Antitumor Effect of SN-38–Releasing Polymeric Micelles, NK012, on Spontaneous Peritoneal Metastases from Orthotopic Gastric Cancer in Mice Compared with Irinotecan

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Abstract

7-Ethyl-10-hydroxy-camptothecin (SN-38), an active metabolite of irinotecan hydrochloride (CPT-11), has potent antitumor activity. Moreover, we have reported the strong antitumor activity of NK012 (i.e., SN-38–releasing polymeric micelles) against human cancer xenografts compared with CPT-11. Here, we investigated the advantages of NK012 over CPT-11 treatment in mouse models of gastric cancer with peritoneal dissemination. NK012 or CPT-11 was i.v. administered thrice every 4 days at their respective maximum tolerable doses (NK012, 30 mg/kg/day; CPT-11, 67 mg/kg/day) to mice receiving orthotopic transplants of gastric cancer cell lines (44As3Luc and 58As1mLuc) transfected with the luciferase gene ($n = 5$). Antitumor effect was evaluated using the photon counting technique. SN-38 concentration in gastric tumors and peritoneal nodules was examined by high-performance liquid chromatography (HPLC) 1, 24, and 72 hours after each drug injection. 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. High concentrations of SN-38 released from NK012 were detected in gastric tumors and peritoneal nodules up to 72 hours by HPLC. Only a slight conversion from CPT-11 to SN-38 was observed from 1 to 24 hours. Fluorescence originating from NK012 was detected up to 72 hours, whereas that from CPT-11 disappeared until 24 hours. NK012 also showed antitumor activity against peritoneal nodules. Thus, NK012 showing enhanced distribution with prolonged SN-38 release may be ideal for cancer treatment because the antitumor activity of SN-38 is time dependent. [Cancer Res 2008;68(22):9318–22]

Introduction

Gastric cancer is the second most common cause of death from cancer in the world. The survival rate has remained low in patients with advanced gastric cancer, with a median survival rate of 13 months having been recently reported in a phase III trial, which has been the best outcome thus far (1). Patients with gastric cancer with scirrhous type stroma particularly showed poor prognosis even after curative resection, as well as highly progressed peritoneal dissemination (2). Because 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 (3). 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 (4, 5). On the other hand, angiogenesis was reported to be an essential factor in the development of peritoneal metastasis, and the 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 (6–10). In addition, several factors such as kinins and nitric oxide are involved in tumor vascular permeability (11–13). Polymer-conjugated drugs and nanoparticles categorized under drug delivery system agents are favorably extravasated from the vessels into the interstitium of tumors due to the enhanced permeability and retention effect (EPR effect; refs. 14, 15). The EPR effect is based on the following pathophysiologic characteristics of solid tumor tissues: hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors stimulating extravasation within cancer tissue, and absence of effective lymphatic drainage from the tumors that impedes the efficient clearance of macromolecules accumulated in solid tumor tissues. Moreover, macromolecules cannot freely leak out from normal vessels, and thus, the side effects of an anticancer agent can be reduced. Very recently, we have shown that NK012 (i.e., SN-38–releasing polymeric micelles) exerted superior antitumor activity and less toxicity than CPT-11 (15–17). In a series of studies, we showed that NK012 markedly enhanced the antitumor activity of SN-38, particularly against highly VEGF-secreting SBC-3/VEGF tumors compared with SBC-3/Neo tumors. On the other hand, it is conceivable that satisfactory drug delivery cannot be achieved in less-vascularized and highly fibrotic tumors, particularly for macromolecules. However, we observed that NK012 showed a strong antitumor activity even in the xenograft of Capan1 cells, which are pancreatic cancer cells with abundant stromal tissue, compared with CPT-11. This result suggests that NK012 can selectively accumulate in both hypervascular and hypovascular tumors with high interstitial pressure, and then induce sustained

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release of SN-38, followed by SN-38 distribution throughout the entire tumor tissues. In the present study, we evaluated the antitumor activity of NK012 against peritoneal tumor dissemination compared with that of CPT-11 using mouse models orthotopically transplanted with scirrhous gastric cancer cells, as well as against spontaneously progressing peritoneal dissemination (18, 19).

Materials and Methods

Cell cultures. 44As3 and 58As1m were previously reported as human signet-ring cell gastric cancer cell lines that spontaneously metastasize to the peritoneal cavity and produce large volumes of bloody ascites after orthotopic implantion in the gastric wall (18–21). Here, 44As3 and 58As1m cells were transfected with a complex of 4 μg of pEGF-PLuc plasmid DNA (Clontech) and 24 μL of GeneJammer reagent (Stratagene; Cloning Systems) in accordance with the manufacturer's instructions. Stable transfectants were selected in geneticin (400 μg/mL; Invitrogen), and bioluminescence was used to screen transfected clones for luciferase gene expression using the IVIS system (Xenogen). Clones expressing the luciferase gene were named 44As3Luc and 58As1mLuc. 44As3Luc and 58As1mLuc cells were maintained in RPMI 1640 supplemented with 10% FCS (Sigma), 100 IU/mL penicillin G sodium, and 100 mg/mL streptomycin sulfate (Immuno-Biological Laboratories) in a humidified atmosphere containing 5% CO_2 at 37°C.

