

Figure 1 Cellular metabolism and mechanism of gemcitabine. For explanation of symbols and metabolic routes, see text.

(SLC29A1) and ENT2 (SLC29A2)) and three concentrative nucleoside transporters (CNTs; CNT1 (SLC28A1), CNT2 (SLC28A2), and CNT3 (SLC28A3)) (Mini et al, 2006). Kinetic studies of human cell lines have shown that gemcitabine intracellular uptake is mediated mainly by ENT1 and, to a lesser extent, by CNT1 and CNT3.

The reported allele frequencies of nucleoside transporter gene variants are generally low except *ENT1* -706 G>C in Caucasians and *ENT1* -1050 G>A in Africans, as shown in Table 1 (Osato et al, 2003; Damaraju et al, 2005; Kim et al, 2006; Myers et al, 2006). To date, it is unclear whether these genetic variants of nucleoside transporter genes including *ENT1* contribute to interindividual differences in response to gemcitabine. The functional analyses of the two nonsynonymous SNPs of *ENT1* (SLC29A1 647T>C and 1171G>A) and the three nonsynonymous SNPs of *CNT3* (SLC28A3 14G>A, 391 C>T, and 1538A>T) failed to demonstrate functional diversity (Osato et al, 2003; Damaraju et al, 2005). On the other hand, a recent study found that individuals with CGG/CGC haplotypes based on the three SNPs in the promoter region of *ENT1* (SLC29A1 -1345C>G, -1050 G>A, -706G>C) showed 1.37-fold higher median expression of the *ENT1* transcript than those with the common CGG/CGG haplotypes, suggesting that *ENT1* promoter region variants may influence gene expression and alter gemcitabine chemosensitivity (Myers et al, 2006).

As to expression, several studies have suggested that *ENT1* expression of mRNA/proteins in tumour tissues may be a good predictive marker of outcome in cancer patients receiving gemcitabine. Spratlin et al (2004) performed an immunohistochemical study on paraffin-embedded tumour tissues from 21 patients with pancreatic cancer and reported that overall survival was significantly longer in those expressing detectable amounts of *ENT1* in tumour blocks than in those with low or absent *ENT1* following gemcitabine treatment (median, 13 months vs 4 months; $P=0.01$). Polymerase chain reaction analysis of 81 patients with pancreatic cancer also showed that those with high *ENT1* mRNA expression in the tumour specimens had significantly longer survival after gemcitabine therapy than patients with low *ENT1* levels (median, 25.7 vs 8.5 months; $P<0.001$) (Giovannetti et al, 2006). Similar results were obtained in a study of 12 bladder cancer patients treated with gemcitabine, which demonstrated that the mean level of *ENT1* mRNA in tumour specimens was significantly higher in patients achieving a complete pathological response than in those with stable disease (1.166 vs 1.021; $P=0.040$) (Mey et al, 2006). These results

suggest that tumour-specific expression of *ENT1* may be a promising predictive biomarker of outcome after gemcitabine treatment, although formal validation in prospective studies is needed.

CYTIDINE DEAMINASE

Cytidine deaminase is involved in the salvaging of pyrimidines, and plays a key role in detoxifying gemcitabine. Therefore, patients with impaired CDA activity might develop strong toxicities after administration of gemcitabine, while CDA overexpression in tumour tissues might reduce the antitumour efficacy of this drug. An *in vitro* study has demonstrated resistance to gemcitabine in cells overexpressing CDA (Neff and Blau, 1996).

So far, two nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), have been identified in the coding region of the human CDA gene (Yue et al, 2003; Fukunaga et al, 2004; Gilbert et al, 2006; Sugiyama et al, 2007). Ethnic or racial differences in the allele frequencies of these SNPs have been reported, as shown in Table 1. Remarkable reduction in activity of 70Thr CDA was reported *in vitro* (Yue et al, 2003) and *in vivo* (Sugiyama et al, 2007), while only marginal reduction in activity of 27Gln CDA was observed *in vitro* (Yue et al, 2003; Gilbert et al, 2006). On the other hand, Fitzgerald et al (2006) investigated SNPs in the promoter region of *CDA* *in vitro* and *in vivo*, and found that some promoter *CDA* haplotypes might affect CDA activity.

With regard to the correlation between CDA SNPs and clinical outcome, we have recently carried out a prospective pharmacogenomic study in cancer patients treated with gemcitabine (Sugiyama et al, 2007). In that study, 256 Japanese patients who had not previously received gemcitabine were enrolled. In our study, we defined the haplotype without amino-acid changes as the *1 group, and haplotypes harbouring the 79A>C and 208G>A were designated *2 and *3, respectively. The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are summarised in Table 2. The data clearly showed a haplotype *3-dependent decrease in gemcitabine clearance (CL_m^{-2}) and increases in peak concentration (C_{max}) and area under the curve (AUC) values, although these parameters were not significantly influenced by haplotype *2. The values of AUC and CL_m^{-2} observed in the patient with 208AA (*3/*3) were five-fold and one-fifth of the median of the 208GG (*non**3/*non**3) group, respectively (Figure 2). Then, associations of haplotype *3 with toxicities were analysed. Nadir grades of neutrophil counts were compared between the patient groups with or without haplotype *3 under individual therapeutic regimens. Although there were no significant differences in the incidences of grade ≥ 3 neutropenia between the two groups receiving gemcitabine monotherapy, grade ≥ 3 neutropenia occurred more frequently in the group with haplotype *3 than in the group without haplotype *3 when gemcitabine was administered with carboplatin, cisplatin, or 5-fluorouracil. We concluded that haplotype *3 harbouring 208G>A decreased the clearance of gemcitabine, and increased the incidence of neutropenia when patients were coadministered platinum-containing drugs or 5-fluorouracil. Indeed, the patient with *CDA* 208AA developed severe myelosuppression with severe gastrointestinal toxicities after gemcitabine plus cisplatin combination therapy (Yonemori et al, 2005). Extra caution may be necessary if patients carrying a *3 allele, especially those who are homozygous for *3, are treated with gemcitabine. On the other hand, Vasile et al (2006) recently examined the correlation between CDA SNPs and clinical efficacy in 61 NSCLC patients treated with gemcitabine alone or gemcitabine plus cisplatin, and reported that the patients with *CDA* 79AA ($n=21$) showed a significantly better

Table 1 Reported SNPs of genes involved in the gemcitabine pathways and their allele frequencies in three ethnic groups

Gene	Variant nucleotide location ^a	Amino-acid change ^b	Functional study	Allele frequencies					
				Africans	Ref.	Caucasians	Ref.	Asians	Ref.
ENT1 (SLC29A1) (NT_007592.14)	-1345 C>G		Different mRNA expression among haplotypes ¹	0.08	1	0	1	0.002	2
	-1050 G>A			0.19	1	0	1	0	2
	-706 G>C			0.05	1	0.21	1	0	2
	177 C>G	Asp59Glu	No functional change ³					0.002	2
	647 T>C	Ile216Thr		0.005	3	0.021	3	0	2, 3
	1171 G>A	Glu391Lys	No functional change ³	0.01	3	0	3	0	2, 3
1288 G>A	Ala430Thr						0.002	2	
CNT3 (SLC28A3) (NT_023935.17)	14 G>A	Ser5Asn	No functional change ⁴			0.01	4		
	391 C>T ^c	Leu131Phe	No functional change ⁴			0.01	4		
	1538 A>T	Try513Phe	No functional change ⁴			0.06	4		
CDA (NT_004610.18)	-897 C>A		Different activity among haplotypes ⁵	0.02 (ethnic group unknown)			5		
	-451 C>T			0.26 (ethnic group unknown)			5	0.199	6
	-92 A>G		0.15 (ethnic group unknown)			5	0.205	6	
	79 A>C	Lys27Gln	Reduced ⁸ or unaltered ⁹ activity	0.04–0.108	7, 8	0.298–0.36	7, 8	0.201–0.207	6, 9
208 G>A	Ala70Thr	Reduced activity ⁹	0–0.13	7, 8	0	7, 8	0.037–0.043	6, 9	
DCK (NT_006216.14)	-360 C>G		Increased mRNA expression ¹¹			0.02	10	0.156	11
	-201 C>T					0.02	10	0.156	11
	364 C>T	Pro122Ser ^d				0.015	10	0	11
	727 A>C	Lys243Gln ^d				0.005	10	0	11
DCTD (NT_022792.17)	172 A>G	Asn58Asp	Reduced activity ⁸	0	8	0.008	8		
	315 T>C	Val105Val		0.475–0.48	7, 8	0.25–0.333	7, 8		
RRM1 (NT_009237.17)	-524 T>C		Different activity among genotypes ¹²	0.277	12	0.361	12	0.360	12
	-37 C>A			0.133	12	0.263	12	0.271	12
	850 C>A ^e	Arg284Arg	No different mRNA expression among genotypes ¹³	0.452 (cancer cell lines obtained from ATCC ^f)					13
	2223 A>G ^e	Thr741Thr		0.597 (cancer cell lines obtained from ATCC ^f)					13
2232 G>A ^e	Ala744Ala		0.790 (cancer cell lines obtained from ATCC ^f)					13	

^aA of the translation initiation codon ATG is numbered 1. ^bThe first methionine of a protein is numbered 1. ^cOriginally reported as 1159 C>T. ^dOriginally reported as Pro121Ser and Lys242Gln. ^eOriginally reported as 1082 C>A, 2455 A>G, and 2464 G>A. ^fAmerican Type Culture Collection, Rockville, Maryland, USA. References: ¹Myers et al (2006), ²Kim et al (2006), ³Osato et al (2003), ⁴Damaraju et al (2005), ⁵Fitzgerald et al (2006), ⁶Sugiyama et al (2007), ⁷Fukunaga et al (2004), ⁸Gilbert et al (2006), ⁹Yue et al (2003), ¹⁰Joergler et al (2006), ¹¹Shi et al (2004), ¹²Bepler et al (2005), ¹³Kwon et al (2006).

response rate and progression-free survival than those with CDA 79AC or 79CC ($n=40$) (response rate: 52.4 vs 20%; median progression-free survival: 8.0 vs 2.5 months; $P=0.0136$). Further functional and clinical studies focusing on these CDA SNPs are required.

With regard to gene expression, Ganti et al (2006) investigated the gene expression of CDA in bone marrow mononuclear cells in 71 patients with advanced solid tumours, and reported that patients with a lower relative gene expression of CDA tended to show a higher incidence of grades 2–4 haematological toxicity during gemcitabine therapy. Recently, some additional interesting results have been reported by Bengala et al (2005),

who performed a phase I study of gemcitabine infusion at a fixed dose rate in patients with pancreatic cancer, and also investigated the relationship between CDA mRNA expression in peripheral blood mononuclear cells and clinical outcome. They reported that patients with a lower gene expression level of CDA showed significant longer overall survival than those with a higher expression level (median, 8.5 vs 3.7 months; $P=0.03$). On the other hand, as to expression in tumour tissues, Giovannetti et al (2006) reported that multivariate analysis failed to show any prognostic significance of CDA mRNA expression in 81 patients with pancreatic cancer receiving gemcitabine.

