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Phase I Study of NK012, a Novel SN-38–Incorporating Micellar Nanoparticle, in Adult Patients with Solid Tumors

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Abstract

Purpose: We conducted a first-in-human phase I study to determine the dose-limiting toxicity (DLT), evaluate the pharmacokinetic profile, and document any antitumor activity of NK012, a novel SN-38–incorporating micellar nanoparticle.

Experimental Design: Patients with solid tumors refractory to standard therapy, or for which no standard therapy is available, were enrolled. NK012 was administered as a 30-minute infusion every 3 weeks. The starting dose was 2 mg/m^2 as SN-38 equivalent, and an accelerated titration schedule was used. Pharmacokinetic analysis was conducted in cycles 1 and 2.

Results: Twenty-four patients were enrolled in the study. No *UGT1A1*28* homozygous patients were enrolled. Predominant toxicity was neutropenia. Nonhematologic toxicity, especially diarrhea, was mostly grade 1 or 2 during study treatments. Two of nine patients had DLT during cycle 1 at the 28 mg/m^2 dose level. DLTs were mostly neutropenia or a related event. Polymer-bound SN-38 (NK012) and SN-38 released from NK012 were slowly eliminated from the plasma, with a terminal-phase half-life of approximately 140 and 210 hours, respectively. Systemic exposure to both polymer-bound SN-38 and SN-38 increased in proportion to the dose. A refractory esophageal cancer patient and a lung carcinoid tumor patient had an objective response and continued the study treatment for 5 and 12 months, respectively.

Conclusions: NK012 was well tolerated and showed antitumor activity including partial responses and several occurrences of prolonged stable disease across a variety of advanced refractory cancers. Phase II studies are ongoing. *Clin Cancer Res; 16(20); 5058–66. ©2010 AACR.*

Irinotecan hydrochloride (CPT-11) has proven to be active against colorectal, lung, and ovarian cancers (1–5). CPT-11 is a prodrug that is converted to a biologically active metabolite, 7-ethyl-10-hydroxy-CPT (SN-38), by carboxylesterase (CE) enzymes. SN-38 is an analogue of the plant alkaloid camptothecin, which targets DNA topoisomerase I. Compared with CPT-11, SN-38 exhibits up to 1,000-fold more potent cytotoxic activity against various cancer cells *in vitro* (6). Although CPT-11 is converted to SN-38 in the liver and tumor, the metabolic conversion rate is <10% of the original volume of CPT-11 (7, 8). Moreover, the conversion of CPT-11 to SN-38 depends on the genetic interindividual variability of CE activity (9). Thus, more efficient use of SN-38 might be highly advantageous and quite attractive for cancer treatment.

Drugs categorized under the drug delivery system (DDS) are made primarily by using nanotechnology (10). In the field of oncology, DDS drugs have been produced and evaluated in preclinical or clinical trials, with some already approved for clinical use (11, 12). NK012 categorized in DDS is a micelle-forming macromolecular prodrug prepared by binding SN-38 to the polyglutamate of a block copolymer via an ester bond (Fig. 1). The amphiphilic block copolymers consist of polyethylene glycol and partially SN-38–bound polyglutamate. Polyethylene glycol is hydrophilic and would form the outer shell of the micelle, producing a "stealth" effect that allows NK012 to avoid uptake by the reticuloendothelial system, and SN-38–bound polyglutamate is hydrophobic and would form the inner core of the micelle. The ester bond between glutamic acid and SN-38 is gradually cleaved by hydrolysis under physiologic conditions. In other words, SN-38 can gradually be released from NK012 in a nonenzymatic

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Translational Relevance

NK012 is an SN-38–loaded polymeric micelle constructed in an aqueous milieu via self-assembly of an amphiphilic block copolymer. NK012, which combines enhanced distribution with prolonged sustained release of SN-38 within tumors, may be ideal for treating solid tumors because the antitumor activity of SN-38 is time dependent. This phase I study was conducted to determine the maximum tolerated dose, dose-limiting toxicities, and pharmacokinetics of NK012 administered as an i.v. infusion every 3 weeks. Two patients achieved partial response, indicating preliminary evidence of antitumor activity. Hematologic toxicities were manageable, and notably, none of the patients experienced grade 3 diarrhea during any cycle. In the pharmacokinetic study, polymer-bound SN-38 (NK012) clearance was significantly lower and released SN-38 concentration in the plasma was maintained for a long time compared with those of conventional CPT-11 at a dose of 250 mg/m^2. Moreover, systemic exposure to both polymer-bound SN-38 and SN-38 increased in proportion to the dose. NK012 warrants further evaluation to assess its efficacy, alone or in combination with other agents, in tumors showing sensitivity to CPT-11.

manner. Therefore, unlike CPT-11, NK012 is expected to exhibit stable drug efficacy regardless of differences in CE activity among patients. NK012 has a diameter of ~20 nm. In preclinical experimental tumor models such as lung cancer (13, 14), pancreatic cancer (15), renal cancer (16), glioma (17), gastric cancer (18), and colorectal cancer (19), NK012 exerted significantly superior antitumor activity and induced longer survival compared with CPT-11. In preclinical pharmacokinetic (PK) studies (13–18), CPT-11 and SN-38 converted from CPT-11 rapidly disappeared from the plasma. On the other hand, polymer-bound SN-38 (NK012) exhibited a lower clearance rate. In the tumor tissues, polymer-bound SN-38 and released SN-38 concentration were also maintained for a long time following injection. Thus, NK012, which combines enhanced distribution with sustained release of SN-38 within tumors, may be ideal for the treatment of solid tumors because the antitumor activity of SN-38 is time dependent (15).

