*	222380_s_at		PCR	53	0.6
0.0029	3.8	ERGIC and golgi 2	*ERGIC2*	226422_at			54	0.6
0.0029	0.4	Erythrocyte membrane protein band 4.1 like 5	*EPB41L5*	225855_at			55	0.6
0.003	6.5	Chromosome 14 open reading frame 32	*C14orf32*	212644_s_at			56	0.6

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Table 2. (*Continued*)

P-value	Hazard ratio	Description	Gene	Probe set	Pass	PCR		
0.0031	0.2	Transcribed locus		239437_at			57	1.8
0.0031	0.3	DOT1-like, histone H3 methyltransferase (*S. cerevisiae*)	*DOT1L*	231297_at			58	1.9
0.0031	2.2	Transcription elongation factor A (SII)-like 8	*TCEAL8*	224819_at			59	1.9
0.0031	0.3	Laminin, β 1	*LAMB1*	236437_at			60	2.0
0.0032	2.7	FK506 binding protein 5	*FKBP5*	224840_at			61	2.0
0.0033	0.5	Integrin, α 6	*ITGA6*	244665_at			62	2.1
0.0034	2.7	COMM domain containing 9	*COMMD9*	218072_at			63	2.2
0.0034	0.2	Eukaryotic translation initiation factor 4 γ, 3	*EIF4G3*	201936_s_at			64	2.3
0.0035	0.5	235616_at	*235616_at*	235616_at			65	2.6
0.0036	1.9	Metallothionein 1X	*MT1X*	204326_x_at		PCR	66	2.6
0.0036	2.7	Peroxiredoxin 5	*PRDX5*	1560587_s_at			67	2.7
0.0037	0.3	Core-binding factor, runt domain, α subunit 2; translocated to, 2	*CBFA2T2*	207625_s_at			68	2.7
0.0037	0.4	Transcribed locus, moderately similar to XP_531878.2		230168_at			69	2.7
0.0038	0.3	Zinc finger protein 346	*ZNF346*	236267_at			70	2.8
0.0038	2.0	Metallothionein 1H-like protein /// hypothetical protein LOC650610	*LOC645745 /// LOC650610*	211456_x_at			71	2.9
0.0039	0.2	Hypothetical protein DKFZp586I1420	*DKFZp586I1420*	213546_at			72	3.4
0.0039	2.0	Adrenergic, β-2-, receptor, surface	*ADRB2*	206170_at			73	3.5
0.0039	0.3	CTD-binding SR-like protein rA9	*KIAA1542*	234952_s_at			74	3.5
0.0039	2.6	Peroxiredoxin 5	*PRDX5*	222994_at			75	3.6
0.004	0.2	ATPase, H^+ transporting, lysosomal 42kDa, V1 subunit C1	*ATP6V1C1*	226463_at			76	3.8
0.004	8.0	XK, Kell blood group complex subunit-related family, member 8	*XKR8*	218753_at			77	3.8
0.004	0.3	Caspase 6, apoptosis-related cystein peptidase	*CASP6*	242323_at			78	4.0
0.0041	0.4	Coagulation factor XII (Hageman factor)	*F12*	205774_at			79	4.0
0.0041	0.3	Centaurin, γ 2	*CENTG2*	240758_at			80	4.2
0.0042	0.6	LR8 protein	*LR8*	220532_s_at			81	4.2
0.0042	0.2	WD repeat domain 42A	*WDR42A*	243318_at			82	4.5
0.0042	2.6	Potassium channel tetramerisation domain containing 14	*KCTD14*	219545_at			83	4.7
0.0043	2.8	6-Phosphogluconolactonase	*PGLS*	218388_at			84	4.9
0.0044	3.8	Bruno-like 6, RNA binding protein (*Drosophila*)	*BRUNOL6*	227775_at			85	4.9
0.0044	2.3	Zinc finger protein 415	*ZNF415*	205514_at			86	5.0
0.0045	0.5	HIR histone cell cycle regulation defective homolog A (*S. cerevisiae*)	*HIRA*	240451_at			87	5.1
0.0046	0.5	Cardiolipin synthase 1	*CRLS1*	241741_at			88	5.9
0.0046	0.3	c-mer proto-oncogene tyrosine kinase	*MERTK*	233079_at			89	6.1
0.0047	0.2	Additional sex combs like 2 (*Drosophila*)	*ASXL2*	218659_at			90	6.5
0.0047	3.6	Platelet endothelial aggregation receptor 1	*PEAR1*	228618_at			91	6.9
0.0047	0.3	Core-binding factor, runt domain, α subunit 2; translocated to, 2	*CBFA2T2*	238549_at			92	7.4
0.005	0.6	Lysosomal associated protein transmembrane 4 β	*LAPTM4B*	208029_s_at			93	8.0

Pass, number of overlapped probes; PCR, the genes that were subsequently examined using real-time RT-PCR.

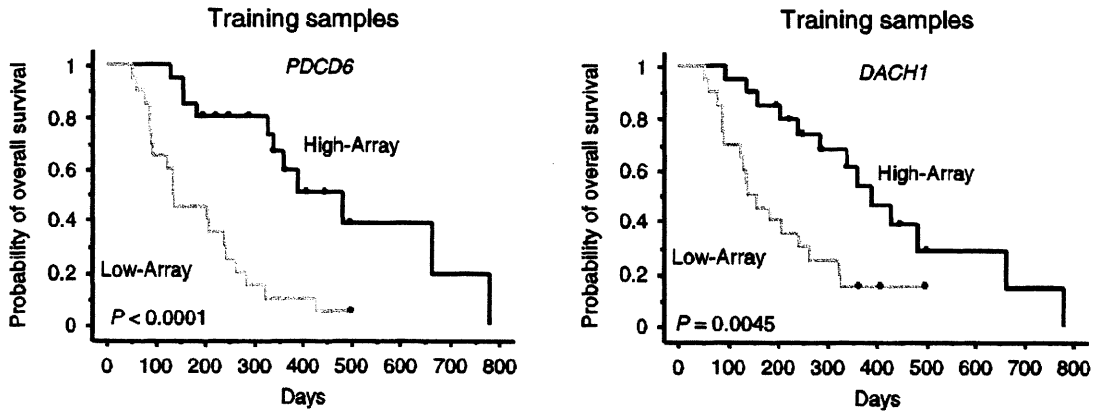

Fig. 2. Results of microarray data and patient survival in the training set of 40 patients. The Kaplan–Meier method was used for *DACH1* and *PDCD6*. The patients were divided into high and low expression groups by median values. The low *PDCD6* and *DACH1* expression groups had significantly poorer outcomes ($P < 0.0001$ and $P = 0.0045$). High-Array, group with high expression levels as determined by signal intensity of microarray data. Low-Array, group with low expression levels as determined by signal intensity of microarray data.

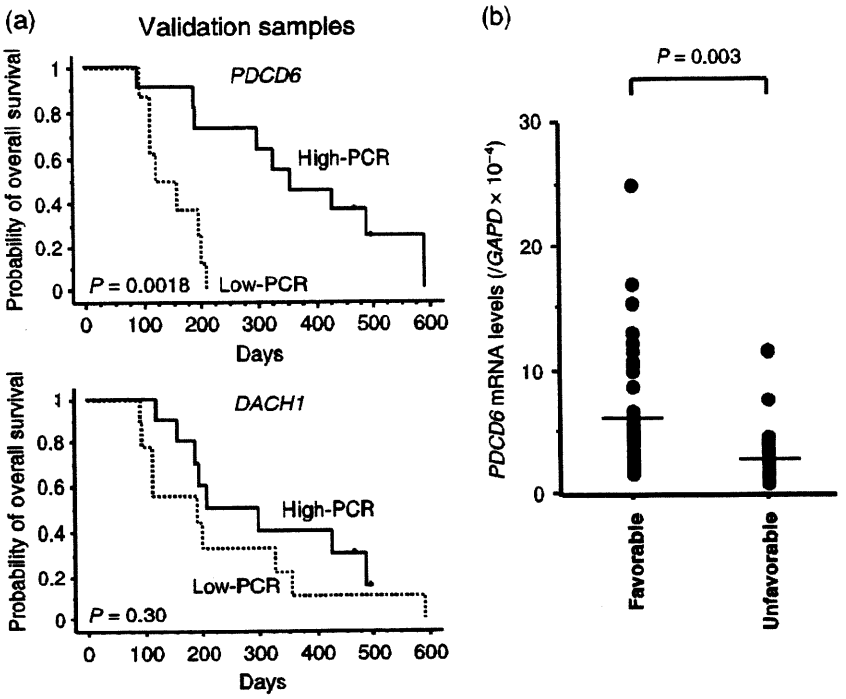

Fig. 3. Results of real-time reverse transcription polymerase chain reaction (RT-PCR) analysis and patient survival in the independent validation set of 19 samples. (a) The Kaplan–Meier method was used to estimate the overall survival. The low *PDCD6* expression groups had significantly poorer outcomes ($P = 0.0018$). High-PCR, group with high expression levels as determined by PCR. Low-PCR, group with low expression levels as determined by PCR. (b) All quantified expression levels of real time RT-PCR data are shown. The mRNA expressions of *PDCD6* were significantly lower in unfavorable group ($P = 0.003$) and varied ~25 fold (range, 0.98–25.1). Favorable, the patients with survival time over 180 days. Unfavorable, the patients with a survival time less than 180 days.

Table 3. Multivariate analysis of prognosis-related genes

Variable	Hazard ratio	95% confidence interval	*P*-value
Age (≥65)	1.78	0.570–5.559	0.3212
Sex (male)	3.26	0.732–14.489	0.1210
Performance status (≥1)	2.36	0.687–8.078	0.1728
Metastasis (≥3)	1.58	0.450–5.561	0.4739
Chemotherapy (5-FU)	1.48	0.402–5.475	0.5541
DACH1	0.38	0.175–0.817	0.0134
EGFR	1.41	0.992–2.001	0.0553
MT1X	0.71	0.317–1.600	0.4111
YWHAE	1.91	0.401–9.061	0.4169
GPX3	1.62	0.869–3.007	0.1293
PDCD6	0.06	0.010–0.334	0.0015
WDR33	1.38	0.268–7.067	0.7017
C14orf43	0.64	0.122–3.407	0.6045
MYLIP	0.67	0.221–2.042	0.4826
GKAP1	2.31	0.751–7.106	0.1440

Cox regression model was performed for multivariate analysis against each of the variables.