Table 2 Pharmacokinetic parameters of gemcitabine in the patient groups categorized according to diplotypes

Diplotype	Mean \pm s.d.			P-value ^a
	*1/*1 (n = 148)	*2/*1 (n = 69)	*2/*2 (n = 15)	
PK parameter				
C_{max} (mg ml ⁻¹)	22.7 \pm 6.3	22.9 \pm 6.4	24.1 \pm 5.5	0.52
AUC (h μ g ml ⁻¹)	10.1 \pm 2.5	9.8 \pm 2.3	9.8 \pm 1.5	0.46
CL m ⁻² (l h ⁻¹ m ⁻²)	105.8 \pm 31.1	107.2 \pm 27.2	103.3 \pm 19.2	0.99
	Mean \pm s.d.			
Diplotype	*1/*1 (n = 148)	*3/*1 (n = 13)	*3/*3 (n = 1)	
PK parameter				P-value ^a
C_{max} (mg ml ⁻¹)	22.7 \pm 6.3	26.8 \pm 5.9	46.4	5.94E-04
AUC (h μ g ml ⁻¹)	10.1 \pm 2.5	12.7 \pm 2.6	52.9	6.66E-13
CL m ⁻² (l h ⁻¹ m ⁻²)	105.8 \pm 31.1	82.6 \pm 24.9	18.9	7.77E-04

^aP-value of a correlation test. Multiplicity is adjusted by the false-discovery rate.

DEOXYCYTIDINE KINASE

Deoxycytidine kinase is the rate-limiting enzyme for the intracellular phosphorylation of gemcitabine to its active phosphate form. Therefore, DCK may play an important role in sensitivity to gemcitabine. A clear correlation between DCK activity and gemcitabine sensitivity in tumour xenografts has been reported (Kroep *et al*, 2002).

Haplotype analysis in the 5' regulatory region (-360C>G and -201C>T) suggested that -360C/-201C and -360G/-201T had almost complete linkage disequilibrium, and a functional study revealed that patients carrying the -360CG/-201CT and -360GG/-201TT genotypes expressed significantly higher levels of DCK mRNA than patients carrying the -360CC/-201CC genotype (Shi *et al*, 2004). Then the relationship between DCK SNP haplotypes and event-free survival in 122 patients with acute myeloid leukaemia treated with cytarabine was analysed, and slight but statistically significant prolongation of event-free survival time in the group with -360CG/-201CT and -360GG/-201TT over the group with -360CC/-201CC (2-year event-free survival rate, 30.7 vs 23.2%; $P=0.0423$) was observed. Recently, Joerger *et al* (2006) detected two nonsynonymous SNPs in a Caucasian population, 364C>T (Pro122Ser) and 727A>C (Lys243Gln), but their clinical relevance has not yet been clarified.

Recent clinical studies have also shown an association between tumoral DCK expression level and clinical outcome. Sebastiani *et al* (2006) investigated the relationship between the clinical outcome of pancreatic cancer patients treated with gemcitabine-based chemotherapy and immunohistochemical expression of DCK in cancer tissues. They reported that patients whose tumours showed low DCK expression ($n=9$) had significantly shorter overall survival than those whose tumours showed high expression ($n=23$) (median, 14.6 vs 21.7 months; $P<0.009$). They also sequenced the entire DCK-encoding gene in 17 human pancreatic cancer cell lines and nine samples of cancer tissue from patients, but no mutations were identified. Mey *et al* (2006) administered gemcitabine intravesically to 12 patients with bladder cancer, and reported that the mean expression of mRNA in the tumours was significantly higher in patients who achieved a complete pathological response than in those who did not. On the other hand, Seve *et al* (2005) reported that immunohistochemical expression of DCK protein in tumours was not significantly correlated with the survival of NSCLC patients treated with gemcitabine-based chemotherapy.

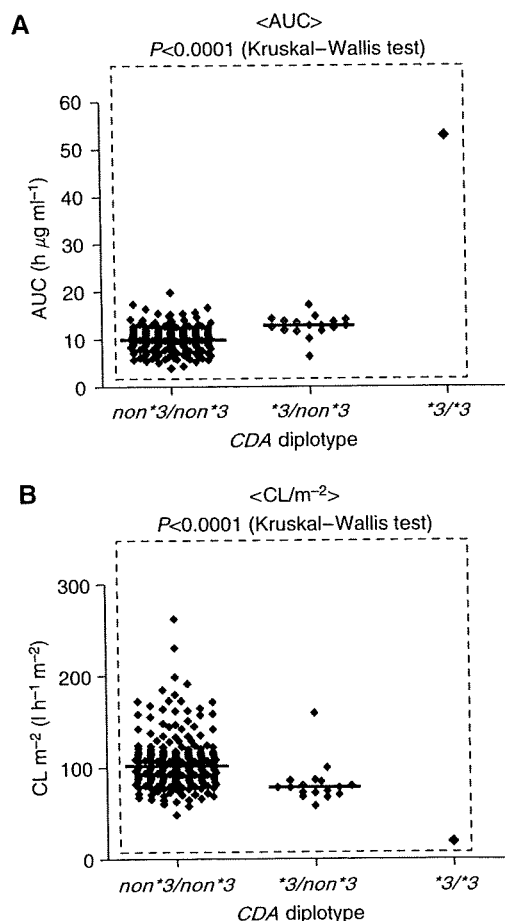


Figure 2 Effects of CDA *3 on the pharmacokinetic parameters of gemcitabine. (A) Area under the curve (AUC) and (B) clearance (CL m⁻²). Each point corresponds to an individual patient. The bars denote the median values.

5'-NUCLEOTIDASE

Since phosphorylated metabolites of gemcitabine are reduced by cellular 5'-NT, the activity level of 5'-NT may be one of the factors affecting the clinical outcome of gemcitabine therapy. Using malignant cells obtained from 43 NSCLC patients receiving gemcitabine-based chemotherapy, Seve *et al* (2005) applied immunohistochemical methods to assess the abundance of proteins involved in gemcitabine pathways, including cN-II, one of the cytosolic nucleotidases that have been shown to be predictive factors in patients with acute myeloid leukaemia (AML) receiving cytarabine. They reported that cN-II was expressed in 86% of the patients, and that among various proteins investigated, only the level of cN-II was significantly correlated with overall survival ($P=0.02$). Since low levels of cN-II were associated with a poor prognosis in NSCLC patients receiving gemcitabine and with a better prognosis in AML patients receiving cytarabine (Seve *et al*, 2005), further studies are necessary to confirm the usefulness of cN-II as a prognosis factor.

RIBONUCLEOTIDE REDUCTASE

Ribonucleotide reductase is the rate-limiting enzyme of the DNA synthesis pathway and converts ribonucleoside diphosphate to deoxyribonucleoside diphosphate, which is essential for DNA synthesis and repair. Ribonucleotide reductase consists of two

subunits, ribonucleotide reductase M1 (RRM1) and ribonucleotide reductase M2 (RRM2).

Kwon *et al* (2006) investigated the association between polymorphisms of *RRM1* and gemcitabine chemosensitivity *in vitro* using 62 human cancer cell lines. When the association between these SNPs and gemcitabine IC_{50} was examined, only cell lines with *RRM1* 2232G>A showed a tendency to be more chemosensitive to gemcitabine, although none of the differences reached a statistically significant level. Bepler *et al* (2005) analysed the *RRM1* promoter for polymorphism, and discovered two SNPs, *RRM1* -37C>A and -524T>C. There was a strong linkage between these SNPs, and -37CC in combination with -524TT was the most frequently observed allelotype, accounting for 42.4% of the ethnically diverse population of 1129. They investigated *RRM1* promoter allelotypes and the outcomes of patients who had undergone surgical resection for NSCLC. It was found that patients with the -37CC/-524TT allelotype had better overall and disease-free survival than patients with the -37AC/-524CT allelotype (median overall survival, 80 vs 46 months; $P=0.06$, median disease-free survival, 74 vs 36 months; $P=0.03$). However, no association between allelotype and tumoral *RRM1* expression was found.

Rosell *et al* (2004b) examined the potential correlation of *RRM1* mRNA expression in specimens of NSCLC resected from 67 patients who had been treated with neoadjuvant gemcitabine/platinum. They found a good correlation between *RRM1* expression in tumours and survival: significant differences in median survival were observed between the 17 patients in the bottom quartile of *RRM1* expression and the 15 in the top quartile (median, 52 vs 26 months; $P=0.018$). They also reported similar results in patients with advanced NSCLC treated with gemcitabine/cisplatin therapy (Rosell *et al*, 2004a). Patients with low *RRM1* mRNA expression levels had significantly longer median survival than those with high levels (median, 13.7 vs 3.6 months; $P=0.009$). Bepler *et al* (2006) also reported that increased *RRM1* expression resulted in resistance to gemcitabine both *in vitro* and clinically. They found that the gemcitabine IC_{50} of lung cancer cell lines with increased *RRM1* expression was higher than that of cell lines with decreased *RRM1* expression, and the results they obtained in a prospective phase II clinical trial in patients with advanced NSCLC showed a significant inverse correlation between *RRM1* expression and disease response to gemcitabine and carboplatin therapy ($P=0.002$ and $r=-0.498$). Therefore, tumoral *RRM1* expression may be a useful marker of outcome in NSCLC patients receiving gemcitabine-based chemotherapy.

With regard to *RRM2*, the association between its genetic polymorphisms and resistance to gemcitabine has not been reported. Duxbury *et al* (2005) demonstrated an association of *RRM2* overexpression with gemcitabine chemoresistance in pancreatic adenocarcinoma cells: the gemcitabine IC_{50} was four times higher in *RRM2* recombinant than with an empty vector ($P<0.05$). Goan *et al* (1999) selected a gemcitabine-resistant cell line KB-GEM ($IC_{50}=32 \mu M$) from human oropharyngeal epidermoid carcinoma KB cells ($IC_{50}=0.3 \mu M$), and found that *RRM2* mRNA (nine-fold) and protein (two-fold) were overexpressed in KB-GEM in comparison with the parental KB cells.

DEOXYCYTIDYLATE DEAMINASE AND UMP/CMP KINASE

Gemcitabine monophosphate is inactivated to dFdUMP by DCTD. A few SNPs including a nonsynonymous one, *DCTD* 172A>G (Asn58Asp), have been reported (Table 1; Fukunaga *et al*, 2004; Gilbert *et al*, 2006). Recombinant Asp58 DCTD was reported to have 11% of wild-type activity for dFdCMP. dFdCMP is further phosphorylated to dFdCDP by UMP/CMP kinase, which is ubiquitously present in human tissues (van Rompay *et al*, 1999). To date, neither association of genetic polymorphisms nor

expression of either DCTD or UMP/CMP kinase with clinical outcome of gemcitabine treatment has been demonstrated.