The primary endpoints of this study were to determine the maximum tolerated dose (MTD) and recommended phase II dose of NK012 administered as an i.v. infusion every 3 weeks, evaluate the toxicity profile and PK, and identify any dose-limiting toxicity (DLT). Evidence of antitumor activity was also evaluated.

Materials and Methods

This trial was a two-center (National Cancer Center Hospital, Tokyo and National Cancer Center Hospital East, Chiba), first-in-human, open-label, phase I, dose-escalation study of NK012 in patients with advanced tumors, sponsored by Nippon Kayaku Co. Ltd. (Tokyo, Japan). This study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Patients

Eligible patients had histologically or cytologically confirmed malignant tumors for which standard curative or palliative measures did not exist. Further requirements were as follows: age ≥20 and <75 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; life expectancy ≥2 months; and adequate bone marrow, hepatic, renal, and pulmonary function within 1 week before commencing treatment [absolute neutrophil count ≥2,000/μL, platelet count ≥100,000/μL, hemoglobin ≥9 g/dL, total bilirubin ≤1.5 mg/dL, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 times the upper limit of normal, creatinine ≤1.5 mg/dL, PaO_2 ≥60 mmHg]. Treatment with radiotherapy, endocrine therapy, or chemotherapy must have ceased at least 4 weeks before commencing treatment. Patients with severe, clinically significant, and/or uncontrolled medical conditions were excluded. Patients who had previously been treated with CPT-11 were accepted for enrollment. Our institutional review board granted approval for the study, and written informed consent from each patient was obtained.

Treatment plan

NK012 was supplied by Nippon Kayaku Co. Ltd. The drug was a sterile lyophilized powder and was diluted with 5% glucose for a total volume of 250 mL. This solution was administered by i.v. infusion for 30 minutes every 21 days. This schedule was set based on the nadir point and the period for recovery after the dosing of NK012 according to data from the preclinical study.

No prophylactic agents for emesis or cholinergic reaction were administered. Patients received up to four cycles of NK012, except in the case of unacceptable toxicity, withdrawal of consent, or disease progression. Patients could continue treatment beyond four cycles if the investigator determined that additional treatment would be of further benefit to the patient, as long as toxicity remained acceptable.

Patients were screened for *UGT1A1* polymorphism (*UGT1A1*28* and *UGT1A1*6*) before enrollment. Based on the screening results, patients were separated into two groups: (group 1) patients with wild-type (*wt/wt*), those with *UGT1A1*28* heterozygous genotype (*wt/*28*), or those with *UGT1A1*6* genotype (*wt/*6*, **6/*6*, or **28/*6*), and (group 2) those with *UGT1A1*28* homozygous genotype (**28/*28*). Patients of group 1 received a starting dosage of NK012 of 2 mg/m^2, which is one third the toxic dose low in dogs. As a safety measure, patients of group 2 were treated at a lower dose (confirmed tolerable dose in group 1) to avoid any anticipated severe toxicity in this trial.

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Assessments, follow-up, and monitoring

Toxic events were observed until resolution to baseline or less than grade 1. Before entry into the study, patients underwent a clinical history and physical examination, performance status assessment, complete blood count, chemistries, urinalysis, pregnancy test (if applicable), chest X-ray, electrocardiogram including assessment of QTc interval, and disease assessment by computed tomography (CT) scan. During therapy, patients were assessed at least weekly for adverse events (AE). CT scanning of disease sites was repeated every two cycles. AEs were classified/graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3). Response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors.

DLT was defined as any drug-related grade 4 hematologic toxicity (grade 4 neutropenia ≥5 days, grade 4 thrombocytopenia) and other toxicity grade ≥3 with the exception of nausea, vomiting, loss of appetite, or hypersensitivity. We conducted this dose finding study according to the accelerated titration method described by Simon et al. (20). Namely, because many patients in phase I clinical trials are treated at doses of chemotherapeutic agents that are below the biologically active doses, they have a reduced chance of receiving therapeutic benefit. Therefore, we decided to adopt an accelerated titration followed by a