Table 4. Results of real-time *RT-PCR* for *PDCD6* and *DACH1* in an independent valiation set

Genes	Hazard ratio	95% confidence limits		*P*-value
		Upper	Lower	
*PDCD6**	0.29	0.12	0.71	0.007
DACH1	0.79	0.56	1.13	0.199

*, $P < 0.05$.

the uncontrollable factors, we aimed to avoid controllable factors with our best efforts. In this sense, we believe that the present study has succeeded in stratifying potential controllable variables.

Based on the results of the series of analyses conducted in the current study, we validated *PDCD6* as a molecular biomarker of the prognosis in gastric cancer.

PDCD6, also known as ALG-2 (apoptosis-linked gene-2), was first identified in a study on T-cell apoptosis conducted by Vito *et al.*[29] *PDCD6* encodes a calcium-binding protein that belongs to the penta-EF-hand protein family. The gene product participates in T-cell receptor-, Fas- and glucocorticoid-induced programmed cell death and cell proliferation. The stimulation of cells to enter the cell cycle is thought to drive the cellular apoptotic program, and the presence of additional survival or pro-apototic signals determines whether a cell proliferates or commits suicide. Krebs *et al.* indicated that the deregulation of such an obviously delicate balance could lead to pathological developments, such as cancer.[30] Detailed biological function of *PDCD6* genes in gastric cancer is still unclear. The speculated function may lead us to hypothesize that the expression is generally downregulated in cancer.

Our ultimate goal is to use real-time RT-PCR or immunohistochemical examination to identify patients with a poor prognosis prior to undertaking chemotherapy. We are now planning a large-scale prospective study based on the evidence obtained in the current study.

In conclusion, we identified prognostic biomarkers in patients with unresected gastric cancer, and our PCR-based single gene prediction strategy successfully predicted the overall survival of patients with gastric cancer. Our findings may provide a novel insight into the treatment of gastric cancer and may lead to a better understanding of this disease subgroup.

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References

1 Sastre J, Garcia-Saenz JA, Diaz-Rubio E. Chemotherapy for gastric cancer. *World J Gastroenterol* 2006; **12**: 204–13.
2 Vanhoefer U, Rougier P, Wilke H *et al.* Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin versus etoposide, leucovorin, and fluorouracil versus infusional fluorouracil and cisplatin in advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. *J Clin Oncol* 2000; **18**: 2648–57.
3 Ohtsu A, Shimada Y, Shirao K *et al.* Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol* 2003; **21**: 54–9.
4 Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T. Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1M tegafur-0.4M gimestat-1M otastat potassium) in advanced gastric cancer patients. *Eur J Cancer* 1998; **34**: 1775–20.
5 Koizumi W, Kurihara M, Nakano S, Hasegawa K. Phase II study of S-1, a novel derivative of 5-fluorouracil, in advanced gastric cancer. *Oncology* 2000; **58**: 191–7.
6 Shirao K, Shimada Y, Kondo H *et al.* Phase I–II study of irinotecan hydrochloride combined with cisplatin in patients with advanced gastric cancer. *J Clin Oncol* 1997; **15**: 921–7.
7 Boku N, Ohtsu A, Shimada Y *et al.* Phase II study of combination of irinotecan and cisplatin against metastatic gastric cancer. *J Clin Oncol* 1999; **17**: 319–23.
8 Boku N, Yamamoto S, Shirao K *et al.* Gastrointestinal Oncology Study Group/Japan Clinical Oncology Group. Randomized phase III study of 5-fluorouracil (5-FU) alone versus combination of irinotecan and cisplatin (CP) versus S-1 alone in advanced gastric cancer (JCOG9912) (Abstract). *Proc Am Soc Clin Oncol* 2007; **25**: 18S.
9 Chau I, Norman AR, Cunningham D, Waters JS, Oates J, Ross PJ. Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer – pooled analysis from three multicenter, randomized, controlled trials using individual patient data. *J Clin Oncol* 2004; **22**: 2395–403.
10 Metzger R, Leichman CG, Danenberg KD *et al.* ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and 5-fluorouracil chemotherapy. *J Clin Oncol* 1998; **16**: 309–16.
11 Boku N, Chin K, Hosokawa K *et al.* Biological makers as a predictor for response and prognosis of unresectable gastric cancer patients treated with 5-fluorouracil and cis-platinum. *Clin Cancer Res* 1998; **4**: 1469–74.
12 Yonemura Y, Ninomiya I, Yamaguchi A *et al.* Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. *Cancer Res* 1991; **51**: 1034–8.
13 Sanz-Ortega J, Steinberg SM, Moro E *et al.* Comparative study of tumor angiogenesis and immunohistochemistry for p53, c-ErbB2, c-myc and EGFr as prognostic factors in gastric cancer. *Histol Histopathol* 2000; **15**: 455–62.
14 Shibata A, Parsonnet J, Longacre TA *et al.* CagA status of *Helicobacter pylori* infection and p53 gene mutations in gastric adenocarcinoma. *Carcinogenesis* 2002; **23**: 419–24.
15 Okusa Y, Ichikura T, Mochizuki H. Prognostic impact of stromal cell-derived urokinase-type plasminogen activator in gastric carcinoma. *Cancer* 1999; **85**: 1033–8.
16 Linder N, Haglund C, Lundin M *et al.* Decreased xanthine oxidoreductase is a predictor of poor prognosis in early-stage gastric cancer. *J Clin Pathol* 2006; **59**: 965–71.
17 Resnick MB, Gavilanez M, Newton E *et al.* Claudin expression in gastric adenocarcinomas: a tissue microarray study with prognostic correlation. *Hum Pathol* 2005; **36**: 886–92.
18 Kido S, Kitadai Y, Hattori N *et al.* Interleukin 8 and vascular endothelial growth factor – prognostic factors in human gastric carcinomas? *Eur J Cancer* 2001; **37**: 1482–7.
19 Xiangming C, Natsugoe S, Takao S *et al.* The cooperative role of p27 with cyclin E in the prognosis of advanced gastric carcinoma. *Cancer* 2000; **89**: 1214–9.
20 Yasui W, Oue N, Ono S, Mitani Y, Ito R, Nakayama H. Histone acetylation and gastrointestinal carcinogenesis. *Ann NY Acad Sci* 2003; **983**: 220–31.
21 Oue N, Motoshita J, Yokozaki H *et al.* Distinct promoter hypermethylation of p16INK4a, CDH1, and RAR-beta in intestinal, diffuse-adherent, and diffuse-scattered type gastric carcinomas. *J Pathol* 2002; **198**: 55–9.
22 Yamanaka R, Arao T, Yajima N *et al.* Identification of expressed genes characterizing long-term survival in malignant glioma patients. *Oncogene* 2006; **25**: 5994–6002.
23 Hasegawa S, Furukawa Y, Li M *et al.* Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23 040 genes. *Cancer Res* 2002; **62**: 7012–17.
24 Hippo Y, Taniguchi H, Tsutsumi S *et al.* Global gene expression analysis of gastric cancer by oligonucleotide microarrays. *Cancer Res* 2002; **62**: 233–40.
25 Inoue H, Matsuyama A, Mimori K, Ueo H, Mori M. Prognostic score of gastric cancer determined by cDNA microarray. *Clin Cancer Res* 2002; **8**: 3475–9.
26 Leung SY, Yuen ST, Chu KM *et al.* Expression profiling identifies chemokine (C-C motif) ligand 18 as an independent prognostic indicator in gastric cancer. *Gastroenterology* 2004; **127**: 457–69.
27 Leung SY, Chen X, Chu KM *et al.* Phospholipase A2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. *Proc Natl Acad Sci USA* 2002; **99**: 16203–8.
28 Chen CN, Lin JJ, Chen JJ *et al.* Gene expression profile predicts patient survival of gastric cancer after surgical resection. *J Clin Oncol* 2005; **23**: 7286–95.
29 Vito P, Lacana E, D'Adamio L. Interfering with apoptosis: $Ca^{(2+)}$-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* 1996; **271**: 521–5.
30 Krebs J, Saremaslani P, Caduff R. ALG-2: a Ca^{2+}-binding modulator protein involved in cell proliferation and in cell death. *Biochim Biophys Acta* 2002; **1600**: 68–73.

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A Phase I Study of Bolus 5-Fluorouracil and Leucovorin Combined with Weekly Paclitaxel (FLTAX) as First-line Therapy for Advanced Gastric Cancer

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Objective: To determine the dose-limiting toxicity (DLT) and the maximum-tolerated dose (MTD) of combination chemotherapy with leucovorin-modulated weekly bolus 5-fluorouracil (5-FU) and weekly paclitaxel in patients with advanced gastric cancer (GC).
Methods: Chemotherapy-naïve patients with histologically proven metastatic or recurrent GC were enrolled. Paclitaxel was administered as a 1-h intravenous (i.v.) infusion followed by 5-FU as a bolus i.v. infusion on Days 1, 8 and 15. A 2-h i.v. infusion of *l*-leucovorin was started at the same time as the paclitaxel infusion on Days 1, 8 and 15. Treatment cycles were repeated every 28 days until disease progression or unacceptable toxicity occurred. Patients were scheduled to receive 5-FU, *l*-leucovorin and paclitaxel at four dose levels (mg/m^2/week): 500/250/60 (level 1), 500/250/80 (level 2), 600/250/80 (level 3) and 600/250/100 (level 4), respectively.
Results: Eighteen patients were enrolled. During the first cycle of the highest dose level (level 4), two of the six patients had DLT involving Grade 3 diarrhea and Grade 3 skin rash. Furthermore, three of the four patients who received the second consecutive cycle of treatment at dose level 4 had Grade 4 neutropenia. Dose level 3 was thus determined to be the MTD. Eleven (61%) of the 18 patients had partial responses, and the median progression-free survival time was 6.8 months.
Conclusions: The MTD and the recommended dose for phase II studies of this regimen were determined to be 5-FU 600 mg/m^2/week, *l*-leucovorin 250 mg/m^2/week and paclitaxel 80 mg/m^2/week.

