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MONOGRAPH

NK012

Apoptosis Inducer
Oncolytic

Nanosized-micellar particle containing 7-ethyl-10-hydroxycamptothecin (EHC), consisting of poly(ethylene glycol)-poly(glutamic acid)block copolymer chemically bound to EHC

EN: 417411

ABSTRACT

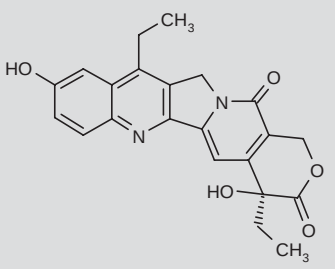
Selective tumor targeting by therapeutic agents is a long-standing pharmacological goal to improve selectivity and therapeutic indices. Most scientists have sought to use active receptor-mediated tumor-targeting systems, although the passive targeting afforded by the enhanced permeability and retention (EPR) effect provides a versatile and nonsaturable opportunity for tumor-selective delivery. Polymeric micelles are ideally suited to exploit the EPR effect, and they have been used for the delivery of a range of anticancer drugs in preclinical and clinical studies. NK012 is an SN-38-loaded polymeric micelle constructed in an aqueous milieu by the self-assembly of an amphiphilic block copolymer, PEG-PGlu(SN-38). Recently, we have demonstrated that NK012 exerts significantly more potent antitumor activity against various human tumor xenografts than irinotecan (CPT-11). Preclinical and clinical studies of NK012 up to the present are reviewed here.

BACKGROUND

Nanotechnology is one of the fastest moving technologies and is presently contributing significantly to the progress of medical science. Drugs categorized under the drug delivery system (DDS) are prepared primarily by utilizing nanotechnology. In the field of oncology, DDS drugs have been prepared and evaluated in preclinical and/or clinical trials, with some already approved for clinical use (Table I). More specifically, DDS can be used for active or passive targeting of tumor tissues. Active targeting refers to the development of monoclonal antibodies directed against tumor-related molecules, allowing targeting of a tumor from the specific binding of antibodies with respective antigens. However, the application of DDS using monoclonal antibodies is restricted to tumors expressing high levels of related antigens. Passive targeting can be achieved by utilizing the enhanced permeability and retention (EPR) effect (1, 2). This effect is based on the pathophysiological characteristics of solid tumor tissues, namely, hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors stimulating extravasation within cancer tissue, and absence of effective lymphatic drainage from tumors that impedes the efficient clearance of macromolecules accumulated in solid tumor tissues (Fig. 1A, B).

Several techniques have been developed to maximally utilize the EPR effect, including modification of drug structures and the development of drug carriers. Polymeric micelle-based anticancer drugs were originally developed by Kataoka et al. in the late 1980s and early 1990s (3-5). Polymeric micelles were expected to increase the accumulation of drugs in tumor tissues by utilizing the EPR effect, as well as to incorporate various kinds of drugs into their inner core with relatively high stability by chemical conjugation or physical entrapment. Also, the size of micelles can be controlled within the diameter range of 20-100 nm to ensure that they do not penetrate normal vessel walls. With this development, it is expected that the incidence of drug-induced side effects may be decreased owing to reduced drug distribution in normal tissues.

Irinotecan hydrochloride (CPT-11) has been demonstrated to be active against colorectal, lung and ovarian cancers (6-10). CPT-11 is a prodrug that is converted to 7-ethyl-10-hydroxycamptothecin (SN-38, **1**), a biologically active metabolite of CPT-11, by carboxylesterases. SN-38 is an analogue of the plant alkaloid camptothecin, which targets DNA topoisomerase I. SN-38 exhibits up to 1,000-fold more potent cytotoxic activity against various cancer cells in vitro than CPT-11 (11). Although CPT-11 is converted to SN-38 in the liver and tumors, the metabolic conversion rate is less than 10% of the original volume of CPT-11 (12, 13). Moreover, the conversion of CPT-11 to SN-38 depends on the genetic interindividual variability of car-



SN-38 (**1**)

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Table I. *Examples of DDS in oncology and their stage of development.*