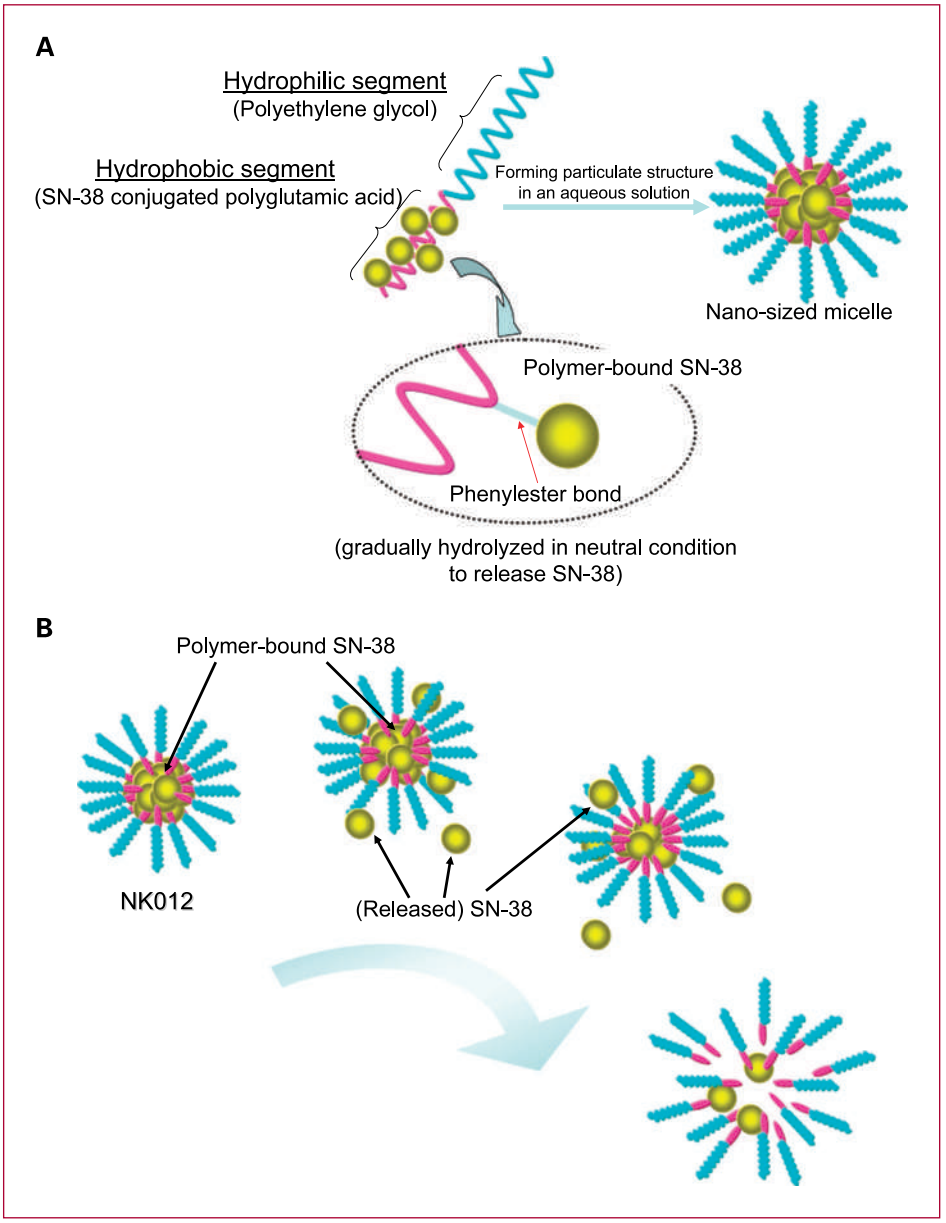

Fig. 1. A, schematic structure of NK012. B, release of SN-38 from NK012.

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modified Fibonacci method to reduce the number of such patients as described previously (21). In this two-stage design, the first stage allows for a single patient to be enrolled at each dose level. The dose of NK012 is doubled in each successive patient until grade 2 toxicity is observed. If grade 2 toxicity occurs in one patient, that dose level is given to another two patients. This marks the start of the second stage of the design, which is a modified Fibonacci method.

The recommended phase II dose was defined by the Efficacy and Safety Assessment Committee based on the results of this trial. Determination of the MTD/recommended dose was based on the patients of group 1.

PK analysis

Blood samples for PK analysis were obtained on days 1, 2, 3, 4, 8, 15, and 22 of cycle 1 and on days 1 and 2 of cycle 2. Urine samples were collected and pooled over before dosing and 0 to 24 and 24 to 48 hours after the start of infusion in cycle 1. Blood samples were immediately centrifuged, and then a portion of the obtained plasma sample was mixed with an equivalent volume of ice-cold 0.1 mol/L HCl to prevent hydrolysis of NK012. Plasma and urine samples were stored at −80°C until analysis.

The concentration of total SN-38 (the sum of polymer-bound SN-38 and released SN-38), SN-38, and its glucuronide (SN-38G) in the plasma and that of total SN-38 and SN-38G in the urine were assayed by modified reversed-phase high-performance liquid chromatography (HPLC) using fluorescence detection (13). Polymer-bound SN-38 was not quantitated in the urine, as NK012 is unstable in urine. For the respective analytes, proteins were precipitated with an ice-cold mixture of methanol/H_2O/$HClO_4$ (10:9:1, v/v/v). The sample was vortexed for 10 seconds, filtered through a MultiScreen Solvinert (Millipore Corp.), and analyzed. We had previously confirmed that the filtered solution did not contain polymer-bound SN-38. Total SN-38 was determined after alkali hydrolysis. For the plasma matrix, the values for the lower limit of quantitation for total SN-38, SN-38, and SN-38G were 1.0, 0.1, and 0.5 ng/mL, respectively. For the urine, the value for the lower limit of quantitation for both total SN-38 and SN-38G was 10 ng/mL. Polymer-bound SN-38 was determined by subtracting the SN-38 from the total SN-38.

The PK parameters [observed peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}), half-life of the terminal phase ($t_{1/2z}$), area under the concentration-time curve ($AUC_{inf.}$), total clearance (CL_{tot}), volume of distribution at steady state (V_{ss}), and the mean residence time ($MRT_{inf.}$)] were calculated for each patient using the noncompartmental analysis module of the software program WinNonlin Professional (Pharsight Corp.).