Key words: gastric cancer – chemotherapy – weekly paclitaxel – bolus 5-fluorouracil – leucovorin

INTRODUCTION

Globally, gastric cancer (GC) is the second most common cause of cancer death. Even though the incidence of GC is declining, ~930 000 cases are newly diagnosed each year (1). Because of the vague and non-specific symptoms associated with GC, the disease is often advanced at the time of diagnosis. Despite the identification and development of several new classes of anticancer agents, GC remains an aggressive malignancy, with a median survival of 9–13 months in patients with metastatic or recurrent disease (2–5).

Paclitaxel, a unique antimicrotubule agent derived from a type of Western yew, *Taxus brevifolia*, is effective against a variety of cancers, including breast cancer, ovarian cancer, lung cancer and GC (6–10). Recent studies have shown that a weekly regimen of paclitaxel is less toxic than paclitaxel given once every 3 weeks (11,12). Weekly regimens of paclitaxel have thus become popular in Japan, producing good results in patients with advanced GC (13,14).

5-Fluorouracil (5-FU) remains a key drug for the management of GC. In the last decade, oral fluoropyrimidines, such as S-1 and capecitabine, have been tested in many clinical trials as alternative treatments to intravenous (i.v.) 5-FU (3–5,15–17). However, patients with advanced GC often have gastrointestinal symptoms, negatively

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affecting their general condition and sometimes precluding the administration of oral drugs. Since patients who are in poor condition or who have peritoneal dissemination cannot tolerate aggressive hydration and are at increased risk for intestinal obstruction, treatment with cisplatin or irinotecan is not indicated. New regimens of infusional chemotherapy that are less toxic and more effective than conventional therapy are thus required for patients in poor condition or with poor oral intake, as well as those in good condition.

A sequence-dependent, synergistic cytotoxic effect of paclitaxel followed by 5-FU has been demonstrated *in vitro* (18,19), and these drugs are relatively free of overlapping toxic effects. Many clinical trials have thus evaluated combination chemotherapy with 5-FU and paclitaxel (20–22). To our knowledge, however, no study has previously examined the safety and efficacy of leucovorin-modulated weekly bolus 5-FU combined with weekly paclitaxel, a regimen that can be given on an outpatient basis.

In this phase I study, 5-FU and *l*-leucovorin were administered weekly as a bolus i.v. infusion of 5-FU and a 2-h i.v. infusion of *l*-leucovorin in combination with a 1-h i.v. infusion of paclitaxel (FLTAX regimen). The primary objectives were to define the dose-limiting toxicity (DLT) and the maximum-tolerated dose (MTD) of this regimen in patients with metastatic or recurrent GC. Secondary objectives were characterization of the safety profile and assessment of the antitumor activity of the FLTAX regimen.

PATIENTS AND METHODS

Patient Eligibility

Patients registered in this trial were treated at National Cancer Center Hospital, Tokyo, Japan. To be eligible, patients had to meet the following criteria: histologically proven metastatic or recurrent GC, an age of 20–75 years, a performance status of two or less according to the Eastern Clinical Oncology Group (ECOG) scale, an estimated life expectancy of >8 weeks after study entry, no prior chemotherapy for metastatic disease, adequate hematological function (a white blood cell count between 3000 and 12 000/mm^3, a platelet count of ≥100 000/mm^3), adequate hepatic function (a serum total bilirubin level of ≤2.0 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of ≤100 IU/l), adequate renal function (a serum creatinine level of ≤1.5 mg/dl), a serum C-reactive protein level of ≤10 mg/dl and written informed consent. Patients also had to have radiographically measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (23).

The exclusion criteria were watery diarrhea, marked pleural effusion or ascites, active infection, severe comorbidity such as heart disease or renal disease, metastasis to the central nervous system, mental disorder, a history of alcoholic hypersensitivity, active concomitant malignancy, pregnant or nursing women and women of childbearing age unless they were practicing effective contraception. This phase I study was approved by the Institutional Review Board of the National Cancer Center and conducted in accordance with the Declaration of Helsinki.

DLT and MTD

DLT was defined as follows: Grade 4, neutropenia lasting for ≥4 days; Grade 3–4, thrombocytopenia; Grade 3–4, febrile neutropenia; Grade 3–4, diarrhea despite adequate antidiarrheal medication; any Grade 3–4, non-hematological toxicity (excluding anorexia, nausea, vomiting, electrolyte abnormalities and alopecia), treatment interruption for ≥2 weeks and a delay of the start of the second cycle by ≥8 days because of toxicity.

The MTD was evaluated on the basis of toxic effects during the first cycle. If three or more patients had DLT at a given dose level, then the previous dose level was defined to be the MTD. However, if two or less patients had DLT during the first cycle at the maximum dose level (dose level 4), the MTD was evaluated on the basis of toxic effects during the second treatment cycle. The recommended dose (RD) for phase II studies was defined as the MTD.

Treatment Schedule and Dose Escalation Schedule

Paclitaxel (Taxol; Bristol-Myers K.K., Tokyo, Japan) was administered as a 1-h i.v. infusion followed by 5-FU (Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan) as a bolus i.v. infusion on Days 1, 8 and 15 of a 28-day cycle. A 2-h i.v. infusion of *l*-leucovorin (Isovorin; Wyeth K.K., Tokyo, Japan) was started at the same time as the paclitaxel infusion in the same days. This treatment was repeated until disease progression or unacceptable toxicity occurred. Short-term premedication was given to prevent paclitaxel-associated hypersensitivity reactions as follows: dexamethasone, 8 mg; ranitidine, 50 mg and chlorpheniramine, 10 mg administered 30 min before the infusion of paclitaxel. The initially administered dose of paclitaxel was 60 mg/m^2 (dose level 1) and escalated to 100 mg/m^2 (dose level 4) in increments of 20 mg/m^2 (Table 1). The dose of 5-FU was 500 mg/m^2 (dose levels 1 and 2) or 600 mg/m^2 (dose levels 3 and 4, Table 1). *l*-Leucovorin was given at a fixed dose of 250 mg/m^2 in 250 ml of normal saline solution.

If patients had leukopenia of <2500/mm^3, thrombocytopenia of <100 000/mm^3, total bilirubin of >2.0 mg/dl, AST and ALT of >100 IU/l or serum creatinine of >1.5 mg/dl, both 5-FU/leucovorin and paclitaxel were withheld until recovery. To receive a subsequent cycle of chemotherapy, patients had to have a leukocyte count of ≥3000/mm^3 and the recovery of any treatment-related non-hematological toxicity to grade ≤1 (except alopecia and neuropathy). If patients had DLT, the dose was reduced by one level for the subsequent cycle of treatment (Table 1). If DLT recurred at the reduced dose level, the patient was withdrawn from the study.

Table 1. Dose escalation scheme of 5-FU, *l*-leucovorin and paclitaxel and DLT during the first cycle

Dose level	5-FU (mg/m^2)	*l*-Leucovorin (mg/m^2)	Paclitaxel (mg/m^2)	Number of patients	DLT events	
1	500	250	60	3	0	
2	500	250	80	3	0	
3	600	250	80	6	2	Treatment interruption for longer than 2 weeks because of prolonged Grade 2 leukopenia; Grade 3 infection (pneumonia) with normal neutrophils
4	600	250	100	6	2	Grade 3 diarrhea; Grade 3 skin rash

Toxicity was assessed according to the Common Terminology Criteria for Adverse Events, version 3.0. DLT, dose-limiting toxicity; 5-FU, 5-fluorouracil.

At least three patients received each dose level. If one or two of the three patients assigned to a given dose had any DLT, three additional patients were assigned to receive the same dose. If one or two of the resulting six patients had DLT, the dose could be increased to the next level. Since the toxicity profiles of both weekly bolus 5-FU and weekly paclitaxel are well known and easy to manage, we used the criterion of three of six DLT for halting dose escalation rather than the standard criterion of two of six DLT.

Toxicity and Response Evaluation

Treatment-related toxic effects were assessed according to the Common Terminology Criteria for Adverse Events, version 3.0. During treatment, patients' histories were obtained, and physical examinations, complete blood counts with differential counts, serum chemical analyses and urinalyses were carried out at least once a week. Tumor response was evaluated according to the RECIST guideline (23) every 8 weeks until tumor progression.

Statistical Analysis

Progression-free survival time was defined as the time from the date of starting treatment to the date of the first documentation of disease progression or death. Progression-free survival time in patients with protocol treatment cessation for toxicity was calculated as the time to the date of the first documentation of disease progression in subsequent therapies. Time-to-treatment failure was measured from the date of starting treatment to the date of treatment cessation for any reason. Progression-free survival time and time-to-treatment failure were calculated by the Kaplan–Meier method. If patients were receiving treatment according to the protocol at the time of analysis, data were censored at the time of the last evaluation.

RESULTS

Patient Characteristics

Eighteen patients were enrolled in this study between June 2006 and April 2007 at National Cancer Center Hospital, Tokyo, Japan. All patients received at least two cycles of chemotherapy. Toxicity and response were assessable in all patients. The clinical characteristics of the patients are given in Table 2. The median age was 63 years (range: 40–75). A total of 107 cycles of chemotherapy were administered, with a median of 6.5 treatment cycles per patient (range: 2–13). As of November 2007, one patient was receiving the seventh cycle of the protocol treatment and another was receiving the eighth cycle. No patient was lost to follow-up.

Table 2. Patient characteristics

Characteristics	No. of patients	%
Patients enrolled	18	100
Sex		
Male	15	83
Female	3	17
Age (years)		
Median	63	
Range	40–75	
ECOG performance status		
0	8	44
1	10	56
Histological type		
Intestinal	6	33
Diffuse	12	67
Prior surgery		
None	15	83
Gastrectomy	3	17
Site of metastasis		
Lymph nodes	18	100
Liver	10	56
Peritoneum	5	28
Lung	3	17
Ovary	1	6
Subcutaneous tissue	1	6

ECOG, Eastern Clinical Oncology Group.

After a 1-week-observation period in the hospital, all patients could receive treatment on an outpatient basis.