Name	Platform	Compound	Clinical stage
Passive targeting			
NK-105	Micelles	Paclitaxel	II
NC-6004	Micelles	Cisplatln	I/II
NK012	Micelles	SN-38	II
Zinostatin stimalamer (SMANCS)	Polymer conjugate	Neocarzinostatin	Launched
Doxil	Liposome	Doxorubicin	Launched
Abraxane	Albumin-coated nanoparticle	Paclitaxel	Launched
Opaxio	Polymer conjugate	Paclitaxel	Prereg.
CT-2106	Polymer conjugate	Camptothecin	II
EndoTAG	Cationic liposome	Paclitaxel	II
Mureletecan	Polymer conjugate	Camptothecin	I
LE-SN-38	Liposome	SN-38	II
PK1	Polymer conjugate	Doxorubicin	II
IT-101	Polymer conjugate	Camptothecin	II
SP-1049C	Micelles	Doxorubicin	II
CPX-1	Liposome	CPT-11, floxuridine	II
Active targeting			
Mylotarg	Anti-CD33 antibody	Calicheamicin	Launched
Zevalin	Anti-CD20 antibody	90Y	Launched
Bexxar	Anti-CD20 antibody	131I	Launched
PK2	Galactose–polymer	Doxorubicin	I
MCC-465	Antibody liposome	Doxorubicin	I
MBP-426	Transferrin–liposome	Oxaliplatin	I
CALAA-01	Transferrin–polymer	siRNA	I
Trastuzumab-DM1	Anti-HER2 antibody	DM1	III

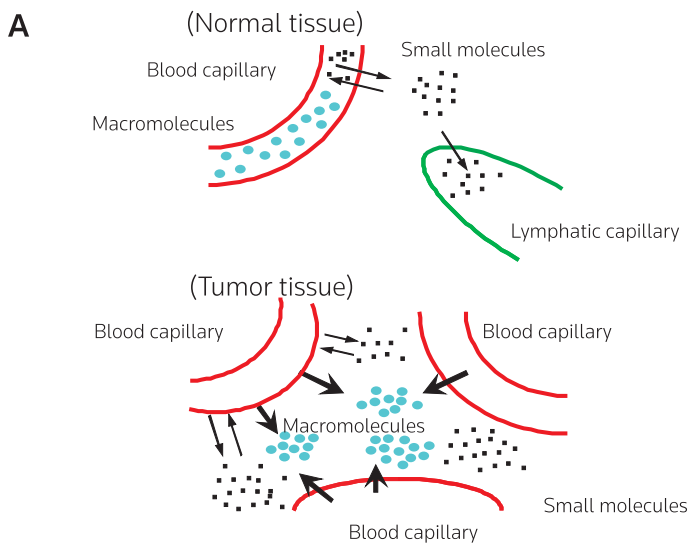
boxylesterase activity (14). Thus, further efficient use of SN-38 might be of great advantage and may be attractive for cancer treatment. The progress in the manufacturing technology of "micellar nanoparticles" may make it possible to use SN-38 for in vivo experiments and further clinical use.

NK012 is an SN-38-incorporating polymeric micelle that can accumulate selectively in solid tumor tissues utilizing the EPR effect. The micelle is constructed in an aqueous milieu by the self-assembly of an amphiphilic block copolymer, PEG-PGlu(SN-38) (15). NK012 was obtained as a freeze-dried formulation and contained about 20% (w/w) of SN-38. The mean particle size of NK012 is 20 nm in diameter, with a relatively narrow range (Fig. 2A). The release rates of SN-38 from NK012 in phosphate-buffered saline (PBS) at 37 °C were 57% and 74%, respectively, at 24 and 48 h, and in 5% glucose solution at the same temperature and times 1% and 3%, respectively (Fig. 2B). These results indicate that NK012 can release SN-38 under neutral conditions even without a hydrolytic enzyme, and is stable in 5% glucose solution. Thus, NK012 is suggested to be stable before administration and starts to release SN-38 gradually under physiological conditions following administration.