Results

Patient characteristics

Between February 2006 and February 2008, 24 patients with advanced solid tumors were enrolled in the study (Table 1). All the patients except one lung carcinoid patient had received chemotherapy before enrollment. Prior therapies ranged from 1 to 11 regimens of chemotherapy. Fifteen patients, especially all colorectal patients, had previously received CPT-11–based chemotherapy. All the patients were included in the safety analyses. A total of 108 cycles of the study drug was delivered (median, 3.5 cycles; range, 1-12 cycles). Twenty-two patients received more than two administrations. The maximum number of treatments was 12 cycles at 28 mg/m^2. No patients were *UGT1A1*28* homozygous.

Table 1. Patient characteristics

No. patients	24
Sex	
Male	18
Female	6
Age (y)	
Median	61.5
Range	41-74
ECOG performance status	
0	15
1	9
Primary tumor site	
Colorectal	12
Pancreas	4
Small cell lung cancer	3
Esophageal	3
Lung carcinoid	1
Non–small cell lung cancer	1
Prior treatment	
Chemotherapy regimens	
Median	2
Range	0-11
UGT1A1 genotype	
wt/wt	10
*wt/*28*	3
*wt/*6*	10
**6/*28*	1
**6/*6* or **28/*28*	0

Dose-escalation process

Dosage escalation started at 2 mg/m^2 and was gradually increased up to 28 mg/m^2 (Table 2). Clinically meaningful grade 2 NK012-related toxicity was not observed up until 8 mg/m^2 during cycle 1. However, the Efficacy and Safety Assessment Committee recommended raising the dosage by 50% instead of 100% at 12 mg/m^2 and that a modified Fibonacci escalation method should be implemented because the neutrophil count had decreased by 50% compared with the baseline. Therefore, we restarted the dose identification study using a modified Fibonacci method. At a dose level of 20 mg/m^2, one patient experienced grade 4 neutropenia lasting for <5 days. As a safety measure, it was decided that the dose of later cohorts would be increased in increments of 4 mg/m^2, although this AE was

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not considered DLT. Although 9 of 15 patients at a dose level of 20 to 28 mg/m^2 had either a delay or a reduction in treatment as a result of toxicity (especially neutropenia), only 3 of these patients required dose reduction after DLT; even in the 28 mg/m^2 cohort, 6 of 8 patients successfully continued treatment with NK012 without dose reduction.

Toxicity

NK012 was generally well tolerated. All patients reported AEs considered to be related to NK012, most of which were asymptomatic and grade 1 to 2 in severity: nausea, anorexia, vomiting, fatigue, elevated AST/ALT, elevated γ-glutamyl transpeptidase (γ-GTP), and thrombocytopenia. Three infusion-related reactions were observed at a dose level of 2, 12, and 24 mg/m^2. No cholinergic reactions were observed; such reactions sometimes occur during CPT-11 administration. Fifteen patients reported 36 grade 3 to 4 drug-related AEs (Table 3). The most common grade 3 to 4 events were leukopenia and neutropenia. The median time to the neutrophil nadir at a dose level of 28 mg/m^2 was 12 days (range, 9-21 days), with recovery to grade 1 within 4 to 16 days in cycle 1. No grade 3 or 4 diarrhea was observed.

Four patients reported five serious AEs, three of which were deemed possibly related to NK012: grade 3 infection with neutropenia, grade 4 neutropenia, and grade 3 atrial flutter.

Two of the nine patients treated at a dose level of 28 mg/m^2 experienced DLT during cycle 1. In another independent phase I study of NK012 in the United States, only one of six patients experienced DLT at 28 mg/m^2 during cycle 1, but two of five patients experienced DLT at 37 mg/m^2 during cycle 1 (22). Although the protocol definition of MTD had not been reached, the Efficacy and Safety Assessment Committee recommended discontinuing dose escalation according to the hematologic toxicity profile of our study and another independent phase I study (22). The recommended phase II dose was determined to be 28 mg/m^2 with at least a 3-week interval between treatment cycles.

Table 2. Dose-escalation schema and DLT

NK012 dose (mg/m^2)	Total	DLT			
		Cycle 1	Cycle 2	Cycle 3	Cycle 4
2	1	0	0	0	0
4	1	0	0	0	0
8	1	0	0	0	0
12	3	0	0	0	0
16	3	0	0	0	1*
20	3	0	0	0	0
24	3	0	0	1†	0
28‡	9	2†	1†	1†	1§

*γ-GTP increased.
†Neutropenia or a related event.
‡Recommended dose for phase II studies.
§Atrial flutter.

Efficacy

Twenty-three patients were assessable for response. Two patients had confirmed partial response at a dose of 28 mg/m^2. The first patient, who received CDDP + 5-fluorouracil combination chemotherapy followed by docetaxel monotherapy and had a previously progressing esophageal cancer, achieved a partial response confirmed by CT and continued this therapy for 5 months (serial CT scans can be seen in Supplementary Data). The second patient had a recurrence of lung carcinoid tumor. He developed multiple liver and bone metastases and enrolled in this phase I study because there is no standard systemic chemotherapy for his disease. After four cycles, a partial response was documented. He continued to receive this chemotherapy for 12 months until disease progression. Nine patients had stable disease. In 12 colorectal cancer patients, who were refractory to CPT-11 and oxaliplatin, 5 patients had stable diseases, 4 of whom successfully received six cycles of treatment or more.