DNA REPAIR

As the main mechanism of action of gemcitabine is potent inhibition of DNA synthesis, DNA repair may play an important role in gemcitabine-mediated cell death. Recently, Li *et al* (2006) investigated 13 SNPs of eight DNA damage response and repair genes in 92 patients with resectable pancreatic cancer treated with neoadjuvant gemcitabine-based chemotherapy. They found that *RecQ1* 1596(*6), *Rad54L* 2190C>T, and *ATM* IVS20-77 T>C genotypes had a significant effect on overall survival. The strongest genetic effect on survival was observed for *RecQ1* 1596(*6), with median overall survival times of 18.9 and 13.1 months for the AC and CC genotypes, respectively, compared with a mean survival time of 46.9 months for the AA wild type ($P=0.001$). De las Penas *et al* (2006) investigated the association of survival with genetic polymorphisms of various DNA repair genes in 135 cisplatin/gemcitabine-treated NSCLC patients at stage IIIB and IV. After adjusting for performance status, a significantly low hazard ratio (0.44) for carriers of *XRCC3* 722TT (241Met/Met) compared to carriers of 722CT (241Thr/Met) was demonstrated ($P=0.01$). With regard to the expression levels of DNA repair genes, Lord *et al* (2002) investigated the relationship between excision repair cross-complementing group 1 *ERCC1* expression in tumours with response or overall survival in NSCLC patients treated with cisplatin/gemcitabine. They failed to show any significant association between *ERCC1* expression and response, but reported that low expression of *ERCC1* in tumours was associated with longer survival (61.6 vs 20.4 weeks in the low and high expression groups, respectively). Bepler *et al* (2006) also found a similar trend for the relationship between *ERCC1* expression and NSCLC response. Cytotoxic synergism has been demonstrated between gemcitabine and cisplatin through downregulation of *ERCC1* activity by gemcitabine (Lord *et al*, 2002).

CONCLUSION AND FUTURE DIRECTIONS

In this article, we have reviewed recent genetic studies of gemcitabine. The impact of genetic polymorphisms as well as tumour-specific expression of mRNA/proteins on gemcitabine efficacy and toxicity has been described. Looking at these data, tumour-specific expression of *ENT1*, *RRM1* or *ERCC1*, or some DNA repair genetic polymorphisms appear to be promising indicators of prognosis in patients receiving gemcitabine chemotherapy, although prospective pharmacogenetic-based clinical studies will be necessary to clarify the usefulness of these biomarkers in patients receiving gemcitabine-based chemotherapy. With regard to adverse reactions caused by gemcitabine, the expression level or genetic polymorphism of *CDA* seems to be a good predictor. SNP, *CDA* 208A>G, or *CDA* expression level may be candidate biomarkers for individualised gemcitabine-based chemotherapy to avoid severe toxicity, at least in Japanese and some African populations in which considerable numbers of homozygote carriers exist, as is the case for *UGT1A1*28* for irinotecan and *TPMT* genotypes for thiopurine drugs.

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Cost-effectiveness analysis of anastrozole vs tamoxifen in adjuvant therapy for early stage breast cancer in the United Kingdom: the 5-year completed treatment analysis of the ATAC ('Arimidex', Tamoxifen alone or in combination) trial

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Results from the completed treatment analysis of the ATAC (Arimidex, Tamoxifen alone or in combination) trial indicated that anastrozole was significantly superior to tamoxifen in terms of efficacy and safety in the adjuvant treatment of postmenopausal women with hormone receptor-positive (HR+) early breast cancer. On the basis of these results, this study estimated the cost-effectiveness of anastrozole vs tamoxifen, from the perspective of the UK National Health Service (NHS). A Markov model was developed using the 5-year completed treatment analysis from the ATAC trial (ISRCTN18233230), as well as data obtained from published literature and expert opinion. Resource utilisation data and associated costs (2003–4 UK£) were compiled from standard sources and expert opinion. Utility scores for a number of health states were obtained from a cross-sectional study of 26 representative patients using the standard gamble technique. The utility scores were then inserted into the model to obtain cost per quality adjusted life-year (QALY) gained. Costs and benefits were discounted at recommended annual rates of the UK Treasury (3.5%). Modelled for 25 years, anastrozole, relative to generic tamoxifen, was estimated to result in 0.244 QALYs gained per patient at an additional cost of £4315 per patient). The estimated incremental cost-effectiveness of anastrozole compared with tamoxifen was £17 656 per QALY gained. There was a greater than 90% probability that the cost-effectiveness of anastrozole was below £30 000 per QALY gained and of the order of 65% that it was below £20 000 per QALY gained. The results were robust to all parameters tested in sensitivity analysis. Compared with commonly accepted thresholds, anastrozole is a cost-effective alternative to generic tamoxifen in adjuvant treatment of postmenopausal women with HR+ early breast cancer from the UK NHS perspective. *British Journal of Cancer* (2007) **97**, 152–161. doi:10.1038/sj.bjc.6603804 www.bjcancer.com

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Keywords: anastrozole; tamoxifen; aromatase inhibitor; breast cancer; cost-effectiveness analysis; cost-utility analysis

Breast cancer is common among women in the United Kingdom, causing the death of around 70 women per 100 000 aged 50–69 years on an annual basis (Peto *et al*, 2000). Treatment of this common malignancy is a significant economic burden. For example, the annual cost to the UK National Health Service (NHS) of drug therapy for 13 200 patients with hormone receptor-positive (HR+) early breast cancer has been estimated at £64.6 million (Benedict and Christie, 2003). Tamoxifen is an established adjuvant treatment option in this regard (Early Breast Cancer Trialists' Collaborative Group, 1998; Early Breast Cancer Trialists' Collaborative Group, 2005). However, treatment with tamoxifen has been associated with serious safety concerns (Reddy and Chow, 2000), including thromboembolic disorders (Love *et al*, 1991) and increased risk of the development of endometrial cancer

(Fisher *et al*, 2001). Consequently, there is a need for new hormonal treatment options with improved safety and efficacy, particularly in terms of avoiding or delaying costly disease recurrences (Lamerato *et al*, 2004).

Anastrozole is an aromatase inhibitor that has proved significantly superior to tamoxifen as adjuvant treatment for postmenopausal women with HR+ early breast cancer. This was apparent in terms of more favourable disease-free survival (hazard ratio 0.83, $P = 0.005$) and longer time to recurrence (Hazard Ratio 0.74, $P = 0.0002$) in the completed treatment analysis of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial (ISRCTN18233230), which was performed in postmenopausal women with early operable breast cancer who had completed primary therapy and were eligible for adjuvant hormonal therapy (ATAC Trialists' Group, 2005). Anastrozole was also associated with a more favourable safety profile vs tamoxifen, including a significantly lower incidence of endometrial cancer, thromboembolic events and vaginal bleeding/discharge albeit with increased

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FP Therapy for Controlling Malignant Ascites in Advanced Pancreatic Cancer Patients

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ABSTRACT

Background/Aims: Malignant ascites is one of the poor prognostic factors for pancreatic cancer, and causes serious symptoms and treatment-related toxicity. We conducted a retrospective analysis to evaluate the efficacy of 5-fluorouracil (5-FU) plus cisplatin (FP therapy) for controlling malignant ascites in patients with advanced pancreatic cancer.

Methodology: This analysis was based on 28 consecutive chemotherapy-naïve advanced pancreatic cancer patients with cytologically proven malignant ascites who were treated with FP therapy from November 1991 to April 2003.

Results: No patients achieved measurable tumor responses. The objective improvement of ascites was seen in 35.7% of the patients (N=10/28, 95% confidence interval, 18.0 to 53.4%), but there was no

patient with complete disappearance of ascites. The median time to disease progression and the median survival time were 1.7 months and 2.7 months, respectively. In all pretreatment variables, the presence of distant metastasis other than peritoneal dissemination was an unfavorable predictive factor for the objective improvement of ascites (Fisher's exact test: $P=0.002$).

Conclusions: FP therapy was modestly effective for controlling malignant ascites but insufficient in shrinking for measurable metastatic lesions. Systemic chemotherapy for controlling malignant ascites might be worth while for palliative management in advanced pancreatic cancer patients, especially in patients without distant metastasis.

KEY WORDS:

Pancreatic cancer; Malignant ascites; 5-Fluorouracil and Cisplatin

ABBREVIATIONS:

5-Fluorouracil (5-FU); 5-Fluorouracil plus cisplatin (FP); Eastern Cooperative Oncology Group (ECOG); National Cancer Institute Common Toxicity Criteria (NCI-CTC); C-reactive Protein (CRP); Carcinoembryonic Antigen: (CEA)

INTRODUCTION

The prognosis of pancreatic cancer patients is very poor, because most patients have unresectable disease at the time of diagnosis, and there are few effective non-surgical treatments for this disease. Although gemcitabine generated improvements in symptom control and survival, patients with metastatic disease still have a short survival (3 to 6 months) (1). Pancreatic cancer typically spreads to liver and peritoneum. Peritoneal dissemination causes many serious complications, such as malignant ascites, intestinal obstruction and hydronephrosis. In general, malignant ascites is a manifestation of advanced malignant disease that is associated with significant morbidity. The symptoms of these events include abdominal pain, sensation of abdominal fullness, vomiting, and constipation, leading to an extremely poor quality of the patients' remaining life. Although intraperitoneal chemotherapy has been used to treat malignant ascites, the results remain poor (2).

Many chemotherapeutic agents have been studied for treatment of advanced pancreatic cancer. Before gemcitabine became a standard first-line chemotherapy, 5-fluorouracil (5-FU) was the most extensively evaluated chemotherapeutic agent for pancreatic cancer (3). Cisplatin, which itself has marginal activity

against pancreatic cancer, is a chemical modulator of 5-FU (4,5). The combination of 5-FU and cisplatin (FP therapy) was one of the most widely used chemotherapeutic regimens before the introduction of gemcitabine. Rothman *et al.* reported that the objective response rate to FP therapy was 16% (6). In our previous phase II trial of FP therapy for advanced pancreatic cancer patients, the response rate was only 8%, but ascites was eliminated in 2 out of 4 patients (7). The clinical benefit response, according to performance status and pain, to the FP therapy was achieved in 19% of the patients, which bears comparison with that of gemcitabine (1,8). Therefore, in this retrospective study we investigated the efficacy of FP therapy for controlling malignant ascites in patients with advanced pancreatic cancer.

METHODOLOGY

Patient Selection

A total of 119 patients with advanced pancreatic cancer were treated with FP therapy between November 1991 and April 2003 at the National Cancer Center Hospital, Tokyo, Japan. We selected patients from the database who fulfilled the following criteria: 1) cytological confirmation of adenocarcinoma in ascites, 2) no previous chemotherapy or radiotherapy, 3) an

TABLE 1 Patient Characteristics

Total number of patients	28
Male/Female	19 / 9
Age	
Median (range)	62 (39-73)
PS	
0	13 (46.4%)
1	14 (50%)
2	1 (3.6%)
Site of disease	
Primary lesion	26 (93%)
Liver metastasis	10 (35.7%)
Lymph node metastasis	10 (35.7%)
Lung metastasis	3 (10.7%)
Others	2 (7.1%)
Total number of courses	64
Median number of courses (range)	2 (1-6)
Median follow-up time (range)	3 (0.43-11.7 months)

ECOG performance status of 0 to 2, 4) 15 to 75 years of age, 5) adequate bone marrow and organ functions at the start of treatment (leukocytes \geq 4000/ μ L, hemoglobin \geq 11.0g/dL, platelets \geq 100,000/ μ L, total bilirubin \leq 2.0mg/dL, AST $<$ 2.5 times the normal limit, ALT $<$ 2.5 times the normal limit, serum creatinine \leq 1.2mg/dL, creatinine clearance \geq 60mL/min), and 6) obtained written informed consent before the start of treatment.