PRECLINICAL PHARMACOLOGY

Following CPT-11 injection, the plasma concentrations of CPT-11 and SN-38 rapidly decrease with time in a log-linear fashion. On the other hand, NK012 (polymer-bound SN-38) exhibited slower clearance. In tumor xenografts, NK012 clearance was significantly slower and the free SN-38 concentration was maintained for a long time following injection (15, 16). Interestingly, there was no significant difference in the kinetic characteristics of free SN-38 in the small intestine between mice treated with NK012 and CPT-11.

Deviating from the usual experimental tumor models, tumors were allowed to grow until they became very large (around 1.5 cm) and treatment was then initiated. NK012 showed potent antitumor activity against bulky small cell lung cancer SBC-3/Neo tumors compared with CPT-11. Striking antitumor activity was observed in mice treated with NK012 when its antitumor activity was compared with CPT-11 using SBC-3/VEGF cells. In the clinical setting, CPT-11/5-fluorouracil (5-FU) combination therapy is now a standard regimen for colorectal cancer (6, 7). It was therefore speculated that the use of NK012 in place of CPT-11 in combination with 5-FU might yield superior results. As expected, the therapeutic effect of NK012/5-FU was significantly superior to that of CPT-11/5-FU against human colon adenocarcinoma HT-29 xenografts (P = 0.0004) (17). In other tumors, such as renal cancer (18), glioma (19), gastric cancer (20) and pancreatic cancer (16), NK012 exerted significantly superior antitumor activity and was associated with longer survival compared with CPT-11. In an orthotopic glioma xenograft model, both NK012 and CPT-11 appeared to be able to effectively extravasate from the blood–brain tumor barrier but not from normal brain vessels (Fig. 2C) (19). In human pancreatic adenocarcinoma Capan-1 tumor xenografts, a hypovascular tumor model, it was also demonstrated that NK012 showed significantly more potent antitumor activity than CPT-11. Pharmacological examination revealed that only a slight conversion of SN-38 from CPT-11 was observed from 1 h to 24 h, and no SN-38 was detected thereafter. On the other hand, SN-38 released from NK012 continued to be detected from 1 h to 96 h fol-