Pharmacokinetics

The mean plasma concentration-time profile of polymer-bound SN-38, SN-38, and SN-38G at a dose of 28 mg/m^2 (recommended phase II dose) is shown in Fig. 2A. The concentrations of these analytes in the plasma were maintained over an extended period of time, indicating that NK012 achieved prolonged exposure. There was a proportional increase in C_{max} and $AUC_{inf.}$ values with dose (Fig. 2B and C). The PK parameters are summarized in Table 4. The $t_{1/2z}$ of bound SN-38 was 36.0 to 168 hours, CL_{tot} was 98.8 to 150 mL/h/m^2, V_{ss} was 2,020 to 4,050 mL/m^2, and $MRT_{inf.}$ was 15.9 to 28.6 hours. No significant differences in these parameters were seen in the dose range from 2 to 28 mg/m^2. The $t_{1/2z}$ of SN-38 ranged from 70.7 to 266 hours, and $MRT_{inf.}$ ranged from 47.3 to 109 hours. These parameters were dose independent. Therefore, PK of NK012 proved to be linear in the dose range of 2 to 28 mg/m^2. There was no obvious difference between the plasma concentration of the respective analytes in cycles 1 and 2 (data not shown), although the study design could not fully evaluate the cycle dependency of NK012 PK. The cumulative urinary excretion rate (0-48 hours) of total SN-38 and SN-38G at a dose level of 28 mg/m^2 was 7.0% and 6.8%, respectively. These rates were independent of the dose escalation.

Discussion

In this phase I study, NK012 was well tolerated at doses <28 mg/m^2 every 3 weeks.

Observed toxicity was consistent with CPT-11, a prodrug of SN-38. DLT associated with NK012 was mainly neutropenia. Therefore, it is necessary to pay attention to neutrophil count changes after treatment with NK012; patients with a decreased count should be carefully monitored to prevent infection. Infusion-related reactions characterized by flushing, chest discomfort, or itching occur sometimes during the administration of liposomal

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Table 3. Highest hematologic and nonhematologic toxicity per patient

A. Hematologic toxicity

Dose (mg/m^2)	*n*	Leukopenia				Neutropenia				Thrombocytopenia			
		Grade				Grade				Grade			
		1	2	3	4	1	2	3	4	1	2	3	4
2	1	0	0	0	0	0	0	0	0	0	0	0	0
4	1	0	0	0	0	0	0	0	0	0	0	0	0
8	1	0	0	0	0	0	0	0	0	0	0	0	0
12	3	0	1	1	0	1	0	1	0	1	0	1	0
16	3	1	2	0	0	2	0	1	0	1	0	0	0
20	3	1	0	2	0	1	0	0	2	0	1	0	0
24	3	0	2	1	0	0	0	2	1	3	0	0	0
28	9	0	1	5	3	0	0	4	5	3	3	0	0
Total	24	2	6	9	3	4	0	8	8	8	4	1	0

B. Nonhematologic toxicity

	2-20 mg/m^2 (*n* = 12)				24 mg/m^2 (*n* = 3)				28 mg/m^2 (*n* = 9)			
	Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4
Nausea	8	2	0	0	3	0	0	0	3	3	1	0
Anorexia	6	3	0	0	3	0	0	0	3	2	2	0
Diarrhea	3	0	0	0	0	1	0	0	4	4	0	0
Vomiting	3	1	0	0	0	0	0	0	2	3	0	0
Fatigue	7	0	0	0	2	1	0	0	4	2	0	0
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	1	0
Infection	0	1	0	0	0	0	0	0	0	0	1	0
Atrial flutter	0	0	0	0	0	0	0	0	0	0	1	0
Alopecia	7	1	—	—	1	2	—	—	5	3	—	—
γ-GTP	0	1	1	0	1	0	0	0	2	1	0	0
Rash	2	1	0	0	1	1	0	0	1	2	0	0

and antibody drugs. In this study, only three infusion-related reactions were observed at dose levels of 2, 12, and 24 mg/m^2. However, these infusion reactions were grade 1 and deemed non–dose dependent. The factors associated with the infusion-related reactions remain to be explained. Diarrhea, which is known as a DLT of CPT-11, was mild and transient in this study. Fatigue, nausea, and anorexia were also commonly experienced AEs but were mild and transient. Our preclinical study showed that the CPT-11–induced intestinal mucosal change in mice was active inflammation with cellular invasion, deformed glandular alignment, and glandular duct disappearance. On the other hand, the intestinal mucosa in mice in the NK012 treatment group was almost the same as that in the saline treatment group (14). CPT-11, SN-38, and SN-38G are excreted into the bile and eventually reach the small intestinal lumen (23, 24). SN-38G is deconjugated in the cecum and colon to regenerate SN-38 through bacterial β-glucuronidase (25). Because the CPT-11 dose in the preclinical study was 3-fold higher than that of NK012 at the SN-38 equivalent dose, a higher amount of CPT-11 was found in the intestinal lumen. It is speculated that the highly excreted CPT-11 was reabsorbed in the small intestinal epithelium and converted to SN-38, causing damage to the intestinal mucosa (14). It is too early to conclude that NK012 may cause weaker diarrhea than CPT-11, but the present results within a phase I setting may encourage further clinical evaluation about intestinal toxicity of NK012.

