concentration was detected in the small intestinal epithelium. On the other hand, a small amount of NK012 was found in the feces and NK012 was weakly and uniformly distributed in the mucosal interstitium. A portion of SN-38 converted from CPT-11 undergoes subsequent conjugation as induced by UDP-glucuronyltransferase to form SN-38 β-glucuronide (SN-38-Glu) [27]. CPT-11, SN-38, and SN-38-Glu are excreted into the bile and then reach the small intestinal lumen [27,28]. SN-38-Glu is deconjugated in the cecum and colon to regenerate SN-38 through bacterial β-glucuronidase [29]. In our study, CPT-11 was excreted into feces much more than NK012 and a high CPT-11 concentration was detected in the small intestinal epithelium. It is speculated that the highly excreted CPT-11 is reabsorbed in the small intestinal epithelium and converted to SN-38 to damage the intestinal mucosa. On the other hand, NK012 was uniformly distributed in the mucosal interstitium at a lower concentration and this is suggested to be related to the less mucosal damage and diarrhea than those induced by CPT-11, although NK012 was observed for longer period than CPT-11 [30]. Very recently we also found that NK012/S-1, a dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, showed a significantly higher antitumor activity with less intestinal damage than CPT-11/S-1, one of the promising regimens against non SCLC, advanced colorectal cancer, and metastatic advanced gastric cancer [31].

3.3. Renal cell cancer (RCC)

The results of chemotherapy in RCCs have been disappointing, as indicated by the low response rate. However, clinical trials using an antimetabolite, gemcitabine-containing regimens have been encouraging, with major responses occurring in 5–17% of patients [32,33], suggesting the possibility that chemotherapy is promising as a modality for RCC therapy if anticancer agents can be selectively delivered, released, and maintained around tumor tissues. Compared with CPT-11, NK012 had significant antitumor activity against both bulky Renca (mouse renal cell tumor) and SKRC-49 tumors. Notably, NK012 eradicated rapid-growing Renca tumors in 6 of 10 mice [34]. In the pulmonary metastasis treatment model, an enhanced and prolonged distribution of free SN-38 was observed in metastatic lung tissues but not in non-metastatic lung tissues after NK012 administration. NK012 treatment resulted in a significant decrease in metastatic nodule number and was of survival benefit. Our study showed the outstanding advantage of polymeric micelle-based drug carriers and suggests that NK012 would be effective in treating disseminated RCCs with irregular vascular architectures.

3.4. Glioma

Irinotecan hydrochloride (CPT-11), a prodrug of SN-38, shows some antitumor activities in patients with recurrent gliobastoma multiforme (GBM), with response rates of 0 to 17% in several trials [35–38]. CPT-11 activity is thus similar to that of other agents used for recurrent GBM [37]. A recent phase II trial for recurrent GBM demonstrated that the combination of CPT-11 and bevacizumab, an anti-vascular endothelial growth factor (VEGF) monoclonal antibody, is an effective treatment against the neoplasia with a 6-month progression-free survival rate of 46% and a 6-month overall survival rate of 77% [39,40]. However, there is an increased risk of developing venous thromboembolic disease and intracranial hemorrhage with this combination therapy [40]. Therefore, there is an urgent need to develop treatment modalities by which cytotoxic drugs can exert more potent antitumor activity to their full potential with modest adverse effects and thereby reasonably prolong the overall survival in GBM patients. Our study showed that the therapeutic effect of NK012 was superior to that of CPT-11 in terms of antitumor effect and survival. Since the antitumor activity of SN-38 is time dependent, the superiority of NK012 over CPT-11 may be due to the enhanced accumulation of NK012 and the prolonged sustained