Orthotopic models *in vivo*. Six-week-old female BALB/c *nu/nu* mice were purchased from CLEA Japan, Inc., and maintained under specific pathogen-free conditions and provided with sterile food, water, and cages. Ambient light was controlled to provide regular cycles of 12 h of light and 12 h of darkness. A total of 1×10^6 cells of 44As3Luc or 58As1Luc were inoculated into the gastric wall of each mouse after laparotomy, as described previously (18–21). *In vivo* photon counting analysis was conducted on a cryogenically cooled IVIS system using Living Image acquisition and analysis software (Xenogen). All animal procedures were performed in compliance with the Guidelines for the Care and Use of Experimental Animals established by the Committee for Animal Experimentation of the National Cancer Center; these guidelines conform to the ethical standards required by law and also comply with the guidelines for the use of experimental animals in Japan.

Drugs. NK012 was prepared by Nippon Kayaku Co., Ltd. (15). CPT-11 was purchased from Yakult Honsha Co., Ltd.

***In vivo* growth inhibition assay.** After inoculation of 44As3Luc or 58As1mLuc cells into the gastric wall (day 0), mice were randomly divided into test groups consisting of 5 mice per group. 44As3Luc mice were i.v. administered the maximum tolerated dose (MTD) of the 2 drugs via the tail vein on days 20, 24, and 28 as previously reported, that is, at 66.7 mg/kg/d for CPT-11 and 30 mg/kg/d for NK012 (15). 58As1mLuc mice were given the drugs in the same manner on days 18, 22, and 26. Photon counting analysis and body weight were measured twice a week. "Visible ascites," which was evident a few days before death in this mouse model, was used as a surrogate for survival time in consideration of animal welfare. Mice were euthanized when ascites became visible, and colonization of gastric wall by cancer cells and metastasis to the peritoneal cavity were confirmed in all the euthanized mice. Differences in relative photon counts between the treatment groups at day 42 in 44As3Luc mice and at day 81 in 58As1mLuc mice were analyzed using the unpaired *t* test.

Assay of free SN-38 in tissues. We next analyzed the biodistributions of NK012 and CPT-11 to orthotopic gastric tumors and peritoneal nodules. Twenty-six days after the inoculation of 44As3Luc cells into the gastric wall of mice, NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was administered via the tail vein. Under anesthesia, orthotopic gastric tumor and peritoneal nodule samples were excised 1, 24, and 72 hours after injection.

Measurements of tissue concentration of free SN-38 by high-performance liquid chromatography. Samples were rinsed with physiologic saline, mixed with 0.1 mol/L glycine-HCl buffer (pH 3.0)/methanol at 5 w/w%, and then homogenized. To analyze the concentration of free SN-38, 100 μL of the tumor samples were mixed with 20 μL of 1 mmol/L phosphoric acid/methanol (1:1) and 40 μL of ultrapure water, and camptothecin (CPT) was used as the internal standard (10 ng/mL for free SN-38). The samples were vortexed vigorously for 10 s, and then filtered