Treatment Schedule

5-FU was administered by continuous intravenous infusion at 500mg/m²/day over 5 consecutive days. Cisplatin at 80mg/m² was given intravenously over 2 hours on the first day with adequate hydration and anti-emetic drugs. The therapy was repeated every 4 weeks, and was continued for 6 courses or until evidence of disease progression, unacceptable toxicity, or patient refusal.

TABLE 2 Toxicity Per Patient

	Maximum grade (WHO)				Grade 3 \leq (%)
	1	2	3	4	
Leukocytopenia	4	6	1	0	1 (4%)
Neutropenia	4	2	4	0	4 (14%)
Anemia	7	10	6	0	6 (21%)
Thrombocytopenia	3	3	2	0	2 (7%)
Bilirubin	0	6	5	0	5 (18%)
GOT	5	4	0	0	0 (0%)
GPT	6	4	0	0	0 (0%)
Creatinine	6	3	1	0	1 (4%)
Hyponatremia	4	7	1	2	3 (11%)
Anorexia	19	4	1	0	1 (4%)
Nausea	16	7	1	-	1 (4%)
Vomiting	10	9	1	0	1 (4%)
Diarrhea	4	3	0	0	0 (0%)
Stomatitis	3	2	1	0	1 (4%)

Evaluation of Antitumor Effects and Adverse Events

Objective responses in measurable metastatic lesions were evaluated according to the standard WHO criteria. Radiologic objective response assessment by computed tomography was undertaken every 4 weeks and whenever clinical assessment suggested disease progression. To assess the change in ascites volume, we serially measured the thickness of ascites on computed tomography, which was defined as the distance from the parietal surface of the ascites to its visceral surface (i.e. liver surface) in the section containing the hepatic hilum. The objective improvement of ascites was defined as a reduction \geq 50% in the thickness, persisting for at least 4 weeks. No response (NR) was defined as inapplicability for the above criteria. Toxicity was evaluated according to the common toxicity criteria of the NCI-CTC ver. 2.0.

Statistical Analysis

Time to progression was calculated from the date of the first administration of FP therapy to the date of confirmation of disease progression. Survival was determined from the date of the first administration of FP therapy to the date of death by any cause, or to the last date of confirmed survival. Predictive factors for the response of malignant ascites were studied using univariate analysis (Fisher's exact test). The variables were selected in consideration of the potential relationship to the response of malignant ascites, as indicated by previous investigations (9,10) or by our own clinical experience. Analyses of overall survival and time to disease progression were conducted using the Kaplan-Meier method, and differences between the groups were analyzed using the log-rank test.

RESULTS

Patient Characteristics

Twenty-eight patients were selected as the subjects of this study. The patient demographics are summarized in Table 1. Twenty-six patients had at least one measurable lesion.

Response to Therapy

In the 26 patients with measurable lesions, no patient achieved objective tumor response. Seventeen patients showed no change (NC) and the remaining 9 patients had progressive disease (PD). Objective improvement of ascites was observed in 10 of the 28 patients (35.7%, 95%CI: 18.0 to 53.4%), although there was no patient with complete disappearance of ascites. The median duration of objective improvement of ascites was 4.1 months. The median time to disease progression and median overall survival time were 1.7 months and 2.7 months, respectively (Figure 1). The median overall survival time of patients with objective improvement of ascites was longer than that of patients without it (6.5 months vs. 2.2 months, log rank test: $P=0.002$). No patient was alive at 1 year after the initiation of the therapy.

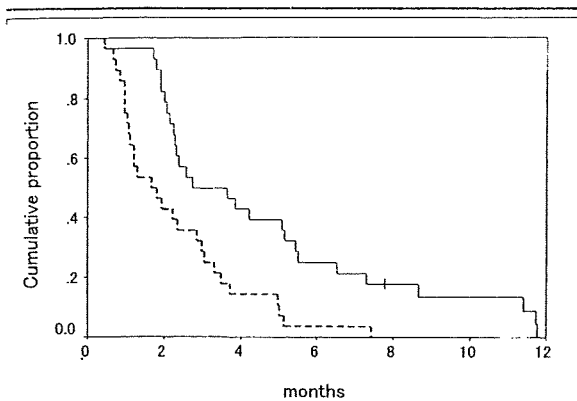


FIGURE 1 Kaplan-Meier analysis of time to progression (dotted line) and overall survival (solid line). The tick mark indicates the censored case.

Toxicity

Toxicity is summarized in **Table 2**. Grade 3 or worse neutropenia, anemia, and thrombocytopenia were observed in 4 (14%), 6 (21%), and 2 (7%) patients, respectively. In non-hematological toxicity, the gastrointestinal toxicity was the most common adverse event. Grade 3 anorexia, nausea and vomiting were seen in 1 patient each. Three patients experienced hyponatremia above grade 3. Four patients discontinued their chemotherapy due to unacceptable toxicity. In these patients, 2 patients experienced grade 2 elevation of serum creatinine after the first course of treatment, and 1 patient experienced grade 3 elevation of serum creatinine after 2 courses. The remaining patient had both grade 3 anorexia and grade 3 vomiting during 3 courses of treatment.

Predictive Factor for the Objective Improvement of Malignant Ascites

Univariate analysis disclosed only the presence of distant metastasis other than peritoneal dissemination as an unfavorable predictive factor for the objective improvement of malignant ascites (**Table 3**; Fisher's exact test: $P=0.002$). The objective improvement rate of ascites was 58.8% in patients without distant metastasis other than peritoneal dissemination, while patients with it had no improvement of ascites.

DISCUSSION

Malignant ascites is one of the poor prognostic impact factors for gastrointestinal and pancreatic cancer (10). Intraperitoneal chemotherapy has been used to treat malignant ascites. However, the efficacy of this therapy has remained poor (2), because the intraperitoneally injected drugs have not been sufficiently delivered to the peritoneal dissemination. Previous report have described that systemic chemotherapy might have little effect on peritoneal dissemination, because of the existence of the peritoneal-plasma barrier, which limits drug penetration into the peritoneum (11). Thus, patients with malignant ascites were considered to be inappropriate candidates for systemic chemotherapy (10). Recently, a 5-FU-con-

taining regimen had modest efficacy and low toxicity for gastric cancer patients with peritoneal dissemination (12). In pancreatic cancer, there have been few reports evaluating the efficacy of systemic chemotherapy for controlling malignant ascites (13).

In present study, FP therapy failed to demonstrate any objective tumor response. However, the improvement rate of malignant ascites was 35.7% according to our evaluation criteria. The prognosis in patients with objective improvement of ascites was favorable; the median survival time in those patients was 6.5 months. Thus, FP therapy may have relieved the patient from the troublesome symptoms related to ascites and maintained a favorable quality of life.

In the present study, it was an interesting finding that the presence of distant metastasis other than peritoneal dissemination was an unfavorable predictor of the objective improvement of ascites. To our knowledge, there has been no report describing predictive factors for the treatment of malignant ascites. Although the etiology is not well understood, patients with distant metastasis might have a more progressive disease and higher tumor burden than patients without it. Thus, the presence of distant metastasis might influence the efficacy of FP therapy for malignant ascites. Our findings need verification in an indepen-

TABLE 3 Univariate Analysis for Objective Improvement of Ascites

Variables	N	Objective improvement of ascites	P value
Sex			1.00
Male	19	7 (36.8%)	
Female	9	3 (33.3%)	
Age			1.00
~59	17	6 (36.4%)	
60~	11	4 (35.3%)	
ECOG Performance status			0.433
0	13	6 (46.2%)	
1-2	15	4 (26.7%)	
Distant metastasis			0.002
Present	11	0 (0%)	
Absent	17	10 (58.8%)	
Serum albumin level			0.677
~3.6g/dL	19	6 (31.6%)	
3.7g/dL~	9	4 (44.4%)	
Serum C-reactive protein			0.626
~0.9mg/dL	23	9 (39.1%)	
1.0mg/dL~	5	1 (20%)	
Carcinoembryonic antigen			0.544
~5.0ng/mL	13	5 (38.5%)	
5.1ng/mL~	15	5 (33.3%)	
Hemoglobin level			0.315
Normal	5	3 (60%)	
Low	23	7 (30.4%)	

ECOG: Eastern Cooperative Oncology group; Hemoglobin level: Normal is ≥ 13.7 g/dL in males, and ≥ 11.3 g/dL in females, and low is < 13.7 g/dL in males and < 11.3 g/dL in females. Distant metastasis indicates metastasis other than peritoneal dissemination.

dent group of patients. We speculated that systemic chemotherapy for controlling malignant ascites would be worth consideration for advanced pancreatic cancer patients without distant metastasis other than peritoneal dissemination, and that it could palliate the symptoms related to malignant ascites, such as abdominal pain and sensation of abdominal fullness.

In patients with ascites, third-space retention of an intravenously administered drug is associated with the prolongation of the terminal drug half-life in plasma, presumably owing to the slow reentry of the sequestered drug into the bloodstream (14). This effect might intensify on the toxicities of patients with ascites, which are frequently observed as more severe than those of patients without ascites.(2). Malignant ascites is one of the clinical presentations of end-stage cancer and may influence the evaluation of non-hema-

tological toxicity (2). Thus, toxicities in present study, especially gastrointestinal and renal toxicity, might have been slightly more severe than those in previous phase II trials of FP therapy (6,7).

In conclusion, FP therapy was modestly effective for controlling malignant ascites, but the objective tumor response was insufficient. This study suggested that patients without distant metastasis other than peritoneal dissemination may be appropriate candidates for palliative chemotherapy. At present, gemcitabine, which is a first-line chemotherapeutic agent for advanced and metastatic pancreatic cancer patients, should be evaluated for efficacy in controlling malignant ascites. Furthermore, it is crucial to develop new drugs or regimens to improve the survival and clinical benefit in such a poor prognostic population.

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A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation

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This phase I study was designed to examine the maximum tolerated dose (MTD), the dose-limiting toxicities (DLTs), the recommended dose (RD) for phase II, and the pharmacokinetics of NK105, a new polymeric micelle carrier system for paclitaxel (PTX). NK105 was administered as a 1-h intravenous infusion every 3 weeks, without antiallergic premedication. The starting dose was 10 mg m⁻², and the dose was escalated according to the accelerated titration method. Nineteen patients were recruited. The tumour types treated included pancreatic (n = 11), bile duct (n = 5), gastric (n = 2), and colonic (n = 1) cancers. Neutropenia was the most common haematological toxicity. A grade 3 fever developed in one patient given 180 mg m⁻². No other grades 3 or 4 nonhaematological toxicities, including neuropathy, was observed during the entire study period. DLTs occurred in two patients given 180 mg m⁻² (grade 4 neutropenia lasting for more than 5 days). Thus, this dose was designated as the MTD. Grade 2 hypersensitivity reactions developed in only one patient given 180 mg m⁻². A partial response was observed in one patient with pancreatic cancer. The maximum concentration (C_{max}) and area under the concentration (AUC) of NK105 were dose dependent. The plasma AUC of NK105 at 150 mg m⁻² was approximately 15-fold higher than that of the conventional PTX formulation. NK105 was well tolerated, and the RD for the phase II study was determined to be 150 mg m⁻² every 3 weeks. The results of this phase I study warrant further clinical evaluation.