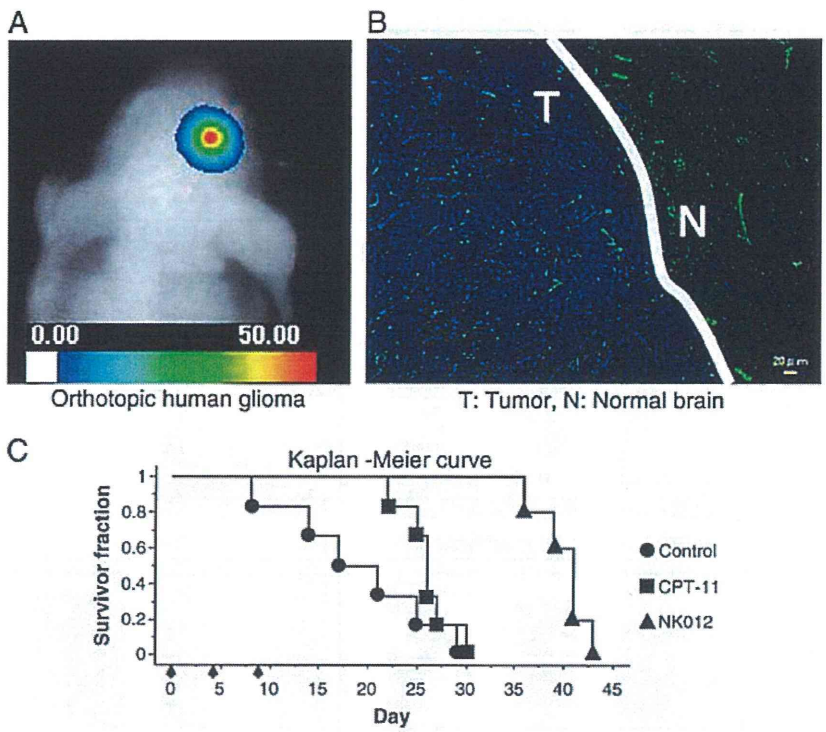

Fig. 4. Antitumor effect of NK012 or CPT-11 on orthotopic xenograft. (A) and survival. 0.9% NaCl solution (●), NK012 (30 mg/kg/day, ▲), or CPT-11 (66.7 mg/kg/day, ■) on days 0 (20 days after tumor inoculation), 4, and 8 (arrows). (B) Distribution of NK012 or CPT-11 in U87MG/Luc glioma xenografts. Tumor tissues were excised 24 h after the intravenous injection of NK012 or CPT-11. Each mouse was administered fluorescein-labeled *Lycopersicon esculentum* lectin 5 min before sacrifice to detect tumor blood vessels. Frozen sections were examined under a fluorescence microscope at an excitation wavelength of 377 nm and an emission wavelength of 477 nm. The same fluorescence conditions can be applied for visualizing NK012 and CPT-11 fluorescence. Free SN-38 could not be detected under these fluorescence conditions. The white lines indicate the border between the tumor and the brain tissue. T, U87MG/Luc tumor; N, normal brain tissue. (Scale bars: 20 μm.). (C) Treatment effects of NK012 on survival. Survival was assessed by Kaplan–Meier analysis. Each group consisted of 6 mice.

release of SN-38 from NK012 within the tumor tissues. Nevertheless, free SN-38 was not detected in the normal brain tissues at any measurement time after intravenous injection of NK012 or CPT-11. It is thus speculated that both NK012 and CPT-11 are unable to cross the blood brain barrier (BBB) in the normal brain, but can pass through the brain tumor vessels effectively [41] (Fig. 4). In addition, CPT-11 in combination with bevacizumab showed significantly more potent antitumor activity and longer survival than CPT-11 monotherapy. However, there was no difference between NK012 monotherapy and NK012 in combination with bevacizumab. Concentration of free SN-38 released from NK012 in the tumor tissue decreased in combination with bevacizumab. NK012 monotherapy or NK012 with bevacizumab showed potent antitumor activity and longer survival than any dosing method of CPT-11 in combination with bevacizumab [42]. Our data warrant a clinical evaluation of NK012 in patients with GBM.

3.5. Stomach cancer

Patients with gastric cancer with scirrhous type stroma particularly demonstrated poor prognosis even after curative resection, as well as highly progressed peritoneal dissemination [43]. Since peritoneal dissemination causes several refractory symptoms such as massive ascites, intestinal obstruction, hydronephrosis and obstructive jaundice, the quality of life of patients at the end stage of cancer is severely impaired. Poor delivery of anticancer drugs to peritoneal metastatic cells may be one of the reasons for the poor prognosis of patients with peritoneal dissemination [44]. In peritoneal nodules, the distribution and eventual diffusion of drugs to cancer cells tend to be impeded because of several obstacles such as severe fibrosis and high interstitial pressure [45,46]. On the other hand, angiogenesis was reported to be an essential factor in the development of peritoneal metastasis, and the