The PK analysis of NK012 suggested that the tissue distribution of SN-38–incorporating micelles is limited. This is consistent with the data obtained in our preclinical study (13). In a phase I study of CPT-11 at doses of 100 to 750 mg/m^2, it was reported that the CL_{tot} and V_{ss} of CPT-11 were 15,000 mL/h/m^2 and 157,000 mL/m^2, respectively (26). Therefore, the present study revealed that the CL_{tot} and V_{ss} of polymer-bound SN-38 were, respectively, approximately 150- and 80-fold lower than those of CPT-11. This suggests that NK012 may have a low distribution in normal tissue after administration. On the other hand, in tumor tissue, we speculate that NK012 accumulates to a greater extent and stays longer in tumor tissue due to the enhanced permeability and retention effect

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(27) because it is stable in circulation and exhibits a markedly higher plasma AUC than CPT-11. Moreover, NK012 seems to induce sustained release of SN-38 inside the tumor following the accumulation of NK012 in the tumor tissue (13–18).

Sustained exposure to SN-38 is required for successful CPT-11–based chemotherapy because SN-38 induced single-strand DNA breaks in the presence of topoisomerase I and is only effective during the relatively short S phase of the cell cycle (28). When compared with SN-38 converted from conventional CPT-11 at a dose of 250 mg/m^2 (29), SN-38 released from NK012 at 28 mg/m^2 exhibited 2.4-fold greater systemic exposure (0.876 versus 2.12 μg·h/mL) and 15-fold slower elimination from plasma (13.9 versus 209 hours). This result was compatible

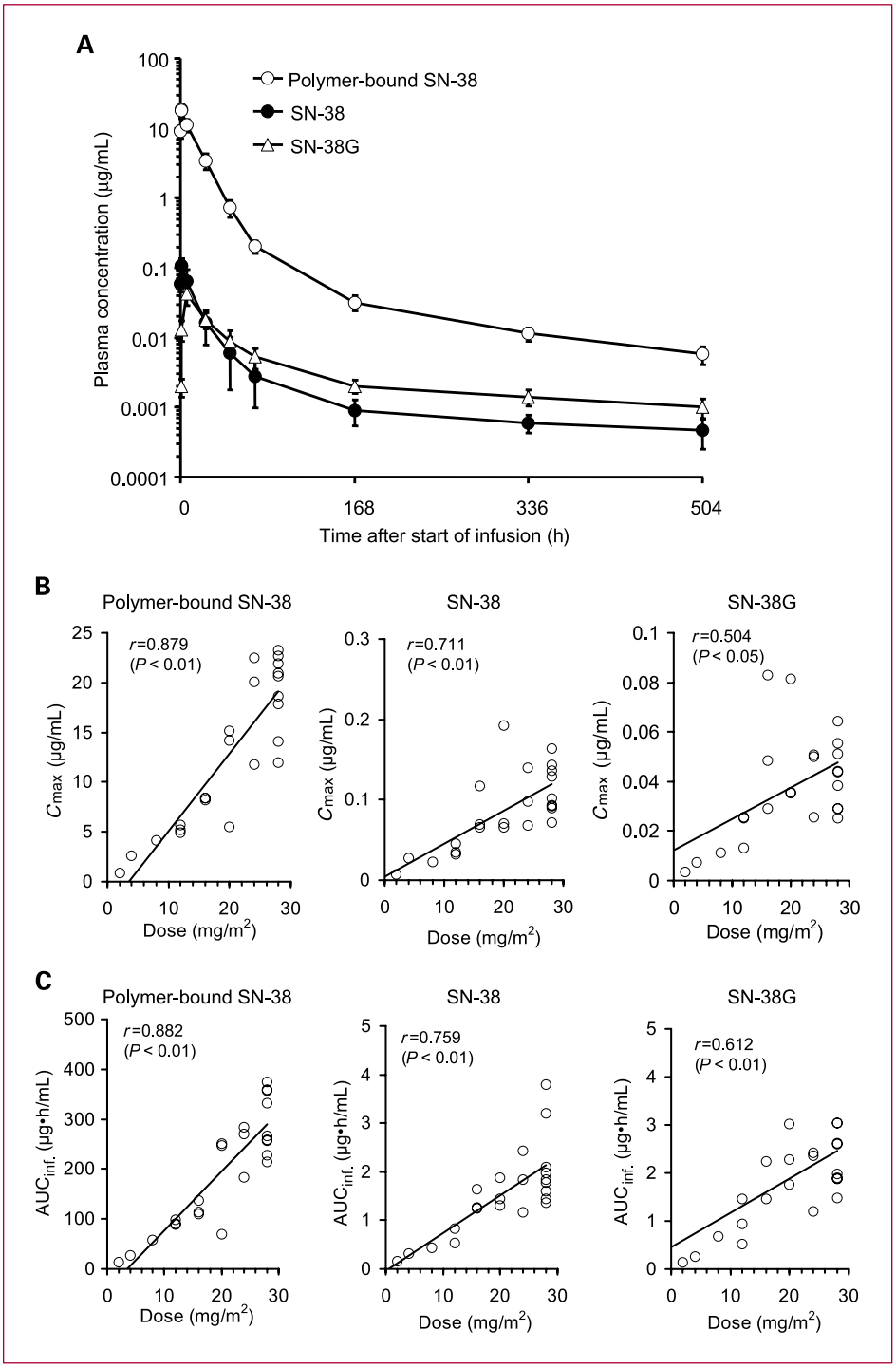

Fig. 2. A, plasma concentration-time profile of polymer-bound SN-38, SN-38, and SN-38G in nine patients after an infusion of NK012 at 28 mg/m^2 in cycle 1. Points, mean; bars, SD. Relationship between dose and C_{max} (B) and between dose and $AUC_{inf.}$ (C) of respective analytes in 23 or 24 patients after an infusion of NK012 in cycle 1.