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Keywords: NK105; paclitaxel; polymer micelles; phase I study; DDS

Paclitaxel (PTX), an antimicrotubule agent, has a wide spectrum of antitumour activity including ovarian, breast, stomach, lung, and head and neck cancers (Rowinsky *et al*, 1990; Carney, 1996; Crown and O'Leary, 2000). The clinically used PTX preparation is a mixture of Cremophor EL and ethanol because of PTX's poor water solubility. However, the use of Cremophor EL is known to be associated with acute hypersensitivity reactions (Weiss *et al*, 1990; Rowinsky and Donehower, 1995; Kloover *et al*, 2004). Other PTX preparations that have been categorised as drug delivery systems (DDS) have also been developed. These preparations include Xyotax (polyglutamate-conjugated PTX; Singer *et al*, 2003; Boddy *et al*, 2005), Abraxane (PTX coated with albumin; Ibrahim *et al*, 2002; Deisai *et al*, 2003; Nyman *et al*, 2005), and Genexol-PM (a PTX micelle in which PTX has been simply solubilised; Kim *et al*, 2004). The common advantage shared by these formulations is that they are injectable intravenously without the mixture of Cremophor EL and ethanol. Among them, Abraxane has been approved for metastatic breast cancer by the Food and Drug Administration in the USA based on the results of a randomised phase 3 trial. In this trial, Abraxane demonstrated significantly higher response

rates, compared with standard PTX, and a significantly longer time to progression (Gradishar *et al*, 2005). In addition, the incidence of grade 4 neutropenia was significantly lower for Abraxane than for PTX. However, peripheral sensory neuropathy was more common in the arm (Gradishar *et al*, 2005).

NK105 is a PTX-incorporating 'core-shell-type' polymeric micellar nanoparticle formulation (Hamaguchi *et al*, 2005). This particle can be injected intravenously without the use of Cremophor EL or ethanol as a vehicle. Therefore, NK105 is expected to possess a clinical advantage similar to that of the above-mentioned PTX formulations. The difference between NK105 and the other PTX dosage forms is that NK105 is expected to yield a markedly higher plasma and tumour area under the concentration (AUC), compared with those for the other PTX formulations. Moreover, regarding the toxic profiles, the repeated administration of NK105 to rats at 7-day intervals produced significantly fewer toxic effects on peripheral nerves than free PTX. Macromolecular drugs, including NK105, have been developed based on the characteristic macroscopic features of solid tumours, such as hypervascularity, the presence of vascular permeability factors stimulating extravasation within cancer, and the suppressed lymphatic clearance of macromolecules. These characteristics, which are unique to solid tumours, constitute the basis of the enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986; Maeda *et al*, 2000; Duncan, 2003). The *in vivo*

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antitumour activity of NK105 was significantly more potent than that of free PTX, probably because of enhanced tumour exposure through the EPR effect (Hamaguchi *et al*, 2005).

We conducted a phase I clinical trial using NK105 in patients with advanced solid tumours. The objectives of this trial were to determine the maximum tolerated dose (MTD), the phase II recommended dose (RD), and the pharmacokinetics of NK105.

PATIENTS AND METHODS

The protocol and all materials were approved by the Institutional Review Board of the National Cancer Center, Tokyo. This study was conducted in compliance with the Good Clinical Practice Guidelines of the International Conference on Harmonization and the Declaration of Helsinki Principles. Written informed consent was obtained from all the patients.

Therapeutic agent

NK105 was supplied by Nippon Kayaku Co. Ltd. (Tokyo, Japan) in 20-ml glass vials containing a dose equivalent to 30 mg of PTX. When reconstituted in 10 ml of 5% glucose solution and diluted with a total volume of 250 ml of 5% glucose, the reconstituted solution was stable for 24 h at room temperature. In our preclinical study, DLS and HPLC analysis showed that less than 2% of PTX incorporated in the micelles was released for 24 h at room temperature (data not shown).

Figure 1 shows the schematic structure of NK105, a PTX-entrapped polymeric micelle formulation. The NK105 polymers were constructed using polyethylene glycol (PEG) as the hydrophilic component and modified polyaspartate as the hydrophobic component. PEG is believed to form the outer shell of the micelle, producing a 'stealth' effect that enables NK105 to avoid being captured by the reticuloendothelial system.

The modified polyaspartate chain is hydrophobic and is believed to form the hydrophobic inner core of the micelles in aqueous media. The hydrophobic inner core enables NK105 to entrap a sufficient amount of PTX. NK105 has a diameter of about 90 nm (Hamaguchi *et al*, 2005).

Patients

Patients with solid tumours refractory to conventional chemotherapy and for whom no effective therapy was available were eligible for enrolment in this study, provided that the following criteria were met: a histologically confirmed malignant tumour; a performance status of ≤ 2 ; an age of ≥ 20 and < 75 years; a normal haematological profile (neutrophil count $\geq 2000 \text{ mm}^{-3}$, platelet count $\geq 100\,000 \text{ mm}^{-3}$, hemoglobin $\geq 9 \text{ g dl}^{-1}$); normal hepatic function (total bilirubin level $\leq 1.5 \text{ mg dl}^{-1}$, AST and ALT ≤ 2.5

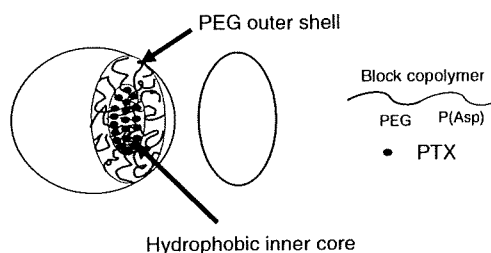


Figure 1 Schematic structure of NK105. A polymeric micelle carrier of NK105 consists of a block copolymer of PEG (molecular weight of about 12 000) and modified polyaspartate. PEG is believed to be the outer shell of the micelle. PEG is believed to form the outer shell of the micelle. NK105 has a highly hydrophobic inner core, and therefore can entrap a sufficient amount of PTX.

times the upper normal limit); normal renal function (serum creatinine $\leq 1.5 \text{ mg dl}^{-1}$); normal cardiac function (New York Heart Association (NYHA) classification of ≤ 1); normal pulmonary function ($\text{PaO}_2 \geq 60 \text{ mm Hg}$); no chemotherapy within 4 weeks (6 weeks for nitrosourea or mitomycin C) of the administration of NK105; and a life expectancy of more than 2 months. Patients with serious infections (including hepatitis B, hepatitis C, or HIV) were ineligible for enrolment in the study. Patients who had been previously treated with a taxane were excluded because of assessing neuropathy. Patients were also excluded if they were pregnant or lactating. Additionally, any patient whom the investigators considered ineligible was excluded.

Drug administration

NK105 was dissolved in 5% glucose solution for injection at room temperature. NK105 was administered intravenously without in-line filtration and without premedication. NK105 solution was infused using an electric pump at a speed of 250 ml h^{-1} .

Dosage and dose escalation

The starting dosage of NK105 was 10 mg m^{-2} , which is one-third of the toxic dose low in dogs. NK105 was administered once every 3 weeks, and the treatment was continued unless a severe adverse event or disease progression was observed. Dose escalation was performed according to the previously described accelerated titration method (Simon *et al*, 1997; Matsumura *et al*, 2004).

Toxicity was graded from 1 to 4 using the National Cancer Institute Common Toxicity Criteria (version 2.0). Inpatient dose escalation was not permitted. The MTD was defined as the level at which two out of six patients experienced dose-limiting toxicities (DLTs). The recommended dosage for a phase II trial was defined by the Efficacy and Safety Assessment Committee based on the safety, pharmacokinetics, and efficacy results of this trial. DLT was defined as grade 4 neutropenia lasting more than 5 days, a platelet count of less than $25\,000 \mu\text{l}^{-1}$, or grade 3 or higher non-haematological toxicity, with the exception of nausea, vomiting, appetite loss, and hypersensitivity.

Pretreatment assessment and follow-up care

A complete medical history and physical examination, performance status evaluation, complete blood cell count (CBC), blood chemistry, urinalysis, electrocardiogram (ECG), and a computed tomography (CT) examination were performed in each patient. Other examinations were performed only in the presence of a specific clinical indication. Patients were physically examined every day until the second administration of NK105; CBC and blood chemistry tests were performed on day 3 and weekly thereafter. An ECG examination was repeated before each administration of NK105. Tumour marker levels were also measured before every administration. Tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors criteria (Therasse *et al*, 2000).

Liquid chromatography/tandem mass spectrometry determination of PTX concentrations

The PTX concentrations determined in the present phase I study represented the total drug concentrations (both micelle-entrapped and released). It was difficult to measure released PTX and micelle-entrapped PTX separately, because the equilibrium between both forms could not keep constant during the separating procedure. PTX was extracted from human plasma (0.2 ml) or urine (0.5 ml) by deproteinisation with acetonitrile. The quantifications of PTX in plasma and urine were performed using liquid chromatography/tandem mass spectrometry. Reversed-phase column-switching

chromatography was conducted using an ODS column and detection was enabled by electrospray ionisation of positive mode.

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated for each patient using a non-compartmental model using the WinNonlin Professional version 4.1 program (Pharsight Corporation, Mountain View, CA, USA). The maximum concentration (C_{max}) was the maximum observed plasma concentration of PTX, and the time-to-the-maximum concentration (T_{max}) was the time corresponding to C_{max} . The area under the concentration (AUC)-time curve from time zero up to the last quantifiable time point (AUC_{0-t}) was calculated using the linear trapezoidal rule, and the area under the concentration-time curve from zero until infinity (AUC_{0-inf}) was calculated as the sum of AUC_{0-t} and the extrapolated area under the zero moment curve from the last quantifiable time point to infinity calculated by dividing the plasma concentration of the last quantifiable time point (observed value) by the elimination rate constant. The half-life of the terminal phase ($t_{1/2z}$) was calculated as $\log_e 2/\lambda z$, where λz is the elimination rate constant calculated from the terminal linear portion of the log of the concentration in plasma. Total clearance (CL_{tot}), the volume of distribution at steady state (V_{ss}), and renal clearance (CL_r) were calculated using the following equations, where D is the dose and $AUMC_{inf}$ the area under the first moment curve from time zero until infinity:

$$CL_{tot} = D/AUC_{inf}$$

$$V_{ss} = AUMC_{inf}/AUC_{inf} \times CL_{tot}$$

$$CL_r = \text{cumulative urinary excretion}/AUC_{inf} \\ / \text{body surface area}$$

RESULTS

Patient characteristics

Nineteen eligible patients were recruited for the study (Table 1). All the patients had received chemotherapy before enrolment. Prior therapies ranged from 1 to 3 regimens of chemotherapy. None of the patients had received taxane chemotherapy. All the patients were included in the safety and response analyses.