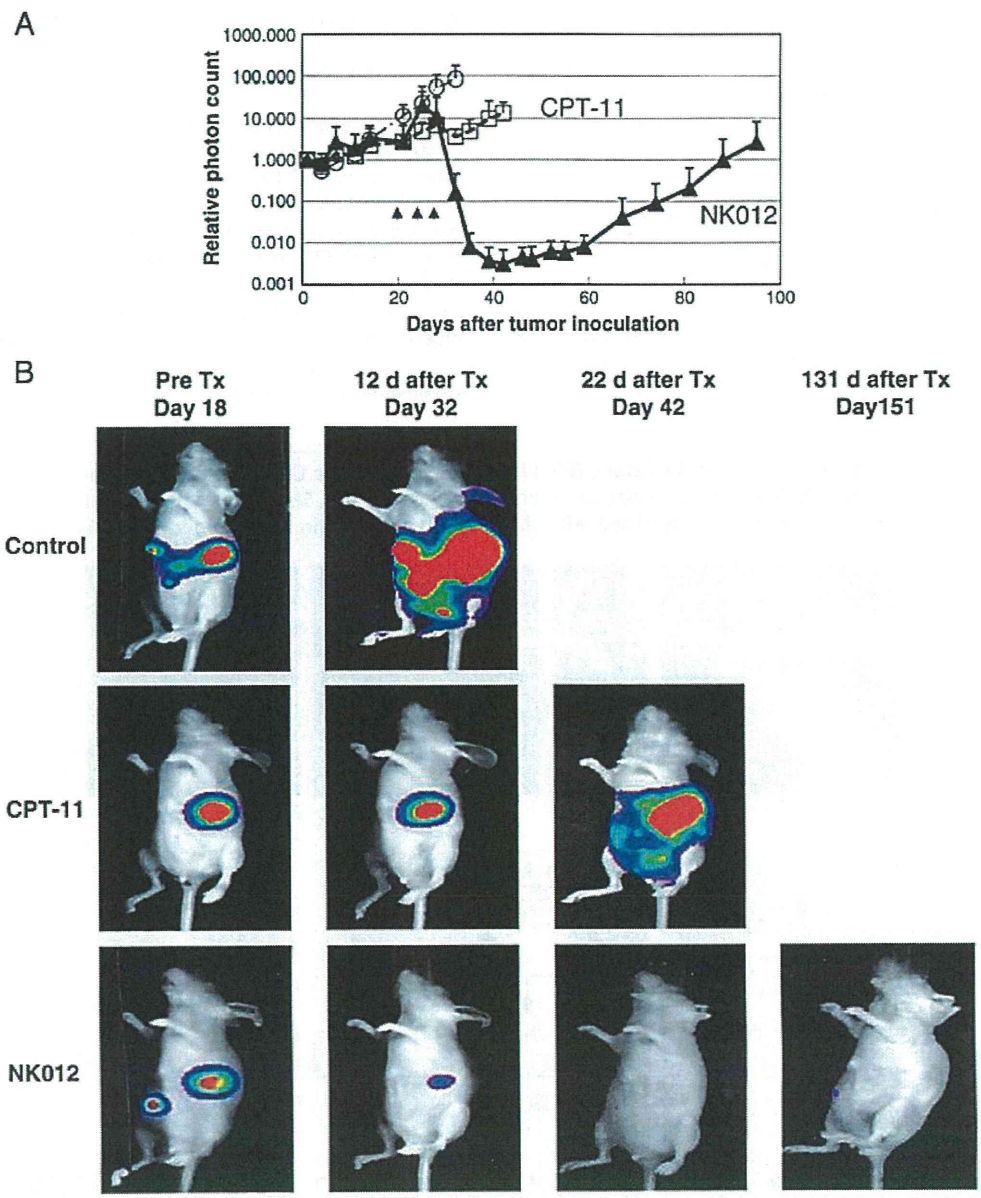

Fig. 5. Effects of NK012 and CPT-11 in 44As3Luc mouse models. Antitumor activity of NK012 or CPT-11 was evaluated by counting the number of photons using the IVIS system (points, mean; bars, SD; arrows, drug injections). A. Antitumor effect of each regimen on days 20, 24 and 28. (○) control, (□) CPT-11 (66.7 mg/kg/day, ×3) and (▲) NK012 (30 mg/kg/day,×3) in 44As3Luc mouse model. B. Images of 44As3Luc mouse model administered NK012 taken using the IVIS system on days 18, 32, 42 and 151 after inoculation of 44As3Luc cells. Data were derived from the same mice as those used in the present study.