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Table 4. Plasma PK parameters of (A) polymer-bound SN-38, (B) SN-38, and (C) SN-38G in cycle 1

Dose (mg/m^2)	No. patients	Mean (SD)						
		C_{max} (μg/mL)	T_{max} (h)	$t_{1/2z}$ (h)	$AUC_{inf.}$ (μg·h/mL)	$MRT_{inf.}$ (h)	CL_{tot} (mL/h/m^2)	V_{ss} (mL/m^2)
A. Polymer-bound SN-38								
2	1	0.91	1	128	14.1	28.6	142	4,050
4	1	2.60	0.6	36.0	27.0	15.9	148	2,370
8	1	4.16	0.5	102	57.8	19.1	138	2,640
12	3	5.32 (0.38)	0.7 (0.3)	135 (8)	92.4 (5.5)	25.3 (4.0)	130 (7)	3,270 (330)
16	3	8.32 (0.11)	0.8 (0.3)	168 (41)	120 (15)	28.2 (4.0)	134 (15)	3,830 (940)
20	3	11.6 (5.3)	0.8 (0.3)	128 (28)	189 (104)	24.3 (2.0)	150 (121)	3,730 (3,190)
24	3	18.1 (5.6)	0.7 (0.3)	128 (13)	246 (55)	24.5 (0.9)	101 (26)	2,480 (600)
28	9	19.1 (3.9)	0.7 (0.3)	137 (19)	294 (62)	22.2 (2.3)	98.8 (20.6)	2,020 (530)
B. SN-38								
2	1	0.01	1	70.7	0.16	47.3	N/A	N/A
4	1	0.03	0.6	179	0.32	99.9	N/A	N/A
8	1	0.02	1	107	0.430	54.5	N/A	N/A
12	3	0.0377 (0.0073)	0.8 (0.3)	193*	0.683*	70.6*	N/A	N/A
16	3	0.0841 (0.0285)	0.8 (0.3)	250 (60)	1.38 (0.23)	91.4 (23.7)	N/A	N/A
20	3	0.110 (0.072)	0.7 (0.3)	266 (40)	1.55 (0.30)	104 (19)	N/A	N/A
24	3	0.102 (0.036)	0.7 (0.3)	216 (24)	1.81 (0.64)	109 (20)	N/A	N/A
28	9	0.114 (0.031)	0.8 (0.3)	209 (25)	2.12 (0.83)	95.7 (16.7)	N/A	N/A
C. SN-38G								
2	1	0	6	42.7	0.15	50.9	N/A	N/A
4	1	0.01	6	30.8	0.27	38.3	N/A	N/A
8	1	0.01	6	72.9	0.68	75.1	N/A	N/A
12	3	0.02 (0.0071)	6 (0)	171 (102)	0.97 (0.471)	148 (92)	N/A	N/A
16	3	0.05 (0.0272)	6 (0)	294 (61)	2.62 (1.39)	221 (80)	N/A	N/A
20	3	0.05 (0.0266)	6 (0)	264 (70)	2.36 (0.63)	212 (49)	N/A	N/A
24	3	0.0420 (0.0143)	6 (0)	222 (18)	2.00 (0.68)	193 (11)	N/A	N/A
28	9	0.04 (0.0132)	6 (0)	205 (20)	2.28 (0.57)	186 (27)	N/A	N/A

NOTE: Values are represented as the mean (SD).

Abbreviation: N/A, not available.

*n = 2. One patient at 12 mg/m^2 was excluded from the analysis due to the presence of an interference peak on the HPLC chromatogram.

with those obtained in preclinical studies (13, 14, 17). Therefore, the longer systemic exposure time of SN-38 achieved with NK012 therapy is also expected to improve therapeutic efficacy.

Overall, our data suggest that polymer-bound SN-38 and released SN-38 exhibit a linear PK in the dose range of 2 to 28 mg/m^2. In several phase I studies of CPT-11, it was reported that the C_{max} and/or the AUC of CPT-11 increased linearly with the dosage, but the AUC of SN-38 was not as dose dependent as that of CPT-11 or it has no correlation with the dose due to considerable interpatient variability (29–32). Several metabolizing enzymes (e.g., CEs, UGT1A, and CYP3A4) are involved in the disposition of CPT-11 (8, 9, 33). NK012, unlike CPT-11, is hydrolyzed nonenzymatically to release SN-38, resulting in a dose-proportional increase in systemic exposure. Thus, NK012 proved to function steadily as a drug carrier in a dose-independent manner and to release SN-38 in a dose-dependent manner in this phase I trial. In the independent phase I trial conducted in the United States, the PK profile and toxic profile including diarrhea were similar to those of our study. The DLT of the U.S. study was neutropenia and pneumonia with neutropenia. The MTD in the U.S. trial was also determined to be 28 mg/m^2 (22).

In conclusion, the recommended phase II dose of NK012 is 28 mg/m^2 with at least a 3-week interval between treatment cycles. The favorable safety profile and promising clinical antitumor activity warrant further clinical evaluation.