Toxicity

The number and the type of DLT that occurred during the first treatment cycle in the 18 patients are listed in Table 1. Major toxic effects occurring during the first cycle are summarized in Table 3 according to the dose level. There was no DLT at dose level 1 or 2 (Table 1). At dose level 2, one patient already had Grade 3 anemia at the start of the protocol treatment. At dose level 3, treatment had to be interrupted for longer than 2 weeks because of the prolonged Grade 2 leukopenia in one patient, and another patient had to be hospitalized because of Grade 3 infection (pneumonia) without neutropenia. Both of these reactions were DLT (Tables 1 and 3). Although the former patient subsequently continued to receive the protocol treatment at a reduced dose level, the latter patient, who had a history of mild emphysema, discontinued the protocol treatment and switched to another chemotherapy regimen because of recurrent pneumonia after the first episode. At dose level 4, one patient had Grade 3 diarrhea and another had Grade 3 skin rash during the first cycle (Tables 1 and 3). Both of these reactions were classified as DLT. Both patients subsequently continued to receive the protocol treatment at a reduced dose level. Although diarrhea (≥Grade 1: 22%) and skin rash (≥Grade 1: 22%) developed in other patients at all dose levels, these reactions were mild and promptly resolved after appropriate medical treatment, such as antidiarrheal agents, antihistamines and steroids. As for hematological toxicity at dose level 4, Grade 3 neutropenia occurred in only one patient during the first cycle of treatment.

The MTD could not be determined on the basis of the toxic effects described above. The MTD was therefore estimated on the basis of toxic effects during the second cycle, as stipulated by the protocol. During the second cycle, no DLT occurred at any dose level. There was no difference in non-hematological toxicity between the first and the second cycles at any dose level. As for hematological toxicity, Grade 4 neutropenia developed in three of the four patients who received the second consecutive cycle of treatment at dose level 4 (Table 4). Although febrile neutropenia did not develop in any of these patients, we decided that dose level 4 was beyond the limits of tolerance. Two patients who had DLT during the first cycle of dose level 4 received subsequent cycles of treatment safely at a reduced dose level. At dose levels 1–3, there were no differences in hematological toxic effects between the first and the second cycles. Thus, the MTD and the RD were defined as dose level 3.

As for cumulative toxicity, four patients (22%) had Grade 2 sensory neuropathy after the second or subsequent cycles. In one of these patients, the protocol treatment was discontinued after the ninth cycle because of the prolonged Grade 2 sensory neuropathy. In another patient, the protocol treatment had to be withdrawn at the end of the second cycle because of Grade 3 sensory neuropathy. No other cumulative toxicity occurred at any dose level.

At dose levels 1–3, 85–90% of the initially planned doses of 5-FU, *l*-leucovorin and paclitaxel were administered (Table 5). At dose level 4, however, only ~75% of the

Table 3. Number of patients with toxicity during the first cycle according to the dose level

Toxicity	Dose level 1 ($n = 3$)				Dose level 2 ($n = 3$)				Dose level 3 ($n = 6$)				Dose level 4 ($n = 6$)			
	Grade															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Hematological																
Leukopenia	0	1	0	0	3	0	0	0	2	3	0	0	1	4	0	0
Neutropenia	1	1	0	0	1	1	0	0	1	2	1	0	0	4	1	0
Anemia	2	1	0	0	2	0	1	0	3	3	0	0	5	1	0	0
Thrombocytopenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Non-hematological																
Nausea	2	0	0	0	1	0	0	0	2	1	0	0	2	0	0	0
Diarrhea	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0
Stomatitis	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0
Rash	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0
Sensory neuropathy	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Hand-foot skin reaction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AST elevation	2	0	0	0	1	0	0	0	3	0	0	0	1	0	0	0
ALT elevation	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Infection (pneumonia)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 4. Number of patients with hematological toxicity during the second cycle according to the dose level

Toxicity	Dose level 1 ($n = 3$)				Dose level 2 ($n = 3$)				Dose level 3 ($n = 4^a$)				Dose level 4 ($n = 4^a$)			
	Grade															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	1	2	0	0	1	1	0	0	1	0	1	0	0	1	3	0
Neutropenia	1	2	0	0	0	1	1	0	0	1	1	0	0	0	1	3
Anemia	3	0	0	0	2	1	0	0	2	2	0	0	2	1	0	0
Thrombocytopenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Since two of the six patients at dose levels 3 and 4 had DLT during the first cycle, they received the second cycle at a reduced dose level. They were excluded from this table to accurately evaluate the toxicity of the second consecutive cycle at each dose level.

Table 5. Drug delivery and antitumor efficacy

Dose level	Number of patients	Total number of cycles administered	Relative dose intensity (median)			Overall response			
			5-FU	*l*-Leucovorin	Paclitaxel	CR	PR	SD	PD
1	3	24	0.90	0.90	0.90	0	2	1	0
2	3	25	0.88	0.88	0.88	0	3	0	0
3	6	28	0.85	0.85	0.85	0	3	2	1
4	6	30[a]	0.75	0.78	0.73	0	3	2	1

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
[a]Two patients at dose level 4 continued to receive the protocol treatment at the time of analysis.

initially planned doses were administered, supporting our decision to designate dose level 3 as the RD.

Efficacy

All patients were included in the evaluation of response. Objective tumor responses at each dose level are given in Table 5. Eleven patients (level 1, two; level 2, three; level 3, three and level 4, three) had partial responses, yielding an overall response rate of 61% (95% confidence interval, 36–83%). Although partial responses were obtained at all dose levels, no patient had a complete response.

The reasons for cessation of the protocol treatment were progressive disease in 12 patients (67%), sensory neuropathy in two (11%), skin rash in one (6%) and infectious pneumonia in one (6%). The median progression-free survival time and time-to-treatment failure were 6.8 and 6.3 months, respectively. At the time of analysis, six patients had died of tumor progression and two patients continued to receive the protocol treatment (the seventh and eighth cycle, respectively). The median survival time was not yet reached, and the median follow-up time was 9.6 months.

DISCUSSION

Recently, several randomized-controlled trials have suggested that the triple-drug combinations (2,4) or oral fluoropyrimidine-based regimens (3,5) are suitable as standard chemotherapy for advanced GC. However, such regimens are very toxic and can be tolerated only by the patients with adequate organ functions, making them unsuitable for many patients with advanced GC because of the poor performance status at initial diagnosis. Thus, further investigations of effective and less toxic regimens are warranted in patients with GC.

The toxicity profile of the FLTAX regimen, a combination of leucovorin-modulated weekly bolus 5-FU and weekly paclitaxel, was acceptable. Grade 3–4 toxicities were infrequent and the only Grade 4 toxicity during the first two cycles was neutropenia at dose level 4 (Tables 3 and 4). As expected, the addition of weekly paclitaxel (13,14) to a leucovorin-modulated weekly bolus 5-FU regimen (24) was associated with a modest increase in adverse events. The toxicity profile of the FLTAX regimen was similar to those in previous studies using different administration schedules for 5-FU/leucovorin and paclitaxel (22,25,26). The good toxicity profile of the FLTAX regimen might make it a viable alternative treatment for patients who cannot receive intensive triple-drug combination regimens. In patients who cannot tolerate more toxic regimens, the good toxicity profile of the FLTAX regimen might also permit the concurrent use of new targeted agents.

We combined a leucovorin-modulated weekly bolus 5-FU regimen with a weekly paclitaxel regimen for several reasons. First, to our knowledge, clinical trials of this combination regimen have not been reported, although it can be used on an outpatient basis. Because metastatic and recurrent GC is incurable, maintenance of an acceptable quality of life and avoiding repeated hospitalization to receive chemotherapy are very important issues. Second, a weekly regimen of paclitaxel is less toxic and has a higher dose-intensity than paclitaxel given once in every 3 weeks (12,27,28). Furthermore, a weekly paclitaxel schedule makes it possible to take advantage of the sequence-dependent synergistic cytotoxic effect of paclitaxel followed by 5-FU (18,19). Third, the leucovorin-modulated weekly bolus 5-FU incorporated in the FLTAX regimen is effective, and 5-FU-associated toxicity can be easily managed (29,30). Although protracted 5-FU infusion might be associated with less toxicity (31), protracted weekly or monthly 5-FU infusions require indwelling venous access devices and ambulatory pumps to permit treatment on an outpatient basis. Such lower toxicity should be balanced against the disadvantages presented by the use of these devices. Our study showed that the FLTAX regimen, which does not need such devices for outpatient treatment, was well tolerated with minimal toxicity. Fourth, although oral fluoropyrimidines, such as S-1 and capecitabine, have been evaluated in many clinical trials to determine whether these newer drugs can replace i.v. 5-FU (3,15–17), they cannot be used in some patients with advanced GC. For examples, tumors associated with obstruction of the pylorus or the cardia or with peritoneal invasion

cause dysphagia, nausea, vomiting, often precluding the administration of oral anticancer drugs as prescribed. Furthermore, diffuse peritoneal spread of disease frequently causes intestinal obstruction at other sites, a common characteristic of advanced GC.

In the present study, five patients (28%) experienced ≥Grade 2 sensory neuropathy and two of them (11%) had to discontinue protocol treatment due to neurotoxicity: one was typical Grade 2 cumulative toxicity after the ninth cycle and another was atypical Grade 3 sensory neuropathy in the second cycle. The incidence of Grade 3 neuropathy (6%) was identical with previous reports (22,28). In general, cumulative neurotoxicity of paclitaxel can be managed by dose reduction or postponement strategy as is successfully applied to the remaining three patients with Grade 2 sensory neuropathy in our study.

In conclusion, our study suggested that the FLTAX regimen would be well tolerated when used for the first-line treatment in patients with metastatic or recurrent GC. The MTD and the RD for phase II studies was dose level 3 (i.e., weekly bolus 5-FU, 600 mg/m^2; *l*-leucovorin, 250 mg/m^2 and weekly paclitaxel, 80 mg/m^2). Eleven of the 18 patients in this study had partial responses, suggesting that the FLTAX regimen is a promising treatment for advanced GC. A multicenter phase II study of the FLTAX regimen is now underway in patients with metastatic or recurrent GC, and a feasibility study of this regimen in patients with diffuse peritoneal spread of GC is being planned.