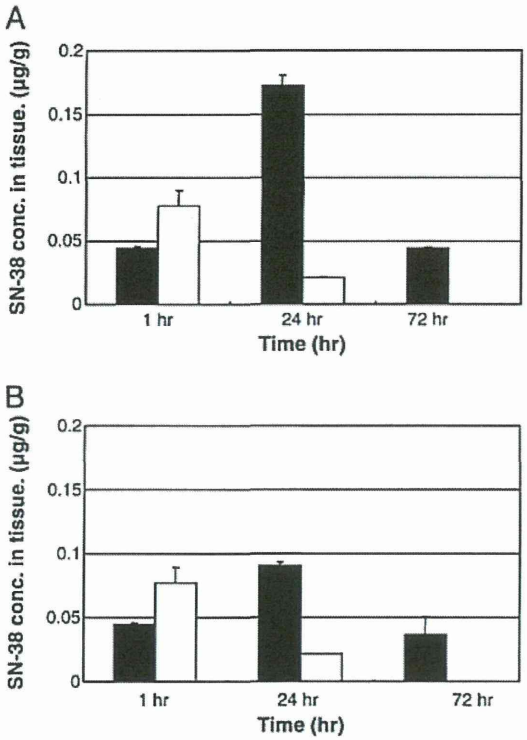

Fig. 6. Concentration-time profile of free SN-38. NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was injected 26 days after implantation of 44As3Luc gastric cancer cells (points, mean; bars, SD). A. Concentration of free SN-38 in orthotopic gastric tumor tissue of 44As3Luc mouse model after administration of NK012 (black column) and CPT-11 (white column). B. Concentration of free SN-38 in peritoneal nodules of 44As3Luc mouse model after administration of NK012 (black column) and CPT-11 (white column).

high expression level of vascular endothelial growth factor (VEGF) in primary gastric tumors or ascitic fluid, which can enhance tumor vascular permeability, was found to be directly associated with the development of ascites and peritoneal dissemination [47–51]. In addition, several factors such as kinins and nitric oxide are known to be involved in tumor vascular permeability [52–54]. We evaluated the antitumor activity of NK012 against peritoneal tumor dissemination as compared with that of CPT-11 using mouse models orthotopically transplanted with scirrhous gastric cancer cells, as well as against spontaneously progressing peritoneal dissemination [55,56]. NK012 or CPT-11 distribution in these tumors was evaluated using a fluorescence microscope on the same schedule. In both models, the antitumor activity of NK012 was superior to that of CPT-11 (Fig. 5A and B). High concentration of SN-38 released from NK012 was detected in gastric tumors and peritoneal nodules up to 72 h by HPLC (Fig. 6A and B). Only a slight conversion from CPT-11 to SN-38 was observed from 1 to 24 h. Fluorescence originated from NK012 was detected up to 72 h, whereas that from CPT-11 disappeared until 24 h. NK012 also showed antitumor activity against peritoneal nodules [57]. Thus, NK012 showing enhanced distribution with prolonged SN-38 release may be ideal for cancer treatment especially in patients with stomach cancer.

3.6. Colorectal cancer (CRC)

In two phase III trials, the addition of CPT-11 to bolus or infusional 5-FU-leucovorin (5FU/LV) regimens clearly yielded greater efficacy than administration of 5FU/LV alone, with a doubling of the tumor response rate and prolongation of the median survival time by 2–3 months [1,2]. We evaluated the antitumor activity of NK012 administered in combination with 5FU as compared with that of CPT-11 administered in combination with 5FU against CRC in an experimental model. And we found that the therapeutic effect of NK012/5FU was significantly superior to that of CPT-11/5FU against HT-29 tumors [58].