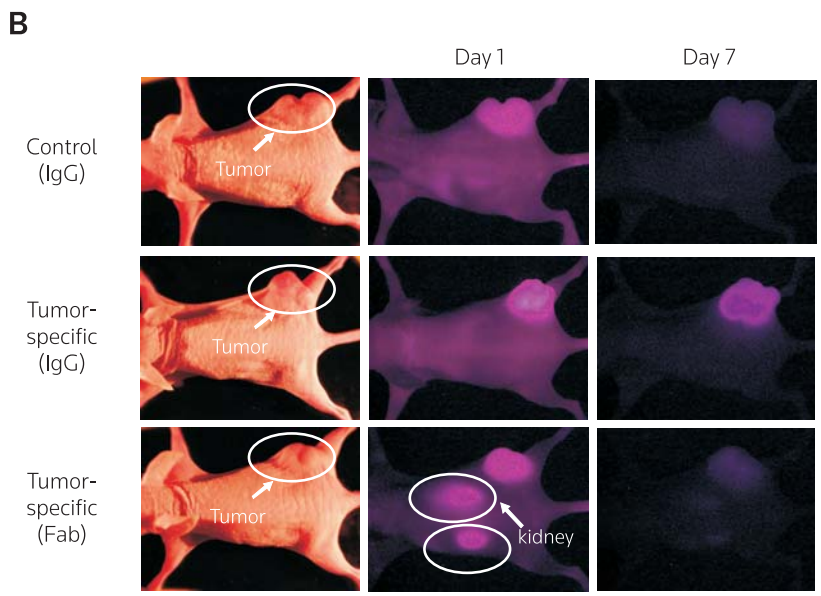


Figure 1. A. A diagram of normal and tumor tissue demonstrating the presence of a lymphatic duct in normal tissue (upper) but the absence of any lymphatic duct in tumor tissue (lower). Small molecules easily leak from normal vessels in the body, which endows them with a short plasma half-life. On the other hand, macromolecules have a long plasma half-life because they are too large to pass through the normal vessel walls, unless they are trapped by the reticuloendothelial system in various organs. In the solid tumor tissues shown in the lower panel, it was found that solid tumors generally possess several pathophysiological characteristics: hypervasculature, secretion of vascular permeability factors stimulating extravasation of macromolecules within the cancer, and absence of effective lymphatic drainage from tumors that impedes the efficient clearance of macromolecules accumulated in solid tumor tissues. These characteristics of solid tumors are the basis of the enhanced permeability and retention effect, or the EPR effect. **B.** In vivo imaging demonstrated that both control whole IgG and specific whole monoclonal antibody (mAb) accumulated selectively in the tumor tissue on day 1 after i.v. injection. On day 7, a greater degree of retention of the specific whole mAb as compared to the control IgG was noted. On the other hand, the F(ab) region of the specific mAb with a molecular weight of 50,000 accumulated in the tumor to the same extent as the control whole IgG. Interestingly, fluorescence of the F(ab) could also be detected in both kidneys, which implied that the F(ab) could easily pass through the kidney glomerulus. This accumulation of the control IgG in the tumor represents the EPR effect. The findings suggest that not only the specific affinity of the mAb, but also the size of the molecules and the stability of the molecules in blood, are important for tumor-selective targeting.

lowing NK012 injection (16) (Fig. 2D). Thus, NK012, which combines enhanced distribution with sustained release of SN-38 within tumors, is ideal for the treatment of hypovascular tumors since the antitumor activity of SN-38 is time-dependent.

CLINICAL STUDIES

Two independent phase I clinical trials have been conducted at the National Cancer Center in Japan (21) and the Sarah Cannon Cancer Center in the U.S. (22) in patients with advanced solid tumors to