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Conflict of interest statement

None declared.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
2. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 2006;24:4991–7.
3. Boku N, Yamamoto S, Shirao K, Doi T, Sawaki A, Koizumi W, et al. Randomized phase III study of 5-fluorouracil (5-FU) alone versus combination of irinotecan and cisplatin (CP) versus S-1 alone in advanced gastric cancer (JCOG9912). *J Clin Oncol* 2007;25:18S. (suppl: abstr LBA4513).
4. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008;358:36–46.
5. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008;9:215–21.
6. Holmes FA, Walters RS, Theriault RL, Forman AD, Newton LK, Raber MN, et al. Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. *J Natl Cancer Inst* 1991;83:1797–805.
7. Einzig AI, Wiernik PH, Sasloff J, Runowicz CD, Goldberg GL. Phase II study and long-term follow-up of patients treated with taxol for advanced ovarian adenocarcinoma. *J Clin Oncol* 1992;10:1748–53.
8. Chang AY, Kim K, Glick J, Anderson T, Karp D, Johnson D. Phase II study of taxol, merbarone, and piroxantrone in stage IV non-small-cell lung cancer: The Eastern Cooperative Oncology Group Results. *J Natl Cancer Inst* 1993;85:388–94.
9. Ohtsu A, Boku N, Tamura F, Muro K, Shimada Y, Saigenji K, et al. An early phase II study of a 3-hour infusion of paclitaxel for advanced gastric cancer. *Am J Clin Oncol* 1998;21:416–9.
10. Yamada Y, Shirao K, Ohtsu A, Boku N, Hyodo I, Saitoh H, et al. Phase II trial of paclitaxel by three-hour infusion for advanced gastric cancer with short premedication for prophylaxis against paclitaxel-associated hypersensitivity reactions. *Ann Oncol* 2001;12:1133–7.
11. Rosenberg P, Andersson H, Boman K, Ridderheim M, Sorbe B, Puistola U, et al. Randomized trial of single agent paclitaxel given weekly versus every three weeks and with peroral versus intravenous steroid premedication to patients with ovarian cancer previously treated with platinum. *Acta Oncol* 2002;41:418–24.
12. Fennelly D, Aghajanian C, Shapiro F, O'Flaherty C, McKenzie M, O'Connor C, et al. Phase I and pharmacologic study of paclitaxel administered weekly in patients with relapsed ovarian cancer. *J Clin Oncol* 1997;15:187–92.
13. Arai T, Hamaguchi T, Shirao K, Shimada Y, Yamada Y, Muro K. Weekly paclitaxel in patients with heavily treated advanced gastric cancer. *Proc Am Soc Clin Oncol* 2003;22:321.
14. Hironaka S, Zenda S, Boku N, Fukutomi A, Yoshino T, Onozawa Y. Weekly paclitaxel as second-line chemotherapy for advanced or recurrent gastric cancer. *Gastric Cancer* 2006;9:14–8.
15. Koizumi W, Tanabe S, Saigenji K, Ohtsu A, Boku N, Nagashima F, et al. Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. *Br J Cancer* 2003;89:2207–12.
16. Inokuchi M, Yamashita T, Yamada H, Kojima K, Ichikawa W, Nihei Z, et al. Phase I/II study of S-1 combined with irinotecan for metastatic advanced gastric cancer. *Br J Cancer* 2006;94:1130–5.
17. Park YH, Kim BS, Ryoo BY, Yang SH. A phase II study of capecitabine plus 3-weekly oxaliplatin as first-line therapy for patients with advanced gastric cancer. *Br J Cancer* 2006;94:959–63.
18. Kano Y, Akutsu M, Tsunoda S, Ando J, Matsui J, Suzuki K, et al. Schedule-dependent interaction between paclitaxel and 5-fluorouracil in human carcinoma cell lines in vitro. *Br J Cancer* 1996;74:704–10.
19. Grem JL, Nguyen D, Monahan BP, Kao V, Geoffroy FJ. Sequence-dependent antagonism between fluorouracil and paclitaxel in human breast cancer cells. *Biochem Pharmacol* 1999;58:477–86.
20. Cascinu S, Ficarelli R, Safi MA, Graziano F, Catalano G, Cellerino R. A phase I study of paclitaxel and 5-fluorouracil in advanced gastric cancer. *Eur J Cancer* 1997;33:1699–702.
21. Bokemeyer C, Hartmann JT, Lampe CS, Clemens MR, Quietzsch D, Forkmann L, et al. Paclitaxel and weekly 24-hour infusion of 5-fluorouracil/folinic acid in advanced gastric cancer. *Semin Oncol* 1997;24:S19-96–S19-100.
22. Yeh KH, Lu YS, Hsu CH, Lin JF, Hsu C, Kuo SH, et al. Phase II study of weekly paclitaxel and 24-hour infusion of high-dose 5-fluorouracil and leucovorin in the treatment of recurrent or metastatic gastric cancer. *Oncology* 2005;69:88–95.
23. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
24. Arbuck SG, Douglass HO Jr, Trave F, Milliron S, Baroni M, Nava H, et al. A phase II trial of 5-fluorouracil and high-dose intravenous leucovorin in gastric carcinoma. *J Clin Oncol* 1987;5:1150–6.
25. Bokemeyer C, Lampe CS, Clemens MR, Hartmann JT, Quietzsch D, Forkmann L, et al. A phase II trial of paclitaxel and weekly 24 h infusion of 5-fluorouracil/folinic acid in patients with advanced gastric cancer. *Anticancer Drugs* 1997;8:396–9.
26. Murad AM, Petroianu A, Guimaraes RC, Aragao BC, Cabral LO, Scalabrini-Neto AO. Phase II trial of the combination of paclitaxel and 5-fluorouracil in the treatment of advanced gastric

cancer: a novel, safe, and effective regimen. *Am J Clin Oncol* 1999;22:580–6.
27. Klaassen U, Wilke H, Strumberg D, Eberhardt W, Korn M, Seeber S. Phase I study with a weekly 1 h infusion of paclitaxel in heavily pretreated patients with metastatic breast and ovarian cancer. *Eur J Cancer* 1996;32A:547–9.
28. Seidman AD, Hudis CA, Albanell J, Tong W, Tepler I, Currie V, et al. Dose-dense therapy with weekly 1-hour paclitaxel infusions in the treatment of metastatic breast cancer. *J Clin Oncol* 1998;16:3353–61.
29. Petrelli N, Herrera L, Rustum Y, Burke P, Creaven P, Stule J, et al. A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* 1987;5:1559–65.
30. Sasaki T, Ota K, Sakata Y, Matsuoka T, Wakui A, Akazawa S, et al. High-dose leucovorin and 5-fluorouracil in advanced gastric and colorectal cancer. High-Dose Leucovorin and 5-FU Study Group. *Gan To Kagaku Ryoho* 1990;17:2361–8.
31. Hsu CH, Yeh KH, Chen LT, Liu JM, Jan CM, Lin JT, et al. Weekly 24-hour infusion of high-dose 5-fluorouracil and leucovorin in the treatment of advanced gastric cancers. An effective and low-toxic regimen for patients with poor general condition. *Oncology* 1997;54:275–80.

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DISEASES OF THE ESOPHAGUS

Original article

Chemosensitivity of patients with recurrent esophageal cancer receiving perioperative chemotherapy

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SUMMARY. **Perioperative chemotherapy (CT) and chemoradiotherapy are widely used for advanced esophageal cancer. We evaluated the chemosensitivity of patients displaying recurrent esophageal cancer after esophagectomy with perioperative CT. From the database at National Cancer Center Hospital in Tokyo, we extracted recurrent esophageal cancer cases after perioperative CT and evaluated the effectiveness of the first CT against the recurrent disease according to the duration between termination of the original perioperative CT and recurrence with treatment-free intervals (TFIs) ≤6 and >6 months. Systemic CT for their recurrent disease was performed for 30 esophageal cancer patients after perioperative CT. All patients received 5-fluorouracil and cisplatin as perioperative CT, with relapses occurring at TFIs £6 months in 11 patients (eight received platinum-containing regimens and three received docetaxel for their recurrent disease) and >6 months in 19 patients (all received platinum-containing regimens). The response rate of patients experiencing a recurrence at TFIs ≤6 and >6 months was 0 and 37% ($P = 0.029$), the median progression-free survival was 2.8 and 4.8 months (log-rank $P = 0.001$) and the median overall survival was 6.1 and 10.2 months (log-rank $P = 0.012$), respectively. Recurrence at the TFI ≤6 months could represent resistance to CT, so regimens may need to be altered depending on a patient's specific TFI.**

KEY WORDS: **esophageal cancer, duration, chemosensitivity, adjuvant chemotherapy, neo-adjuvant chemotherapy, recurrent, perioperative chemotherapy.**

INTRODUCTION

Surgery remains the standard treatment for localized esophageal cancer,[1] but recent surgical series have reported that 5-year survival rates remain modest at <40%.[2-4] Such limited outcomes have prompted an evaluation of perioperative (preoperative or postoperative) chemotherapy (CT) and chemoradiotherapy (CRT).[5-7] Some phase III trials directly comparing either perioperative CT or CRT with surgery alone have shown the clinical benefits of both perioperative CT and CRT.[8-10] Perioperative CT and CRT have thus been increasingly chosen as a standard treatment recently.[11] Although multimodal therapy has been developed for localized esophageal cancer, outcomes have not yet been satisfactory. When recurrence is found, CT would typically be indicated, but response to CT has been poor so far for such patients.

It has been reported that the same regimen used in the first CT is effective when used the second time in treating ovarian cancer,[12,13] Hodgkin's lymphoma,[14] and small-cell carcinoma of the lung.[15] The duration between termination of the first CT and recurrence, or the 'treatment-free interval' (TFI), however, must be sufficiently long. This same consideration may also apply to patients with recurrence after perioperative CT. Patients with colorectal cancer frequently receive 5-fluorouracil (5-FU)-based adjuvant CT, and recurrence >6 months after adjuvant CT is thought to indicate sensitivity to 5-FU, so the same 5-FU-based regimen is generally used for any recurrent disease.

Perioperative CT and CRT are widely used for advanced esophageal cancer, but solid evidence has not yet been presented regarding the optimal choice of treatment when considering the TFI for recurrent esophageal cancer patients. Given this background,

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we evaluated the chemosensitivity of patients with recurrent esophageal cancer who received perioperative CT.

MATERIALS AND METHODS

From the database of the National Cancer Center Hospital in Tokyo, we extracted cases involving distant metastasis of recurrent esophageal cancer (gastroesophageal junction cancer patients were not involved) after perioperative CT and evaluated the response rate (RR), progression-free survival (PFS) and overall survival (OS) for first CT against recurrent disease by dividing cases according to TFIs ≤6 and >6 months. In this study, cases with CRT or radiotherapy alone were excluded for two reasons. First, the purpose of this study was to investigate whether differences existed in sensitivity to CT depending on a patient's TFI. Second, patients who received radiotherapy displayed local recurrences and had prognoses different from the CT cases with distant metastasis evaluated in this study.