4. Phase I clinical trials

Two independent phase I clinical trials were conducted in the National Cancer Center in Japan [59] and the Sarah Canon Cancer Center in the USA [60] in patients with advanced solid tumors to define the maximum tolerated dose (MTD), dose limiting toxicity

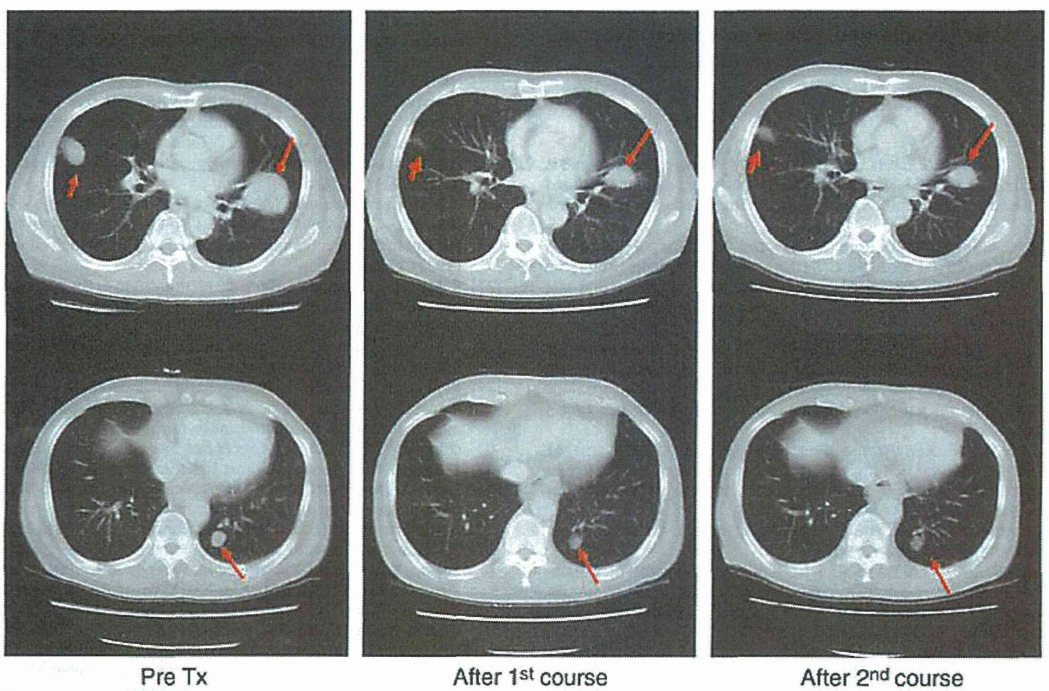

Fig. 7. Computed tomography scan of a patient with conventional-chemotherapy-refractory esophageal cancer with multiple pulmonary metastases showing a reduction in tumor burden after two cycles of NK012 at a dose level of 28 mg/m^2 and continuing study therapy for 5 months until disease progression.

(DLT), and recommended dose for phase II study. NK012 is infused intravenously over 30 min every 21 days until disease progression or unacceptable toxicity occurs. The MTD was 37 mg/m^2 in the US, however 28 mg/m^2 in Japan. The recommended dose (RD) for phase 2 study was the same 28 mg/m^2 in both countries. DLTs were mostly neutropenia or its related events, and diarrhea was mild. The PK profile in the US study was similar to that in the Japanese study. Antitumor activity was also promising. Partial responses (PRs) were obtained in 3 patients with triple negative breast cancer, 1 patient with SCLC, 1 patient with endometrial cancer, and 1 patient with pancreatic neuroendocrine tumor in the USA trial. In Japanese trial, PRs were obtained in 1 patient with esophageal cancer and 1 patient with lung carcinoid (Fig. 7). A phase II study in patients with colorectal cancers is now underway in Japan. In the USA, 2 phase II studies were underway in patients with triple negative breast cancer and patients with SCLC.

5. Conclusion

NK012 is categorized in DDS and the data from several preclinical studies shows that the formulation appears to accumulate selectively and remain for a long time in solid tumor tissues by utilizing the EPR effect.

Our preclinical study showed that the CPT-11-induced mucosal change in mouse intestine was mainly fibrosis considered to be a form of recovery change from erosion. On the other hand, the small intestinal mucosa of the mice in the NK012 treatment group showed only mild shortening and decreased number of villi or mild inflammatory cell invasion. 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 regarding intestinal toxicity of NK012.