Dosing

Dosage escalation started at 10 mg m^{-2} and was increased up to 180 mg m^{-2} . In total, 73 administrations were performed in 19 patients. Eighteen patients received more than two administra-

Table 1 Patient characteristics

Number of patients	19
Male/female	13/6
Age (years)	
Median	57
Range	43-72
ECOG PS	
Median	0
0	10
1	9
Prior treatment	
Chemotherapy regimens	
Median	1
Range	1-3

tions. The maximum number of treatments was 14 courses at 150 mg m^{-2} ; the average number of administrations at all levels was 3.8 courses. Up until 80 mg m^{-2} , grade 2 toxicity was not observed during the first course.

According to the original protocol, the dosage of NK105 should have been doubled for each escalation until grade 2 toxicity. However, the safety committee recommended that the dosage should be raised by 40% instead of 100% at 110 mg m^{-2} and that a modified Fibonacci escalation method should be implemented. Therefore, we recruited three patients at dosage level 5 (110 mg m^{-2}) and re-started the dose identification study using a modified Fibonacci method.

Haematological toxicity

Significant myelosuppression was not observed up to level 4 (80 mg m^{-2}). At level 7 (180 mg m^{-2}), two out of five patients appeared to have acquired DLTs, namely grade 4 neutropenia lasting for more than 5 days. On the basis of these results, 180 mg m^{-2} was considered to be the MTD, with neutropenia as the DLT. Since a dosage of 150 mg m^{-2} was considered to be the recommended dosage for phase II studies, an additional four patients were enrolled at a dosage of 150 mg m^{-2} ; one patient developed DLT, namely grade 4 neutropenia lasting for more than 5 days (Table 2). During the entire period of this study, G-CSF was never used to rescue patients.

Nonhaematological toxicity

The NK105 injection was generally uneventful and well tolerated in terms of nonhaematological toxicities (Table 2). Most of the toxicities were grade 1; none of the patients manifested grade 4 toxicity. A few patients developed a grade 1 elevation in AST or ALT, but these changes were transient. Pain or local toxicity in the area of the injection was not observed in any of the patients treated with NK105. No infusion-related reactions were observed; such reactions sometimes occur during liposomal drug administration. Patients were not premedicated with steroids or antihistamines. Only one patient at 180 mg m^{-2} developed grade 2 hypersensitivity. After the first course, the patient received premedication of hydrocortisone and did not develop such hypersensitivity after that. The other 18 patients did not experience any hypersensitivity during the study. Neuropathy occurred in a typical stocking/glove distribution and was manifested by numbness. Three patients at level 6 (150 mg m^{-2}) and three patients at level 7 (180 mg m^{-2}) experienced grade 1 neurotoxicity during 1 cycle. Of the four patients who received multicycle treatment more than five times, only three patients developed grade 2 neuropathy and the other patient developed grade 1 neuropathy. Even one patient who received 14 cycles of treatment experienced only grade 2 neuropathy.

Pharmacokinetics

The plasma concentrations of PTX after the intravenous infusion of NK105 were determined in each of the patients enrolled at a dose of 150 mg m^{-2} (Figure 2A). The C_{max} (Figure 2B) and AUC (Figure 2C) increased as the doses were escalated from 10 to 180 mg m^{-2} . The pharmacokinetic parameters are summarised in Table 3. The $t_{1/2z}$ ranged from 7.0 to 13.2 h, and a slight tendency towards a dose-dependent extension of this parameter was observed. The CL_{tot} ranged from 280.9 to $880.4 \text{ ml h}^{-1} \text{ m}^{-2}$, and the V_{ss} ranged from 3668.9 to $10400.3 \text{ ml m}^{-2}$. Although these parameters were slightly reduced depending on the dose, linear pharmacokinetics was assumed to have been observed in the dose range from 10 to 180 mg m^{-2} . The AUC of NK105 at 150 mg m^{-2} (recommended phase II dose) was about 15-fold larger than that of conventional PTX at dose of 210 mg m^{-2} (conventional dose for a

Table 2 Haematological and nonhaematological toxicities (cycle I and all cycles)

	10–110 mg m ⁻² (n = 7) grade				150 mg m ⁻² (n = 7) grade				180 mg m ⁻² (n = 7) grade			
	1	2	3	4	1	2	3	4	1	2	3	4
<i>Cycle I</i>												
Leukopenia	2	0	2	0	1	5	1	0	1	1	3	0
Neutropenia	1	0	1	1	0	2	1	3 ^a	0	0	3	2 ^b
Thrombocytopenia	1	0	0	0	2	0	0	0	4	0	0	0
Hemoglobin	1	0	0	0	2	2	0	0	1	0	0	0
Neuropathy	0	0	0	0	3	0	0	0	3	0	0	0
Myalgia	1	0	0	0	3	0	0	0	2	1	0	0
Arthralgia	1	0	0	0	4	0	0	0	3	0	0	0
Hypersensitivity	0	0	0	0	0	0	0	0	0	1	0	0
Rash	1	0	0	0	1	3	0	0	4	0	0	0
Fatigue	1	0	0	0	5	0	0	0	4	0	0	0
Fever	2	0	0	0	2	0	0	0	1	0	1	0
Anorexia	0	0	0	0	3	0	0	0	1	0	0	0
Nausea	1	0	0	0	1	0	0	0	1	0	0	0
Stomatitis	0	0	0	0	1	0	0	0	1	0	0	0
Alopecia	3	0	—	—	5	0	—	—	5	0	—	—
<i>All cycles</i>												
Leukopenia	3	0	2	0	1	4	2	0	1	1	3	0
Neutropenia	1	0	1	1	1	1	1	4	0	0	3	2
Thrombocytopenia	1	0	0	0	3	0	0	0	4	0	0	0
Hemoglobin	1	0	0	0	1	5	0	0	1	0	0	0
Neuropathy	2	0	0	0	1	3	0	0	4	0	0	0
Myalgia	1	1	0	0	3	0	0	0	2	1	0	0
Arthralgia	2	0	0	0	4	0	0	0	3	0	0	0
Hypersensitivity	0	0	0	0	0	0	0	0	0	1	0	0
Rash	1	0	0	0	3	3	0	0	4	0	0	0
Fatigue	3	0	0	0	5	1	0	0	4	0	0	0
Fever	3	0	0	0	3	1	0	0	1	0	1	0
Anorexia	2	1	0	0	2	1	0	0	2	0	0	0
Nausea	1	0	0	0	1	0	0	0	2	0	0	0
Stomatitis	1	0	0	0	2	0	0	0	1	0	0	0
Alopecia	2	2	—	—	4	3	—	—	4	1	—	—

^aOne of three patients developed DLT, namely grade 4 neutropenia lasting for more than 5 days. ^bThese two patients developed DLT, namely grade 4 neutropenia lasting for more than 5 days.

3-week regimen in Japanese patients) (Tamura *et al*, 1995). The V_{ss} and CL_{tot} of NK105 were significantly lower than those of conventional PTX.

The cumulative urinary excretion rates of PTX (0–73 h) after the administration of NK105 were 2.8–9.2%. These values were low, similar to those reported after the administration of conventional PTX (Tamura *et al*, 1995). The CL_r ranged from 11.7 to 66.4 ml h⁻¹ m⁻³, and was slightly decreased with the dose. Since the ratio of CL_r to CL_{tot} was 3–9%, CL_r hardly contributed to CL_{tot} .

Therapeutic response

Six patients (two gastric, two bile duct, one colon, and one pancreatic) were evaluated as having had a stable disease for longer than 4 weeks at the time of the study's completion. A partial response was seen in a patient with metastatic pancreatic cancer who had been treated at 150 mg m⁻², and in whom the size of the liver metastasis had decreased by more than 90%, compared to the baseline scan (Figure 3A). This patient had previously undergone treatment with gemcitabine. The antitumour response was maintained for nearly 1 year. In a patient with stomach cancer who was treated at 150 mg m⁻², about 40% reduction was observed in a peritoneal metastasis, but a liver metastasis remained stable (Figure 3B).

DISCUSSION

The observed toxicities of NK105 were similar to those expected for conventional PTX. The DLT was neutropenia. The recom-

mended phase II dose using a 3-week schedule was determined to be 150 mg m⁻². This recommended dose of NK105 is less than that of conventional PTX (210 mg m⁻²). Since the plasma AUC of the recommended dose of NK105 was 15- to 20-fold higher than that of the recommended dose of conventional PTX (210 mg m⁻²), whether the so-called therapeutic window of NK105 is wider than that of conventional PTX should be determined in a future phases II or III trial, although the therapeutic window of NK105 appears to be wider than that of free PTX in mice experiments (Hamaguchi *et al*, 2005).

In general, haematological toxicity was mild and well managed in this trial. PTX is known to cause cumulative peripheral neuropathy resulting in the discontinuation of treatment with PTX. At a dose of 150 mg m⁻², three out of seven patients experienced only grade 1 neuropathy during the first cycle. Since the patients enrolled in this trial had almost intractable cancer, such as pancreatic or stomach, a relatively small number of patients received multiple cycles of treatment. Therefore, NK105-related neurotoxicity could not be evaluated in this study. However, three out of four patients who received more than five cycles of treatment experienced transient grade 2 peripheral neuropathy, and other patient developed transient grade 1 peripheral neuropathy. Future phase II trials may clarify whether NK105 is less toxic in terms of peripheral neuropathy when compared with conventional PTX, Abraxane, and other PTX compounds. Another characteristic adverse effect of PTX is hypersensitivity, which may be mainly caused by Cremophor EL. Since NK105 is not formulated in a Cremophor EL-containing solvent, we presumed that hypersensitivity would be diminished.