Preoperative chemotherapy

Although we usually administered postoperative adjuvant CT for patients after oesophagectomy, we conducted a trial of neoadjuvant CT for stage II and stage III patients who were included in this study. The treatment protocol was comprised of two cycles of 5-FU 800 mg/m^2 by continuous intravenous administration on days 1–5 and cisplatin (CDDP) 80 mg/m^2 administered intravenously on day 1 every 4 weeks. Surgery was performed within 5 weeks after finishing a patient's preoperative CT.

Surgery

Patients underwent right or left thoracotomy for curative resections by total or subtotal thoracic oesophagectomy in addition to receiving regional lymphadenectomy. Regional lymph nodes included mediastinal (paraesophageal, paratracheal, subcarinal, supradiaphragmatic, and posterior mediastinal) and perigastric. As a result, at least a two-field lymphadenectomy was performed on every patient with the dissection of distant lymph nodes, such as cervical nodes (cervical paraesophageal, deep cervical and supraclavicular lymph nodes) or celiac nodes, which represented a three-field lymphadenectomy, also included in the present study.

Postoperative chemotherapy

We provided adjuvant CT for stage II and stage III esophageal cancer patients who had undergone curative resections. Adjuvant CT was started within 10 weeks after surgery. The treatment protocol was 5-FU 800 mg/m^2 by continuous intravenous administration on days 1–5 and CDDP 80 mg/m^2 administered intravenously on day 1. Two cycles of this regimen were administered every 4 weeks.

Follow-up after perioperative chemotherapy

For 4 years after surgery or CT, patients underwent physical examination every 3 months. Cervical, chest, and abdominal computerized tomography or magnetic resonance imaging were performed every 6 months. If symptoms were present between examinations, additional examinations were added as needed.

Statistical analysis

TFI was defined as extending from the last day of perioperative CT to the actual identification of recurrent disease. The RR for recurrent disease was assessed every 2 months using the Response Evaluation Criteria in Solid Tumors. In cases of partial or complete response, a second assessment 4 weeks later was required for confirmation of such a response.

PFS was measured from the initiation of CT after recurrence to whichever occurred first, either the date of detection of the first regrowth of a subsequent recurrent disease or death resulting from any cause. For any patient who did not relapse or die, PFS was censored as of the last date on which absence of progression was confirmed for such patient. OS was measured from the initiation of CT after recurrence to either the latest follow-up date or death and was censored as of the last date of contact for all surviving patients.

All P-values were two-sided and values of $P < 0.05$ were regarded as significant. Fisher's exact test was used to compare the RR between the two groups, and the exact confidence interval (CI) for binomial proportions was used to estimate intervals. PFS and OS were estimated using the Kaplan-Meier method, and the two groups were compared using the log-rank test. Statistical analyses were performed using SAS version 9.1.3 software (SAS Institute, Cary, NC, USA).

RESULTS

Perioperative CT was administered to a total of 92 patients from January 1999 to January 2007, with all patients receiving 5-FU and CDDP. Recurrence was found in 46 of these patients and 30 of them received systemic CT. The TFIs were ≤6 months for 11 patients and >6 months for 19 patients (Fig. 1). Patient characteristics at the start of CT for recurrence are shown in Table 1. Characteristics were well balanced for the two groups. For the TFI ≤6 months,

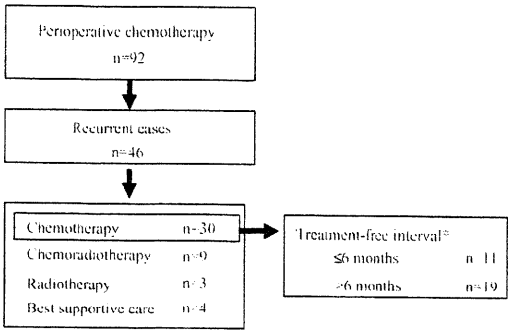


Fig. 1 Treatment schema. *duration from the last day of perioperative chemotherapy to identification of recurrence.

Table 1 Patient characteristics at start of chemotherapy for recurrence

	Treatment-free interval†		
	≤6 months	>6 months	Total
n	11	19	30
Sex			
Male	11	18	29
Female	0	1	1
Age (years)			
Median	59	61	
Range	46–69	48–70	
AJCC clinical stage			
II	2	3	5
III	8	15	23
IV	1	1	2
Histology			
Well-differentiated SCC	4	5	9
Moderately differentiated SCC	4	6	10
Poorly differentiated SCC	3	8	11
Perioperative chemotherapy			
Preoperative	3	3	6
Postoperative	8	16	24
ECOG performance status			
0	3	6	9
1	7	11	18
2	1	2	3
Site of relapse			
Lymph node	8	14	22
Lung	3	4	7
Liver	1	3	4

†duration from last day of perioperative chemotherapy to identification of recurrence. 1 AJCC, American Joint Committee on Cancer; ECOG, European Clinical Oncology Group; SCC, squamous cell carcinoma.

eight patients received a platinum-containing regimen while the other three patients received docetaxel for their recurrent disease. Comparatively, all 19 recurrent disease patients with the TFI >6 months received a platinum-containing regimen, and 12 of these patients received the same 5-FU plus CDDP (FP) regimen administered during perioperative CT (Table 2).

The median follow-up period after recurrence was 47 months (range, 12–78 months). The RR for all patients was 23% (7/30), and all seven responders displayed recurrence with the TFI >6 months. The RR for TFIs ≤6 and >6 months was 0% (0/11) (95%CI, 0–29%) and 37% (7/19) (95%CI, 38.4–83.7%) ($P = 0.029$), respectively (Table 3). The median PFS was 2.3 months (95%CI, 0.8–3.7 months) and 4.8 months (95%CI, 2.6–7.4 months; log-rank, $P = 0.001$), respectively (Fig. 2), and the median OS was 6.1 months (95%CI, 4.3–6.4 months) and 10.2 months (95%CI, 5.8–22 months; log-rank, $P = 0.012$), respectively (Fig. 3).

DISCUSSION

Patients with ovarian cancer, Hodgkin's lymphoma and small-cell carcinoma of the lung who responded to CT and experienced a sufficiently long TFI before initiation of the second-line CT regimen are considered more likely to respond again to the same drug used in the initial treatment.[12–15] Patients with ovarian cancer who responded to CT and then experienced a relapse after a platinum-free interval >6 months were considered platinum-sensitive, and the likelihood of achieving a secondary response increases directly with an increase in the duration of the platinum-free interval.[12,13] No solid clear evidence on the choice of optimal treatment had been reported regarding TFI for advanced esophageal cancer, however, so we decided to evaluate the chemosensitivity of patients with recurrent esophageal cancer who received perioperative CT.

This study demonstrated poor results for patients with recurrence and a TFI ≤6 months. Although

Table 2 Treatment regimen after recurrence

	Treatment-free interval†			
	≤6 months	(*n* = 11)	>6 months	(*n* = 19)
Platinum-containing regimen		8		19
	5FU + CDDP	4	5FU + CDDP	12
	5FU + NDP	3	5FU + NDP	5
	VDS + NDP	1	VDS + NDP	2
Nonplatinum-containing regimen		3	-	0
	docetaxel	3	-	0

†duration from last day of perioperative chemotherapy to identification of recurrence. 5FU, 5-fluorouracil; CDDP, cisplatin; NDP, nedaplatin; VDS, vindesin.

Table 3 Response rate against recurrence

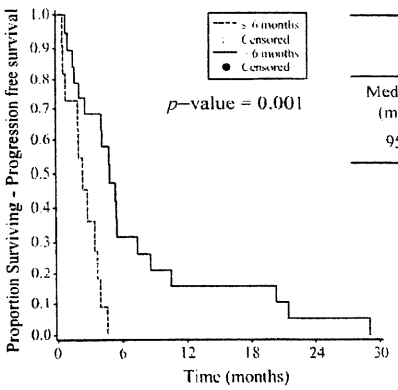
	Treatment-free interval†						
	≤6 months (n = 11)			>6 months (n = 19)			
	total	pre‡	post§	total	pre‡	post§	
CR	0			1		1	
PR	0			6	1	5	
SD	5	1	4	7	2	5	
PD	6	2	4	5	1	4	
Response rate (%)	0			36.8			P = 0.029
95%CI (%)	0–28.5			38.4–83.7			

†duration from last day of perioperative chemotherapy to identification of recurrence; ‡patients who recieved preaopelative chemotherapy; §patients who recieved postopelative chemotherapy.

eight of these patients received the same platinum-containing regimen used for their perioperative CT, and the three other patients received docetaxel as a different regimen from their perioperative CT. the RR was 0% (0/11) for all cases with a TFI ≤6 months. This negative outcome seems to indicate that both are considered to indicate resistance against the same regimen as the perioperative CT. as well as a possible multidrug resistance when the TFI ≤6 months.

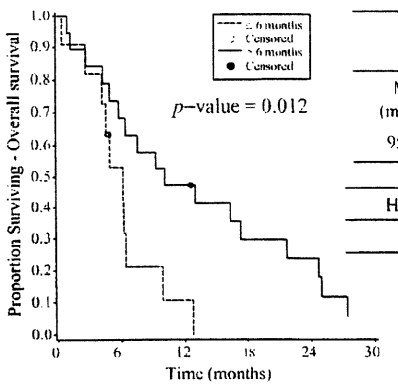
The RR for FP regimens in chemo-naïve metastatic esophageal cancer has been reported to be about 35–40%.[16–18] In this study, the RR for cases of recurrence with a TFI >6 months was 37% (7/19). This outcome indicates that cases with a TFI >6 months may be sensitive to platinum and/or 5-FU with RR results equivalent to the chemo-naïve cases.

Patients who relapsed with a TFI >6 months displayed significantly longer PFS and OS. although one



	Treatment–free interval	
	≤6 months	>6 months
Median PFS (months)	2.3	4.8
95%CI	0.8–3.7	2.6–7.4

Fig. 2 Progression-free survival (PFS). *duration from the last day of perioperative chemotherapy to identification of recurrence.



	Treatment–free interval	
	≤6 months	>6 months
MST (months)	6.1	10.2
95%CI	4.3–6.4	5.8–21.7

Hazard ratio	95%CI
0.330	0.133–0.813

Fig. 3 Overall survival. *duration from the last day of perioperative chemotherapy to identification of recurrence. CI, confidence interval; MST, median survival time.

possibility for this is that earlier relapse may represent another indicator of poor prognosis. These outcomes suggest that chemosensitivity correlates directly to a patient's TFI.