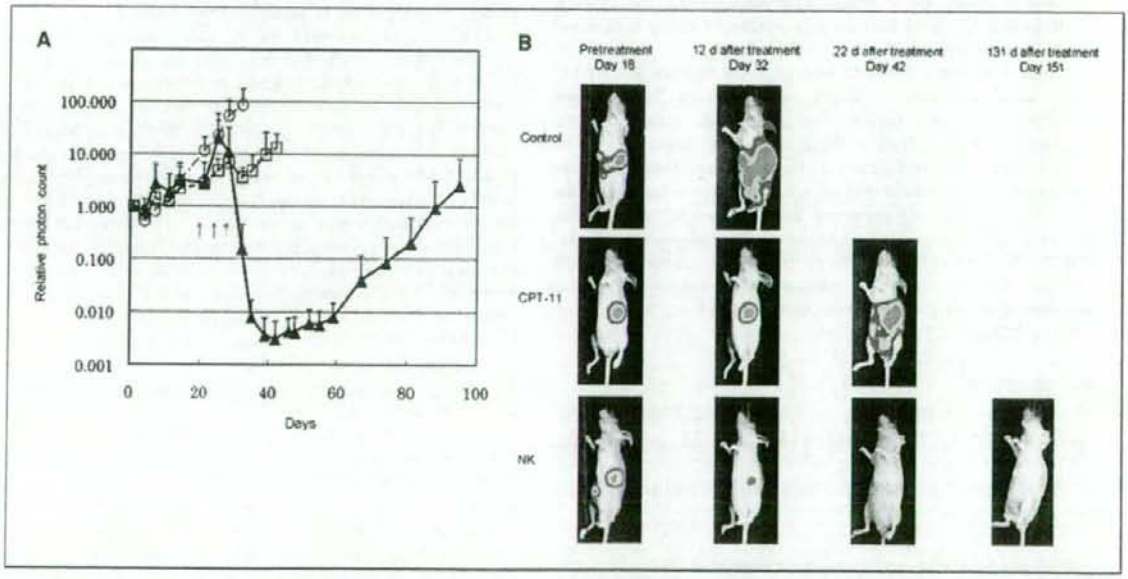

Figure 1. Effects of NK012 and CPT-11 in 44As3Luc mouse models. *A*, antitumor activity of NK012 or CPT-11 was evaluated by counting the number of photons using the IVIS system (*points*, mean; *bars*, SD; *arrows*, drug injections). Antitumor effect of each regimen on days 20, 24, and 28. (○), control; (□), CPT-11 (66.7 mg/kg/d, ×3); and (▲), NK012 (30 mg/kg/d, ×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.

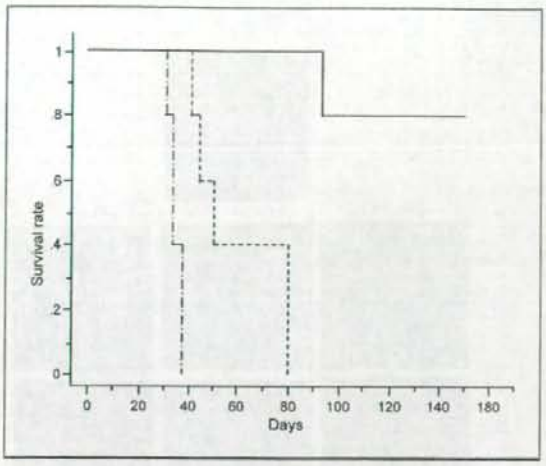

Figure 2. Survival curves of 44As3Luc mouse models. Survival curves of 44As3Luc mouse model in each regimen on days 20, 24, and 28. (–·–·–), control; (- - -), CPT-11 given at 66.7 mg/kg/d ×3; and (——), NK012 given at 30 mg/kg/d ×3.

through Ultrafree-MC Centrifugal Filter Devices with a cutoff molecular diameter of 0.45 μm (Millipore Co.). Reversed-phase high-performance liquid chromatography (HPLC) was conducted at 35°C on a Mightysil RP-18 GP column (150 × 4.6 mm; Kanto Chemical Co., Inc.). Fifty microliters of a sample were injected into an Alliance Water 2795 HPLC system (Waters) equipped with a Waters 2475 multi λ fluorescence detector. Fluorescence originating from SN-38 was detected at 540 nm with an excitation wavelength of 365 nm. The mobile phase was a mixture of 100 mmol/l ammonium acetate (pH 4.2) and methanol [11:9 (v/v)], and the flow rate was 1.0 mL/min. The content of SN-38 was calculated by measuring the relevant peak area and calibrating against the corresponding peak area derived from the CPT internal standard. Peak data were recorded using a chromatography management system (MassLynx v4.0; Waters).

Visualization of distribution of NK012 and CPT-11 by fluorescence microscopy. Mice were given fluorescein *Lycopersicon esculentum* lectin (100 μL per mouse; Vector Laboratories) to visualize tumor vasculature in the samples 5 min before anesthesia. The samples were then excised and embedded in an optimal cutting temperature compound (Sakura Finetechnochemical Co., Ltd.) and frozen at −80°C. Six-μm-thick tumor sections were then prepared using a cryostatic microtome, Tissue-Tek Cryo3 (Sakura Finetechnochemical Co., Ltd.). Frozen sections were examined under a fluorescence microscope, BIOZERO (KEYENCE), at an excitation wavelength of 377 nm and an emission wavelength 447 nm to evaluate the distribution of NK012 and CPT-11. Both drugs could be detected under the same fluorescence conditions because formulations containing SN-38 bound via ester bonds possess a particular fluorescence.

Statistical analyses. Data were expressed as mean ± SD. Data were analyzed using the Student's *t* test when groups showed equal variances (*F* test), or the Welch's test when they showed unequal variances (*F* test). *P* value of <0.05 was considered as significant. All statistical tests were two sided.