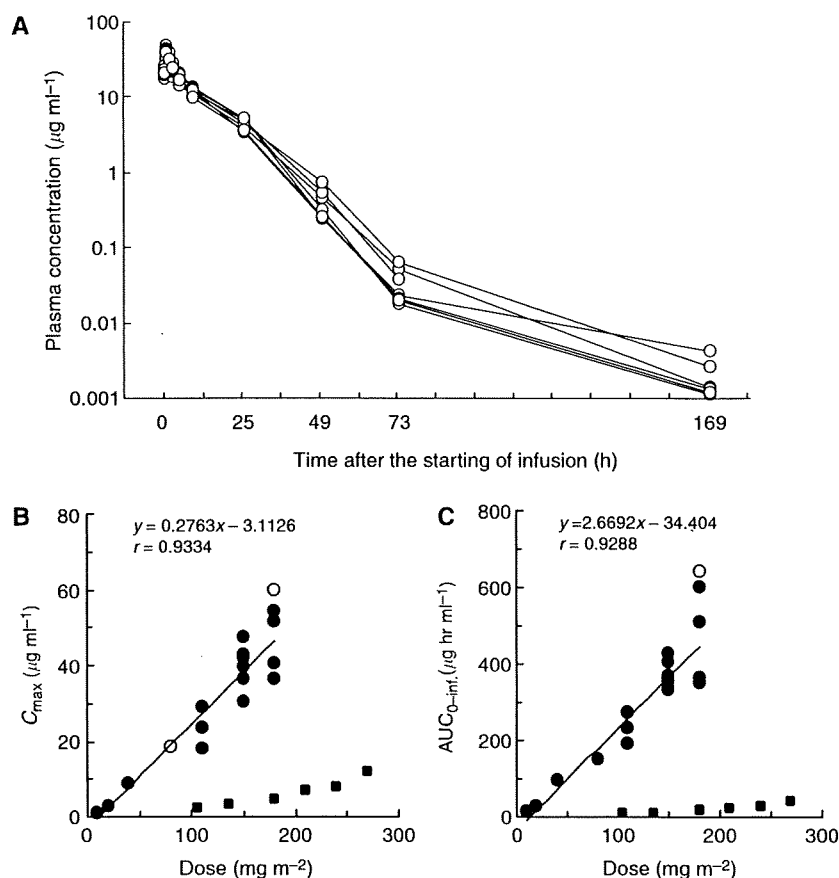


Figure 2 (A) Individual plasma concentrations of PTX in seven patients following 1-h intravenous infusion of NK105 at a dose of 150 mg m⁻². (B) Relationships between dose and C_{max}, and (C) between dose and AUC_{0–inf.} of PTX in patients following 1-h intravenous infusion of NK105. Regression analysis for dose vs C_{max} was applied using all points except one patient at 80 mg m⁻² whose medication time became 11 min longer and one patient at 180 mg m⁻² who had medication discontinuation and steroid medication. (Plots were shown as open circle.) Regression analysis for dose vs AUC_{0–inf.} was applied using all points except one patient who had medication discontinuation and steroid medication. (Plot was shown as open circle.) Relationships between dose and C_{max}, and AUC_{0–inf.} in patients following conventional PTX administration were plotted (closed square, see Tamura *et al*, 1995).

Table 3 Pharmacokinetic parameters

	Dose (mg m ⁻²)	n	C _{max} (μg ml ⁻¹)	AUC _{0–inf.} (μg h ml ⁻¹)	t _{1/2} (h)	CL _{tot} (ml h ⁻¹ m ⁻²)	V _{ss} (ml m ⁻²)	UE ^a (%)	CL _r (ml h m ⁻²)	
NK105	10	1	0.9797	11.4	9	880.4	10 400.3	7.5	66.4	
	20	1	2.8971	29.1	8.5	687.9	8027	8.6	59.4	
	40	1	8.8334	93.9	13.2	426.1	5389.8	5.2	22	
	80	1	18.4533	149.3	7	535.8	5875.8	4.7	25.3	
	110	3	23.3924	232	9.7	483.3	5881.2	7.6	35.6	
				± 5.6325	± 39.1	± 1.6	± 82.7	± 1512.0	± 1.7	± 6.9
	150	7	40.1699	369.8	10.6	408.6	4527.1	5.3	21.6	
			± 5.5334	± 35.2	± 1.3	± 37.3	± 639.5	± 1.5	± 6.5	
	180	4 ^b	45.6278	454.5	11.3	416.5	4983.4	5.9	23.7	
			± 8.6430	± 119.1	± 0.6	± 104.7	± 887.5	± 1.4	± 4.2	

^aUE, urinary excretion. ^bOne patient at 180 mg m⁻² level was omitted from the calculation of summary pharmacokinetic parameters, as there was administrating interruption for developing allergic reactions.

Indeed, the results of this clinical trial show that NK105 can be administered safely as a short infusion (1h) without the administration of antiallergic agents like dexamethasone and antihistamine, although one patient at 180 mg m⁻² developed transient grade 2 hypersensitivity at the first course. Therefore, NK105 may offer advantages in terms of safety and patient convenience and comfort.

The pharmacokinetic analysis of NK105 suggests that the distribution of PTX-incorporating micelles is mostly restricted to the plasma and, in part, to extracellular fluids in the body. This is consistent with data obtained in a preclinical study (Hamaguchi *et al*, 2005) showing that the distribution of NK105 in tissues is characterised by an EPR effect, similar to that of tumour and inflammatory lesions, or by the presence of a reticuloendothelial

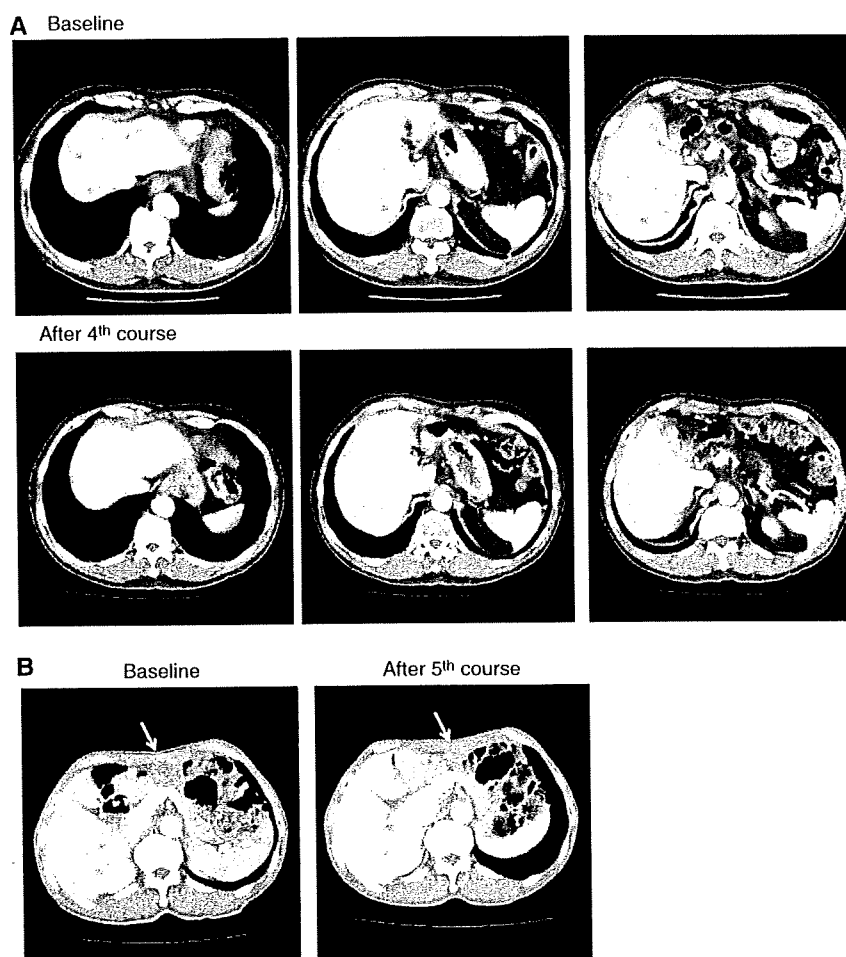


Figure 3 Serial CT scans. **(A)** A 60-year-old male with pancreatic cancer who was treated with NK105 at a dose level of 150 mg m^{-2} . Baseline scan (upper panels) showing multiple metastasis in the liver. Partial response, characterized by a more than 90% decrease in the size of the liver metastasis (lower panels) compared with the baseline scan. The antitumour response was maintained for nearly 1 year. **(B)** A 64-year-old male with stomach cancer who was treated with NK105 at a dose level of 150 mg m^{-2} . Baseline scan (left panel) showing a peritoneal metastasis and liver metastasis. About 40% reduction (right panel) was observed in peritoneal metastasis, but not in the liver metastasis after fifth course.

Table 4 Pharmacokinetic parameters

	Dose (mg m^{-2})	n	C_{max} ($\mu\text{g ml}^{-1}$)	$\text{AUC}_{0-\text{inf}}$ ($\mu\text{g h}^{-1} \text{ml}^{-1}$)	$t_{1/2}$ (h)	CL_{tot} ($\text{ml h}^{-1} \text{m}^{-2}$)	V_{ss} (ml m^{-2})	UE (%)	CL_r ($\text{ml h}^{-1} \text{m}^{-2}$)
NK105	150	7	40.1699 ± 5.5334	369.8 ± 35.2	10.6 ± 1.3	408.6 ± 37.3	4527.1 ± 639.5	5.3 ± 1.5	21.6 ± 6.5
PTX	210	5	6.744 ± 2.733	23.18 ± 10.66	13.3 ± 1.5	10740 ± 4860	58 900 $\pm 24 700$	9.45 ± 3.76	1020 ± 648
XYOTAX ^a	233	4	NA	1583	120	276	6200	NA	NA
Abraxane	300	5	13.52 ± 0.95	17.61 ± 3.70	14.6 ± 2.04	17 700 ± 3894	370 000 $\pm 85 100$	NA	NA
Genoxol-PM	300	3	3.107 ± 1.476	11.58 ± 4.28	11.4 ± 2.4	29 300 $\pm 13 800$	NA	NA	NA

^aConjugated taxanes.

system. When compared with conventional PTX at a dose of 210 mg m^{-2} (conventional dose for a 3-week regimen in Japanese patients), NK105 at a dose of 150 mg m^{-2} (recommended phase II dose) exhibited more than 15-fold larger plasma AUC and a 26-fold lower CL_{tot} . The larger plasma AUC is consistent with the stability of the micelle formulation in plasma. The V_{ss} of NK105

was 13-fold lower than that of conventional PTX. This suggests that PTX may have a relatively lower distribution in normal tissue, including normal neural tissue, following NK105 administration. Regarding the drug distribution in tumours, nanoparticle drug carriers have been known to preferentially accumulate in tumour tissues utilising the EPR effect (Matsumura and Maeda, 1986;

Maeda *et al*, 2000; Duncan, 2003). We speculate that NK105 accumulates more in tumour tissues than free PTX, since NK105 is very stable in the circulation and exhibits a markedly higher plasma AUC than free PTX. Moreover, a polymeric micelle carrier system for a drug has the potential to enable the sustained release of the drug inside a tumour following the accumulation of micelles in the tumour tissue (Hamaguchi *et al*, 2005; Uchino *et al*, 2005; Koizumi *et al*, 2006). Regarding NK105 in particular, this sustained release may begin at a PTX-equivalent dose of $<1 \mu\text{g ml}^{-1}$ (data not shown). Consequently, the released PTX is distributed throughout the tumour tissue where it kills the cancer cells directly.

In the present study, NK105 appeared to exhibit characteristic pharmacokinetics different from those of other PTX formulations including conventional PTX, Abraxane, Genexol-PM, and Xyotax. For example, previous clinical PK data at each phase II

recommended dose shown that plasma AUC and C_{max} were 11.58 and 3.1 in Genexol-PM (Table 4). The antitumour activities seen in two patients with intractable cancers are encouraging. In addition, we recently demonstrated in preclinical study that combined NK105 chemotherapy with radiation exerts a significantly more potent antitumour activity, compared with combined PTX therapy and radiation (Negishi *et al*, 2006). This data on NK105 justifies its continued clinical evaluation.