Few effective medications are available for esophageal cancer, and the same medication is often used in cases of recurrence, but patients displaying recurrence with a TFI ≤6 months may require different medications. We cannot confirm this supposition, however, based on the results with such a small sample size, but the present retrospective study so a prospective clinical trial appears warranted.

We evaluated the chemosensitivity of patients with recurrent esophageal cancer who received perioperative CT. Patients with a TFI >6 months showed the same RR as chemo-naive patients. Comparatively, patients with a TFI ≤6 months, included no responders, suggesting the possibility of resistance against CT particularly for the same FP as used for the perioperative CT. It may be necessary to change regimens, therefore, depending on a patient's specific TFI.

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References

1 Mitchell C P, Arlene A F, Bruce D M. Cancers of the gastrointestinal tract. In: DeVita V T Jr, Hellman S, Rosenberg S A, (eds). Cancer: Principles and Practice of Oncology, 7th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2005: 861–09.
2 Orringer M B, Marshall B, Iannettoni M D. Transhiatal esophagectomy: clinical experience and refinements. Ann Surg 1999; 230: 392–400.
3 Dudhat S B, Shinde S R. Transhiatal esophagectomy for squamous cell carcinoma of the esophagus. Dis Esophagus 1998; 11: 226–30.
4 Bolton JS, Teng S. Transthoracic or transhiatal esophagectomy for cancer of the esophagus – does it matter? Surg Oncol Clin N Am 2002; 11: 365–75.
5 Carey R W, Hilgenberg A D, Wilkins E W *et al.* Long-term follow-up of neoadjuvant chemotherapy with 5-fluorouracil and cisplatin with surgical resection and possible postoperative radiotherapy and/or chemotherapy in squamous cell carcinoma of the esophagus. Cancer Invest 1993; 11: 99–105.
6 Ajani J A, Ryan B, Rich T A *et al.* Prolonged chemotherapy for localized squamous carcinoma of the oesophagus. Eur J Cancer 1992; 28A: 880–4.
7 Bosset J F, Gignoux M, Triboulet J P *et al.* Chemoradiotherapy followed by surgery compared with surgery alone in squamous-cell cancer of the esophagus. N Engl J Med 1997; 337: 161–7.
8 Walsh T N, Noonan N, Hollywood D *et al.* A comparison of multimodal therapy and surgery for esophageal adenocarcinoma. N Engl J Med 1996; 335: 462–7.
9 Urba S G, Orringer M B, Turrisi A, Kelly A, Keeling N, Hennessy TP. Randomized trial of preoperative chemoradiation versus surgery alone in patients with locoregional esophageal carcinoma. J Clin Oncol 2001; 19: 305–13.
10 Ando N, Iizuka T, Ide H *et al.* Surgery plus chemotherapy compared with surgery alone for localized squamous cell carcinoma of the thoracic esophagus: a Japan Clinical Oncology Group study – JCOG9204. J Clin Oncol 2003; 21: 4592–6.
11 Greil R, Stein H J. Is it time to consider neoadjuvant treatment as the standard of care in oesophageal cancer? Lancet Oncol 2007; 8: 189–90.
12 Markman M, Rothman R, Hakes T *et al.* Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin. J Clin Oncol 1991; 19: 389–93.
13 Markman M, Markman J, Webster K *et al.* Duration of response to second-line, platinum-based chemotherapy for ovarian cancer: implications for patient management and clinical trial design. J Clin Oncol 2004; 22: 3120–5.
14 Fisher R I, DeVita V T, Hubbard S P. Prolonged disease-free survival in Hodgkin's disease with MOPP reinduction after first relapse. Ann Intern Med 1979; 90: 761–3.
15 Batist G, Ihde D C, Zabell A *et al.* Small-cell carcinoma of the lung: reinduction therapy after late relapse. Ann Intern Med 1983; 98: 472–4.
16 Hayashi K, Ando N, Watanabe H *et al.* Phase II evaluation of protracted infusion of cisplatin and 5-fluorouracil in advanced squamous cell carcinoma of the esophagus: a Japan Esophageal Oncology Group (JEOG) trial (JCOG9407). Jpn J Clin Oncol 2001; 31: 419–23.
17 Bleiberg H, Conroy T, Paillot B *et al.* Randomised phase II study of cisplatin and 5-fluorouracil (5-FU) versus cisplatin alone in advanced squamous cell oesophageal cancer. Eur J Cancer 1997; 33: 1216–20.
18 Ross P, Nicolson M, Cunningham D *et al.* Norman A: Prospective randomized trial comparing mitomycin, cisplatin, and protracted venous-infusion fluorouracil (PVI 5-FU) with epirubicin, cisplatin, and PVI 5-FU in advanced esophagogastric cancer. J Clin Oncol 2002; 20: 1996–104.

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Original Article

Multicenter Phase II Study of Cetuximab Plus Irinotecan in Metastatic Colorectal Carcinoma Refractory to Irinotecan, Oxaliplatin and Fluoropyrimidines

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Objective: Cetuximab is a chimeric IgG1 monoclonal antibody that specifically blocks the epidermal growth factor receptor. We evaluated the efficacy and safety of cetuximab in combination with irinotecan in patients with metastatic colorectal cancer (CRC) refractory to irinotecan, oxaliplatin and fluoropyrimidines.

Methods: Cetuximab was administered initially at a dose of 400 mg/m^2 followed by weekly infusions at 250 mg/m^2. Irinotecan was administered either weekly at a dose of 100 mg/m^2 or every 2 weeks at 150 mg/m^2.

Results: Between October 2005 and February 2006, 39 consecutive patients were enrolled. The response and disease control rates (complete or partial response, or stable disease) were 30.8% (95% CI, 17.0–47.6) and 64.1% (95% CI, 47.2–78.8), respectively. With a median follow-up of 14.4 months, median time to progression was 4.1 months (95% CI, 2.7–5.1) and median survival time was 8.8 months (95% CI, 5.9–12.8). Patients (5.1%) developed Grade 3 acne-like rash.

Conclusions: Combination therapy of cetuximab and irinotecan is effective and well-tolerated in patients with metastatic CRC refractory to irinotecan, oxaliplatin and fluoropyrimidines.

Key words: cetuximab – irinotecan – colorectal cancer – multicenter phase II study

INTRODUCTION

Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide (1). Palliative treatment of metastatic CRC is based on chemotherapy with 5-fluorouracil, irinotecan and oxaliplatin. Co-administration of these three drugs has substantially increased median overall survival (OS), from 12 months obtained several years ago to about 21–22 months today (2,3). Further, a recent meta-analysis of seven phase III trials in advanced CRC has shown that median OS is significantly correlated with the proportion of patients receiving all active agents during the disease course (4).

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Against this, however, treatment options for metastatic CRC patients with disease refractory to all three antitumor drugs are limited.

The addition of bevacizumab, a recombinant humanized monoclonal antibody, which targets vascular endothelial growth factor, to front- or second-line chemotherapy has improved progression-free survival (PFS) and OS (5). However, the clinical benefits of bevacizumab in third-line therapy have yet to be shown. In this regard, the National Comprehensive Cancer Network (NCCN) clinical practice guidelines (6,7) do not recommend bevacizumab as third-line therapy.

Cetuximab, a chimeric antibody of the IgG1 subclass, blocks the binding of the epidermal growth factor (EGF) and transforming growth factor alpha to the EGF receptor (EGFR) and inhibits ligand-induced activation of this receptor tyrosine kinase (8). Cetuximab has demonstrated antitumor activity in both *in vivo* and *in vitro* study (8). In addition, this agent not only enhanced the effects of irinotecan and radiotherapy (9) but also showed the ability to reverse resistance to irinotecan in preclinical studies (10).

Two previous studies have investigated the use of combination therapy of cetuximab plus irinotecan in subjects with irinotecan-refractory disease; the first, a single-arm Phase II trial, reported a response rate (RR) of 17% in 121 patients (11), the second, a randomized Phase II study of single agent cetuximab or cetuximab plus irinotecan, the BOND trial, reported a RR of 10.8% (111 subjects) versus 22.9% (218), respectively (12). Moreover, response in the combination arm in those who had received oxaliplatin was 22.2%. These results suggest that combination therapy of cetuximab plus irinotecan may benefit subjects refractory to irinotecan-based chemotherapy who have received oxaliplatin-based chemotherapies.

At the time the present study was undertaken, neither cetuximab nor bevacizumab was approved in Japan. Standard management of CRC instead consisted of the use of irinotecan, oxaliplatin or fluoropyrimidines, with no other standard options available after progression on these drugs. We therefore conducted a multicenter phase II study of cetuximab plus irinotecan in metastatic CRC refractory to irinotecan, oxaliplatin and fluoropyrimidines to evaluate the efficacy and safety of this combination.

PATIENTS AND METHODS

Study Design and Patient Eligibility

The study was designed as a phase II, non-randomized, open-label, multicenter trial. Eligibility requirements included histologically confirmed, metastatic CRC that was surgically unresectable, as well as immunohistochemical evidence of EGFR expression measured semiquantitatively (>0 on a scale of 0, 1+, 2+ or 3+) at a single reference laboratory (SRL Medisearch, Inc.). Patients were required to have received irinotecan-based chemotherapy at a weekly irinotecan dose of $\geq$60 mg/m^2 or every 2 weeks at $\geq$100 mg/m^2, both defined as final doses, for at least 6 weeks or more. They were also required to have radiographically documented evidence of disease progression during this previous chemotherapy or within 3 months following the last dose of irinotecan. Further, patients were required to have received and failed fluoropyrimidine- and oxaliplatin-based chemotherapies. For this requirement, failure was defined as progression of disease (clinical or radiological) while receiving the previous oxaliplatin-based chemotherapy or within 6 months following the last treatment of an adjuvant therapy, or intolerance to the oxaliplatin-based chemotherapy. Regarding intolerance, this was defined as discontinuation due to allergic reaction, persistent neurotoxicity or delayed recovery from other toxicity that prevented re-treatment.