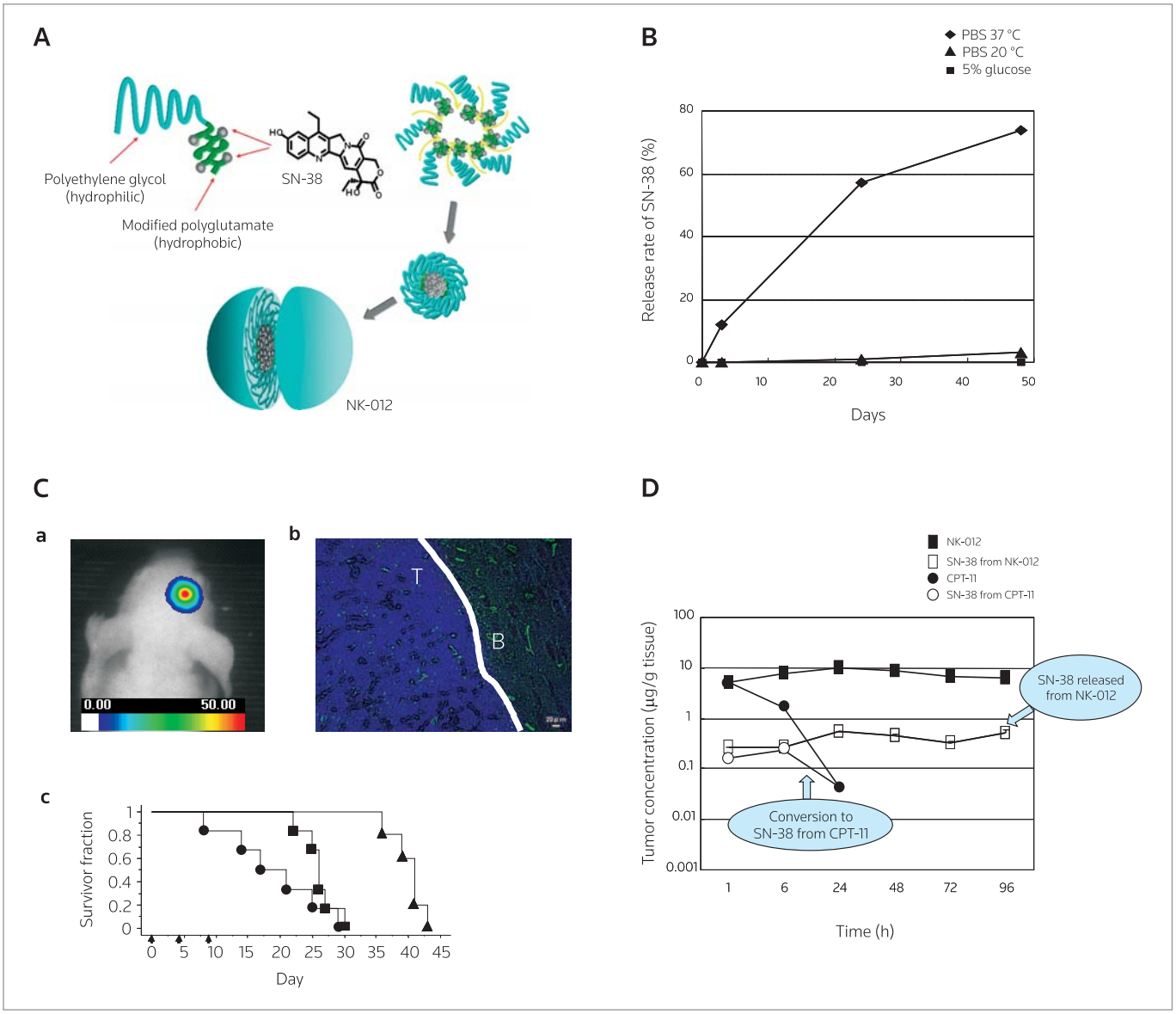


Figure 2. A. Schematic structure of NK012. A polymeric micelle carrier of NK012 consists of a block copolymer of polyethylene glycol (PEG; molecular weight of about 12,000) and partially modified polyglutamate (about 20 units). PEG (hydrophilic) is believed to be the outer shell and SN-38 is incorporated into the inner core of the micelle. **B.** The release rates of SN-38 from NK012 in phosphate-buffered saline (PBS) were 74% at 48 h but only 3% at 48 h in 5% glucose solution. It is therefore suggested that NK012 is stable in 5% glucose solution before administration and starts to release SN-38 gradually under physiological conditions after administration. **C. a**) An orthotopic glioma model. Twenty days after U-87 MG/Luc inoculation, the maximum tolerated dose (MTD) of NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was injected i.v. into the tail vein of mice. **b**) 24 h after NK012 (blue) injection, mice were also administered fluorescein *Lycopersicon esculentum* lectin (100 μL/mouse) to visualize tumor blood vessels. T, tumor, B, normal brain. **c**) NK012 (30 mg/kg/day), CPT-11 (66.7 mg/kg/day) (■) and saline (●) were given i.v. on days 0 (20 days after tumor inoculation), 4 and 8(▼). Kaplan–Meier analysis was performed to determine the effect of drugs on time to morbidity, and statistical differences were ranked according to the Mantel–Cox log-rank test using StatView 5.0. **D.** Tumor distribution of CPT-11, NK012 (or polymer-bound SN-38) and free SN-38 after administration of NK012 and CPT-11 to mice bearing human pancreatic adenocarcinoma Capan-1 (**a**) or PSN-1 (**b**) xenografts. The time profiles of polymer-bound SN-38 (■), free SN-38 released from NK012 (□), CPT-11 (●) and free SN-38 converted from CPT-11 (○) were obtained by high-performance liquid chromatographic (HPLC) analysis. The time points examined were 1, 6, 24, 48, 72 and 96 h after administration of CPT-11 or NK012.

define the maximum tolerated dose (MTD), dose-limiting toxicity (DLT) and recommended phase II dose. NK012 was infused i.v. over 30 min every 21 days until disease progression or unacceptable toxicity. The MTD was 37 mg/m^2 in the U.S. and 28 mg/m^2 in Japan. The recommended dose was the same (28 mg/m^2) in both countries. DLT was mostly neutropenia or related events, and diarrhea was mild. The pharmacokinetic profile in the U.S. study was similar to that in the Japanese study. Antitumor activity was also promising. Partial responses were obtained in three patients with triple-negative breast cancer, one patient with esophageal cancer, one patient with small cell lung cancer and one patient with lung carcinoid. Phase II studies in patients with triple-negative breast and colorectal cancers are being planned in the U.S. and Japan, respectively.

DISCLOSURE

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