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A phase I trial of S-1 with concurrent radiotherapy for locally advanced pancreatic cancer

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This study investigated the maximum tolerated dose of S-1 based on the frequency of its dose-limiting toxicities (DLT) with concurrent radiotherapy in patients with locally advanced pancreatic cancer. S-1 was administered orally at escalating doses from 50 to 80 mg m⁻² b.i.d. on the day of irradiation during radiotherapy. Radiation therapy was delivered through four fields as a total dose of 50.4 Gy in 28 fractions over 5.5 weeks, and no prophylactic nodal irradiation was given. Twenty-one patients (50 three; 60 five; 70 six; 80 mg m⁻² seven patients) were enrolled in this trial. At a dose of 70 mg m⁻² S-1, two of six patients demonstrated DLT involving grade 3 nausea and vomiting and grade 3 haemorrhagic gastritis, whereas no patients at doses other than 70 mg m⁻² demonstrated any sign of DLT. Among the 21 enrolled patients, four (19.0%) showed a partial response. The median progression-free survival time and median survival time for the patients overall were 8.9 and 11.0 months, respectively. The recommended dose of S-1 therapy with concurrent radiotherapy is 80 mg m⁻² day⁻¹. A multi-institutional phase II trial of this regimen in patients with locally advanced pancreatic cancer is now underway.

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Pancreatic cancer (PC) is one of the leading causes of cancer death worldwide. The prognosis of patients with this disease remains extremely poor, with a 5-year survival rate after diagnosis of less than 5%. Despite recent improvements in diagnostic techniques, PC is diagnosed at an advanced stage in most patients. Among these patients, roughly one-third is diagnosed as having locally advanced disease radiographically confined to the pancreas and surrounding tissues. In patients with locally advanced PC, the concurrent external-beam radiation therapy and 5-fluorouracil (5-FU) therapy has been shown to offer a survival benefit in comparison with radiotherapy alone (Moertel *et al*, 1969, 1981) or chemotherapy alone (Gastrointestinal Tumor Study Group, 1988). In an attempt to improve the efficacy of 5-FU with concurrent radiotherapy, various anticancer agents and radiation schedules are being examined in clinical trials, but no significant impact on survival has been accomplished. Because of these results, 5-FU with concurrent radiotherapy remains the predominant chemoradiotherapy for locally advanced PC in clinical use (Willett *et al*, 2005; Yip *et al*, 2006).

S-1 is a novel orally administered drug, which is a combination of tegafur, 5-chloro-2,4-dihydroxypyridine and oteracil potassium in a 1:0.4:1 molar concentration ratio. Tegafur is hydroxylated and converted to 5-FU by the hepatic microsomal enzymes. 5-Chloro-2,4-dihydroxypyridine is a competitive inhibitor of

dihydropyrimidine dehydrogenase, which is involved in the degradation of 5-FU, and acts to maintain effective concentrations of 5-FU in plasma and tumour tissues. Oteracil potassium, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, reducing the serious gastrointestinal toxicity associated with 5-FU (Shirasaka *et al*, 1996a). In athymic nude rats, S-1 has been shown to result in retention of a higher and more prolonged concentration of 5-FU in plasma and tumour tissues in comparison with 5-FU and uracil/tegafur (Shirasaka *et al*, 1996b). The antitumour effect of S-1 has already been demonstrated in a variety of solid tumours, including advanced gastric cancer (Sakata *et al*, 1998), colorectal cancer (Ohtsu *et al*, 2000), non-small-cell lung cancer (Kawahara *et al*, 2001), and head and neck cancer (Inuyama *et al*, 2001). In patients with metastatic PC, a recent early phase II study has demonstrated a response rate of 21% (Ueno *et al*, 2005), and a more favourable tumour response (response rate: 38%) and survival (median: 8.8 months) have been reported in a multi-institutional late phase II trial of S-1 (Furuse *et al*, 2005).

Thus, S-1 has promising antitumour activity against advanced PC, and is much more convenient to administer than intravenous 5-FU infusion, as it is taken orally. Concurrent radiotherapy along with S-1 therapy as an alternative to 5-FU infusion may result in more efficient treatment and improve the quality of life of patients. Therefore, we conducted a phase I trial to determine the maximum tolerated dose of S-1 with concurrent radiotherapy based on the frequency of dose-limiting toxicities (DLT) in patients with locally advanced PC.

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PATIENTS AND METHODS

Eligibility

Patients eligible for study entry had histologically or cytologically confirmed locally advanced nonresectable PC. Eligibility criteria were age 20–74 years; Karnofsky performance status 70–100 points; no evidence of distant metastasis; adequate oral intake; estimated life expectancy ≥ 12 weeks after study entry; no earlier treatment for PC; adequate haematological function (haemoglobin ≥ 10 g dl⁻¹, leucocytes ≥ 4000 mm⁻³, neutrophils ≥ 2000 mm⁻³ and platelets $\geq 100\,000$ mm⁻³); adequate hepatic function (serum total bilirubin ≤ 2.0 times upper normal limit (UNL); serum albumin ≥ 3.0 g dl⁻¹ and serum transaminases (aspartate aminotransferase (AST)/alanine aminotransferase (ALT)) ≤ 2.5 times UNL or ≤ 5 times UNL if biliary drainage present); adequate renal function (serum creatinine ≤ 1.0 mg dl⁻¹); written informed consent.

The exclusion criteria were watery diarrhoea; pleural effusion or ascites; active infection; active gastroduodenal ulcer; severe complication such as heart disease or renal disease; mental disorder; history of drug hypersensitivity; active concomitant malignancy; pregnant and lactating females; females of child-bearing age unless using effective contraception.

Ultrasonography, multidetector row-computed tomography of the abdomen and chest X-ray were performed for pretreatment staging to assess the local extension of the tumour and exclude the presence of distant metastasis. The computed tomography-based criteria for tumour nonresectability included tumour encasement of the celiac trunk, common hepatic artery, superior mesenteric artery or bilateral invasion of the portal vein. All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. 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 Principles.

Treatment schedule

This was an open-label, two-institutional and single-arm phase I study that was performed on an in-patient basis. Radiotherapy was administered by 10 or 25 MV photons using three-dimensional treatment planning. A total dose of 50.4 Gy was delivered in 28 fractions over 5.5 weeks. The clinical target volume (CTV) included only the gross primary tumour and nodal involvement enlarged over 10 mm detected by computed tomography. Elective nodal irradiation was not used. The planning target volume was defined as CTV plus a 10 mm margin in the lateral direction and 10–20 mm margin in the cranio-caudal direction to account for respiratory organ motion and daily set-up error. The four-field technique (anterior, posterior and opposed lateral fields) was used. There was no field reduction. The spinal cord dose was maintained below 45 Gy. The dose received by $\geq 50\%$ of the liver was limited to ≤ 30 Gy, and that received by $\geq 50\%$ of both kidneys was limited to ≤ 20 Gy.

S-1 was administered orally twice daily after breakfast and dinner on the day of irradiation (Monday to Friday) during radiotherapy. The initial dose of S-1 was 50 mg m⁻² day⁻¹, and the dose was escalated to 80 mg m⁻² day⁻¹ in increments of 10 mg m⁻² day⁻¹ (Table 1). The calculated S-1 dose was rounded down to the nearest 60, 80, 100 or 120 mg. S-1 at 50 mg m⁻² day⁻¹ is reported to be almost equivalent to 200 mg m⁻² day⁻¹ intravenously 5-FU (Hirata *et al*, 1999), which has been used in protracted 5-FU infusion with concurrent radiotherapy for locally advanced PC at our institutions (Ishii *et al* 1997). S-1 at 80 mg m⁻² day⁻¹ is the standard dose used as a single agent for systemic therapy (Furuse *et al*, 2005; Ueno *et al*, 2005). Patients maintained a daily journal to record their intake of S-1 and any signs or symptoms that they experienced.

Table 1 Dose schedules of S-1 with concurrent radiotherapy

Dosage level	S-1 dose (mg m ⁻² day ⁻¹)	Number of patients
1	50	3
2	60	5
3	70	6
4	80	7

Patient cohorts had a minimum of three patients at each dose level. If no DLT was observed in the initial three patients, the dosage was escalated in successive cohorts. If DLT was observed in one or two of the initial three patients, three additional patients were evaluated at that dose level. If only one or two of six patients experienced DLT, dose escalation was continued. However, if three or more patients experienced DLT at a given dose level, then the previous dose level was considered as the maximum tolerated dose. Dose-limiting toxicities was defined as the following manifestations of toxicity observed until completion of chemoradiotherapy: grade 3 leucocytopenia and/or neutropenia with a fever $\geq 38^\circ\text{C}$ lasting 3 days or more, grade 3 leucocytopenia and/or neutropenia with infection, grade 4 leucocytopenia and/or neutropenia lasting 3 days or more, grade 4 leucocytopenia and/or neutropenia requiring haematopoietic colony-stimulating factors, platelets $< 25\,000$ mm⁻³, grade 3 thrombocytopenia requiring transfusion, serum AST/ALT ≥ 10 times UNL, grade 3 or 4 nonhaematological toxicities excluding nausea, vomiting, anorexia, fatigue, constipation, hyperglycaemia, and abnormality of sodium, potassium, and calcium or treatment interruption for longer than 12 days.

When grade 3 or greater haematological toxicity, total bilirubin level 2.0–3.0 times UNL, serum AST/ALT 5.0–10.0 times UNL, grade 3 vomiting and/or grade 2 nonhaematological toxicity excluding nausea, vomiting, anorexia, fatigue, constipation, alopecia and pigmentation change, were observed, radiotherapy and S-1 administration was suspended. Treatment was resumed when the toxicities were resolved by one grade or more, compared with these suspension criteria. Dose modification was not performed in this study. When DLT or tumour progression was observed during chemoradiotherapy, this treatment was discontinued. After this treatment, the patients were allowed to receive another anticancer treatment at their physician's discretion.

Toxicity and response evaluation

The primary end point of this trial was to evaluate the frequency of DLT, and the secondary end point was to evaluate the potential antitumour activity. Treatment-related toxicities were assessed using the National Cancer Institute Common Toxicity Criteria version 2.0. During this treatment, complete blood count with differentials, serum chemistry and urinalysis were carried out at least once a week. Tumor response was evaluated at the completion of chemoradiotherapy and every 8 weeks thereafter until tumour progression, according to the Japan Society for Cancer Therapy criteria (Japan Society for Cancer Therapy, 1993) as follows: a complete response was defined as the disappearance of all clinical evidence of the tumour for a minimum of 4 weeks. A partial response was defined as a 50% or greater reduction in the sum of the products of two perpendicular diameters of all measurable lesions for a minimum of 4 weeks. A minor response was defined as a 25% or greater reduction and less than 50% in the sum of the products of two perpendicular diameters of all measurable lesions for a minimum of 4 weeks or a 50% or greater reduction in the sum of the products of two perpendicular diameters of all measurable lesions lasting less than 4 weeks. No change was defined as a reduction of less than 25% or a less than 25% increase