Other eligibility criteria included age 20 to less than 75 years, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0–2, life expectancy of at least 2 months, and adequate organ function (absolute neutrophil count $\geq$1500/mm^3, platelet count $\geq$100 000/mm^3, hemoglobin $\geq$9 g/dl, aspartate aminotransferase and alanine aminotransferase $\leq$2.5 times the upper limit of normal range, serum total bilirubin $\leq$1.5 times the upper limit of normal range and serum creatinine $\leq$1.5 mg/dl). Minimum treatment-free periods between the end of prior therapy and day of registration were 6 weeks for radiation therapy, 4 weeks for major surgery and 4 weeks for chemotherapy. At least one unidimensionally measurable target lesion by Response Evaluation Criteria in Solid Tumors (RECIST) criteria was required. Patients who previously received EGF signal transduction inhibitors or EGFR-targeting therapy were not eligible.

This protocol was reviewed and approved by the institutional review board of each participating center, and all patients gave written informed consent before participation.

Dosage and Drug Administration

The initial dose of cetuximab was administered as a single 2-h intravenous infusion at 400 mg/m^2 followed by weekly 1-h infusions of 250 mg/m^2. All patients were premedicated with an H1 histamine antagonist (e.g. diphenhydramine hydrochloride 50 mg po). Irinotecan was administered under the same schedule as the previous irinotecan-based therapy, namely either weekly at a dose of 100 mg/m^2 on Days 1, 8, 15 and 22, repeated every 6 weeks; or every 2 weeks at 150 mg/m^2 on Days 1, 15 and 29, repeated every 7 weeks.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0.). If a patient experienced Grade 3 or worse skin toxicity, cetuximab therapy was withheld for up to two consecutive infusions with no subsequent change in dose level. If the toxicity resolved to Grade 2 or less by the following treatment period, treatment was resumed. With the second and third occurrences of Grade 3 or worse skin toxicity, cetuximab therapy was delayed again for up to two consecutive weeks with concomitant dose reductions to 200 and 150 mg/m^2, respectively. Treatments were discontinued if

more than two consecutive infusions were withheld or if there was a fourth occurrence of Grade 3 or worse skin toxicity. If a patient experienced a Grade 3 or worse hypersensitivity reaction, treatments were immediately discontinued. If a patient experienced a Grade 2 hypersensitivity reaction, cetuximab infusion was stopped, and if the reaction resolved to Grade 1 or less, it was resumed at half the previous infusion rate. Dose modification and treatment alterations for irinotecan were performed in accordance with hematological and non-hematological toxicities. If a patient experienced a Grade 4 thrombocytopenia or Grade 3 or worse neuropathy, irinotecan therapy was stopped. If a patient experienced a Grade 3 or worse febrile neutropenia, thrombocytopenia, or non-hematological toxicity, or Grade 4 neutropenia, irinotecan dose was reduced by one dose level.

Evaluation of Patients

Medical history, physical examination, laboratory test assessments and safety assessments were performed once before starting treatment and weekly thereafter. Chest X-ray was taken every 6 weeks.

Tumor measurement was performed within 4 weeks prior to starting administration of study therapy. Response was evaluated every 6 weeks thereafter according to the RECIST criteria. All responses were confirmed by an independent review committee.

Statistical Analysis

A sample size of 38 response-evaluable subjects was established based on expected and threshold RRs of 20 and 5%, respectively, under conditions of $\alpha = 0.05$ (one-tailed) if the RR was lower than the threshold RR, and $\beta \geq 0.1$ if higher. The primary endpoint was the RR. If 38 subjects were response-evaluable, the null hypothesis would be rejected if at least five responses were observed. A patient who received at least one dose of study therapy was considered evaluable for response. Secondary endpoints, including duration of response and time to progression (TTP), were estimated using the Kaplan–Meier method.

TTP was defined as the period from the date treatment was started to the first observation of disease progression or to death from any cause before the confirmation of disease progression or after the most recent tumor assessment.

OS time was determined from the date of first administration of chemotherapy to the date of death or the last confirmation of survival. Duration of response was considered the period between the date of first confirmation of response and the date of documented disease progression, or the last confirmation of response. Comparisons among different subgroups of patients were performed using the log-rank test, and RR was the Fisher exact test. All analyses were conducted using SAS software (version 8; SAS Institute, Cary, NC, USA).

RESULTS

Patient Characteristics

Forty-four of 46 centrally screened patients demonstrated detectable expression of EGFR. Of these 44, 39 consecutive patients were enrolled between October 2005 and February 2006. All patients received combination treatment with irinotecan and cetuximab. Patient characteristics are summarized in Table 1 and show a median age of 58 years, male predominance and good performance status. More than 60% ($n = 25$) of patients had received three or more regimens before entry.

All patients had been refractory to irinotecan-based regimens and had received oxaliplatin and fluoropyrimidine before participation in the study. The median duration of

Table 1. Patient characteristics

Patient characteristics	Number of patients (%)
Total number of patients	39
Sex	
Male	27 (69.2)
Female	12 (30.8)
Age	
Median	58.0
Range	32–72
Performance status (ECOG)	
PS 0	26 (66.7)
PS 1	13 (33.3)
Primary tumor site	
Colon	18 (46.2)
Rectum	21 (53.8)
Sites of metastases	
Liver	30 (76.9)
Lung	27 (69.2)
Lymph nodes	17 (43.6)
Peritoneum	5 (12.8)
Rectum	2 (5.1)
Other	6 (15.4)
Number of prior chemotherapy	
2	14 (35.9)
3	11 (28.2)
4	6 (15.4)
>5	8 (20.5)
EGFR expression	
1+	29 (74.4)
2+	10 (25.6)

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.

prior irinotecan-based therapy was 24 weeks (range 6–52 weeks). Responses to irinotecan-based regimen were 2.6% complete response (CR), 23.1% partial response (PR), 41% stable disease (SD) and 30.8% progression of disease (PD). Median time from the last pre-study irinotecan dose to the initiation of cetuximab therapy was 7.2 months, with a range of 1.0–18.3 months. Thirty-five patients (89.7%) discontinued oxaliplatin-based regimens for disease progression, with a median of 2.1 months after last treatment. Response to the most recent oxaliplatin-based regimen was 0% CR, 5.1% PR, 46.2% SD and 46.2% PD.

Among other characteristics, 12 patients (30.8%) had received adjuvant chemotherapy; 34 (87.2%) had undergone surgical resection of the primary tumor; 20 (51.3%) had recurrent disease; and 19 (48.7%) had advanced disease. Two patients had received radiotherapy for CRC.

Efficacy

All patients enrolled were considered evaluable for efficacy (Table 2). An independent review committee determined that 12 patients (30.8%; 95% CI, 17.0–47.6) achieved PRs, and 25 (64.1%; 95% CI, 47.2–78.8) achieved either PRs or SDs (disease control). Median duration of response was 5.4 months (95% CI, 3.9–6.4), and median time to response was 1.4 months (range 1.3–5.3). Median TTP was 4.1 months (95% CI, 2.7–5.1) (Fig. 1). With a median follow-up of 14.4 months, the median OS was 8.8 months (95% CI, 5.9–12.8) (Fig. 2) (Table 2).

No association with response was seen for either age, sex, performance status, number of prior chemotherapy regimens, primary tumor site or degree of EGFR immunostaining. In contrast, RR was significantly higher in patients who had achieved a response in a prior irinotecan regimen than in those who had not [($P = 0.04$ Fisher exact test, 60.0% (95% CI, 26.2–87.8) versus 20.7% (95% CI, 10.2–48.4)]. Moreover, median OS of patients who had achieved a response in a prior irinotecan regimen was slightly longer than that of those who had not, albeit without significance (9.9 versus 8.8 months, $P = 0.92$; log-rank test).

The presence and severity of rash did not correlate with objective response (RR with Grade 0–1 versus Grade 2–3: 31.6 versus 30.0%, $P = 0.73$; Fisher exact test).

Table 2. Efficacy

Best response	Patients ($N = 39$) [No. (%)]
CR	0 (0)
PR	12 (30.8)
SD	13 (33.3)
PD	14 (35.9)
Response	12 (30.8)
95%CI	17.0–47.6
Disease control	25 (64.1)
95%CI	47.2–78.8
Median TTP	4.1 months
95%CI	2.7–5.1
Median OS	8.8 months
95%CI	5.9–12.8

CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease; CI, confidence interval; TTP, time to progression; OS, overall survival.

Adverse Events

Major adverse events are shown in Table 3. Overall, hematological toxicities were generally well tolerated, with Grade 3 or worse neutropenia observed in 23.1% of patients ($n = 9$). Febrile neutropenia was not observed. The incidence of Grade 3 or worse neutropenia was higher in patients receiving irinotecan every 2 weeks than in those on weekly regimens (27.6 versus 10.0%).

Among other adverse events, non-hematological toxicities were also mild, with Grade 3 or worse diarrhea and anorexia observed in seven (17.9%) and six patients (15.4%), respectively. An acne-like rash, which is characteristic of treatment with cetuximab and other EGFR-targeted therapies and included acne, rash, dry skin, pruritus, acneiform dermatitis and papular rash, was observed in 38 patients (97.4%). Median time to the appearance of cetuximab-related acne-like rash was 7.0 days (range 1–31), while the median cumulative dose of cetuximab until appearance was 400 mg/m^2 (range 400–1400) and duration of appearance was 121.5 days. Two patients (5.1%) experienced Grade 3 acne, with a duration of appearance of 10 and 15 days, respectively. All patients received topical treatment and 19 (51.4%) received oral antibiotic drugs, including minocycline. Median duration from the appearance of an acne-like rash to the start of antibiotic drugs, including topical treatment and minocycline, was 5.7 days. No patient experienced allergic reactions leading to the cessation of therapy. One patient discontinued treatment due to Grade 1 lung fibrosis, a condition that has never shown exacerbation without medication.

Duration of Treatment and Dose Intensity of Cetuximab and Irinotecan

The median duration of cetuximab treatment was 18 weeks (range 6–50 weeks), with a median of 16 infusions per patient (range 4–49 infusions). No patient required a dose reduction. Median dose intensity was 232 mg/m^2 per week (range 150–254 mg/m^2); 24 patients received doses at more than 90% of relative dose intensity, nine within 80–90% and six within 60–80%.

Fourteen patients (35.9%) required a dose reduction of irinotecan, primarily due to diarrhea (seven patients) and neutropenia (two patients). The median duration of treatment in all patients was 15 weeks (range 2–49), with a median of seven infusions per patient (range 2–30 infusions). Median