Regarding higher response rate within phase 1 setting, in tumor tissue, we speculate that NK012 accumulates to a greater extent and stays longer in tumor tissue since it is stable in circulation and exhibits markedly higher plasma AUC than CPT-11. Moreover, NK012 appears to induce sustained release of SN-38 inside the tumor following the accumulation of NK012 in the tumor tissue. It is speculated that this pharmacokinetic characteristics of NK012 can produce a higher antitumor activity in clinics.

The favorable safety profile and promising clinical antitumor activity of NK012 warrant further clinical evaluation.

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Cancer Therapy: Clinical

Clinical Cancer Research

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

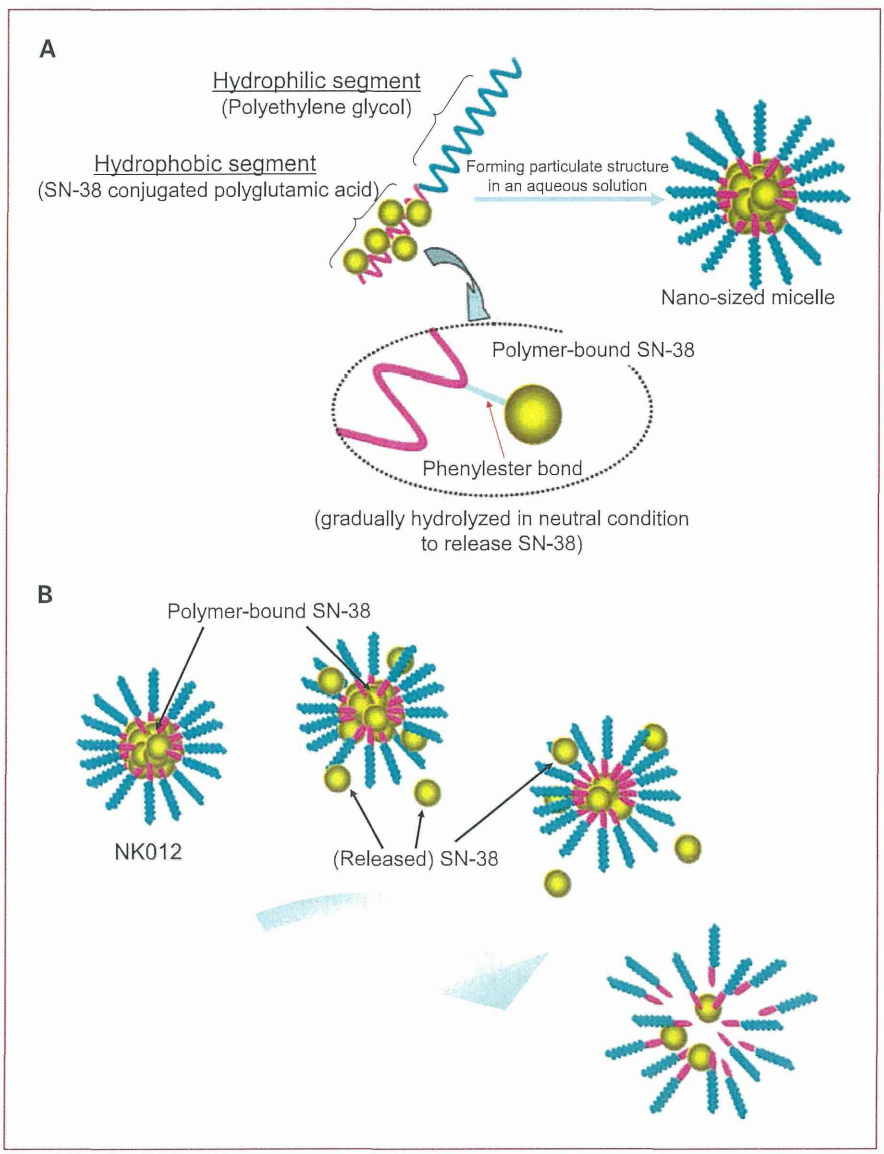

Fig. 1. A, schematic structure of NK012. B, release of SN-38 from NK012.

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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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