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Disclosure of Potential Conflicts of Interest

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References

1. Bodurka DC, Levenback C, Wolf JK, et al. Phase II trial of irinotecan in patients with metastatic epithelial ovarian cancer or peritoneal cancer. J Clin Oncol 2003;21:291–7.
2. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355:1041–7.
3. Negoro S, Masuda N, Takada Y, et al. Randomised phase III trial of irinotecan combined with cisplatin for advanced non-small-cell lung cancer. Br J Cancer 2003;88:335–41.
4. Noda K, Nishiwaki Y, Kawahara M, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 2002;346:85–91.
5. Saltz LB, Cox JV, Blanke C, et al, Irinotecan Study Group. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 2000;343:905–14.
6. Takimoto CH, Arbuck S. Topoisomerase I targeting agents: the camptothecines. In: Chabner BA, Lango DL, editors. Cancer Chemotherapy and Biotherapy: Principles and Practice. 3rd edition, Philadelphia: Lippincott Willams & Wilkins; 2001. p. 579–646.
7. Rothenberg ML, Kuhn JG, Burris HA III, et al. Phase I and pharmacokinetic trial of weekly CPT-11. J Clin Oncol 1993;11:2194–204.
8. Slatter JG, Schaaf LJ, Sams JP, et al. Pharmacokinetics, metabolism, and excretion of irinotecan (CPT-11) following I.V. infusion of [(14)C] CPT-11 in cancer patients. Drug Metab Dispos 2000;28:423–33.
9. Guichard S, Terret C, Hennebelle I, et al. CPT-11 converting carboxylesterase and topoisomerase activities in tumour and normal colon and liver tissues. Br J Cancer 1999;80:364–70.
10. Matsumura Y, Kataoka K. Preclinical and clinical studies of anticancer agent-incorporating polymer micelles. Cancer Sci 2009;100:572–9.
11. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. J Clin Oncol 2001;19:3312–22.
12. Gradishar WJ, Tjulandin S, Davidson N, et al. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol 2005;23:7794–803.
13. Koizumi F, Kitagawa M, Negishi T, et al. Novel SN-38-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors. Cancer Res 2006;66:10048–56.
14. Nagano T, Yasunaga M, Goto K, et al. Antitumor activity of NK012 combined with cisplatin against small cell lung cancer and intestinal mucosal changes in tumor-bearing mouse after treatment. Clin Cancer Res 2009;15:4348–55.
15. Saito Y, Yasunaga M, Kuroda J, Koga Y, Matsumura Y. Enhanced distribution of NK012, a polymeric micelle-encapsulated SN-38, and sustained release of SN-38 within tumors can beat a hypovascular tumor. Cancer Sci 2008;99:1258–64.
16. Sumitomo M, Koizumi F, Asano T, et al. Novel SN-38-incorporated polymeric micelle, NK012, strongly suppresses renal cancer progression. Cancer Res 2008;68:1631–5.
17. Kuroda J, Kuratsu J, Yasunaga M, Koga Y, Saito Y, Matsumura Y. Potent antitumor effect of SN-38-incorporating polymeric micelle, NK012, against malignant glioma. Int J Cancer 2009;124:2505–11.
18. Nakajima TE, Yanagihara K, Takigahira M, et al. Antitumor effect of SN-38-releasing polymeric micelles, NK012, on spontaneous peritoneal metastases from orthotopic gastric cancer in mice compared with irinotecan. Cancer Res 2008;68:9318–22.
19. Nakajima TE, Yasunaga M, Kano Y, et al. Synergistic antitumor activity of the novel SN-38-incorporating polymeric micelles, NK012, combined with 5-fluorouracil in a mouse model of colorectal cancer, as compared with that of irinotecan plus 5-fluorouracil. Int J Cancer 2008;122:2148–53.
20. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst 1997;89:1138–47.
21. Matsumura Y, Hamaguchi T, Ura T, et al. Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. Br J Cancer 2004;91:1775–81.
22. HA Burris I, Infante JR, Spigel DR, et al. A phase I dose-escalation study of NK012 [abstract 2538]. J Clin Oncol 2008;26.
23. Atsumi R, Suzuki W, Hakusui H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. Xenobiotica 1991;21:1159–69.
24. Chu XY, Kato Y, Sugiyama Y. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. Cancer Res 1997;57:1934–8.
25. Takasuna K, Hagiwara T, Hirohashi M, et al. Involvement of β-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. Cancer Res 1996;56:3752–7.
26. Abigerges D, Chabot G, Armand J, Herait P, Gouyette A, Gandia D. Phase I and pharmacologic studies of the camptothecin analog irinotecan administered every 3 weeks in cancer patients. J Clin Oncol 1995;13:210–21.
27. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46:6387–92.
28. Gerrits CJ, de Jonge MJ, Schellens JH, Stoter G, Verweij J. Topoisomerase I inhibitors: the relevance of prolonged exposure for present clinical development. Br J Cancer 1997;76:952–62.
29. Taguchi T, Wakui A, Hasegawa K, et al. Phase I clinical study of CPT-11. Research group of CPT-11. Gan To Kagaku Ryoho 1990;17:115–20.
30. de Forni M, Bugat R, Chabot GG, et al. Phase I and pharmacokinetic study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. Cancer Res 1994;54:4347–54.
31. Pitot HC, Goldberg RM, Reid JM, et al. Phase I dose-finding and pharmacokinetic trial of irinotecan hydrochloride (CPT-11) using a once-every-three-week dosing schedule for patients with advanced solid tumor malignancy. Clin Cancer Res 2000;6:2236–44.
32. Rothenberg ML, Kuhn JG, Schaaf LJ, et al. Phase I dose-finding and pharmacokinetic trial of irinotecan (CPT-11) administered every two weeks. Ann Oncol 2001;12:1631–41.
33. Haaz MC, Rivory L, Riche C, Vernillet L, Robert J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. Cancer Res 1998;58:468–72.

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