Results

Antitumor activities of NK012 and CPT-11. Comparison of the relative photon counts on day 42 in the 44As3Luc mouse model revealed significant differences in counts between mice given with NK012 and those given with CPT-11 ($P = 0.0282$; Fig. 1*A* and *B*). Similar result was obtained in the experiment with 58As gastric tumor (data not shown). The survival rates on day 150 in the 44As3Luc mouse model were 80% and 0% for the NK012 group and CPT-11 group, respectively (Fig. 2). Similar result was obtained in the experiment with 58As gastric tumor (data not shown). No marked toxic effects in terms of body weight changes were observed in any groups for any mouse models (data not shown). Only 1 mouse in the CPT-11 group of 44As1 mouse models showed diarrhea for 3 d, and any other clinical symptoms were not observed.

Tissue concentrations of free SN-38 after administration of NK012 and CPT-11. We examined the concentration-time profile of free SN-38 in orthotopic gastric tumors and peritoneal nodules in the 44As3Luc mouse model after the administration of NK012 and CPT-11 (Fig. 3*A* and *B*). Either orthotopic gastric tumors or peritoneal nodules exhibited the highest concentration of free SN-38 24 hours after NK012 administration, and 1 hour after CPT-11 administration. The highest concentrations of free SN-38 in the NK012 group were much higher than those in the CPT-11 group in either orthotopic gastric tumors or peritoneal nodules. The concentrations of free SN-38 released from NK012 in orthotopic gastric tumors were higher than those in peritoneal nodules.

Tumor tissue distribution of NK012 and CPT-11 as determined by fluorescence microscopy. Results showed that NK012 accumulation in either orthotopic gastric tumors or peritoneal nodules had been maintained from 1 hour to 72 hours after injection (Fig. 4*A*). On the other hand, CPT-11 showed maximum accumulation in either orthotopic gastric tumors or peritoneal nodules 1 hour after injection and disappeared within 24 hours (Fig. 4*B*).

Discussion

The main purpose of this study was to clarify the advantages of NK012 over CPT-11 as treatment against peritoneal metastasis spontaneously disseminated from orthotopically transplanted scirrhous gastric cancer cells in mouse models. We showed that NK012 exerted more potent antitumor activity in the mouse models used than CPT-11. Therefore, NK012 is considered promising in terms of providing clinical benefit to patients with gastric cancer showing progressing peritoneal dissemination.

CPT-11 is converted to SN-38, a biologically active and water-insoluble metabolite of CPT-11, by carboxylesterases (CE) in the liver and tumors. However, only 2% to 8% of administered CPT-11 is converted by CE in the liver and tumors to the active form SN-38 (22, 23). The conversion of CPT-11 to SN-38 also depends on genetic interindividual variability of the activity of CE (24). Thus, the direct use of SN-38 might be of great advantage and is attractive for cancer treatment. We have recently shown that NK012 (i.e., SN-38–releasing polymeric micelles) exerted superior antitumor activity and less toxicity than CPT-11 (15–17). The mean particle size of NK012 is 20 nm in diameter. NK012 can release SN-38 under neutral conditions even in the absence of CE because SN-38, which is bound to the blockcopolymer by phenolic ester binding, is stable under acidic conditions but relatively labile under neutral and mild alkaline conditions. The release rate of SN-38 from NK012 under physiologic conditions is quite high, that is, >70% of SN-38 is gradually released within 48 hours.

In this study, we used mouse models with orthotopically transplanted human scirrhous gastric cancer cells showing spontaneously progressing peritoneal dissemination, which we

reported previously (18, 19, 21). These models can imitate more realistically the progressing mode of human peritoneal dissemination of gastric cancer than conventional experimental models directly transplanted with cancer cells i.p. Moreover, our models enabled us to quantitatively evaluate drug antitumor effect even against peritoneal dissemination without having to sacrifice the animal and perform autopsy through the use of gastric cancer cells transfected with the luciferase gene and by applying photon counting analysis, having already verified the significant correlation between tumor volume and photon counts in a previous report (19).

For *in vivo* growth inhibition assay, drug administration was started on day 18 or 20 after cell inoculation into the gastric wall, when small peritoneal metastatic nodules and a small degree of ascites had appeared. The present results showed that NK012 had more potent antitumor activity than CPT-11 in the mouse models tested, suggesting its effectiveness against peritoneal dissemination of gastric cancer in the clinical setting.

In the pharmacologic evaluation, we could confirm the more enhanced distribution of NK012 than CPT-11 to not only orthotopic gastric tumors but also peritoneal nodules by quantifying SN-38 concentration in the tumors and visualization of fluorescence originating from NK012 or CPT-11 distributed in the tumors. Because CPT-11 or SN-38 has been reported to possess time-dependent growth-inhibitory activity against tumor cells, this prolonged retention of NK012 in the tumors and the sustained release of free SN-38 from NK012 may be responsible for its more potent antitumor activity observed in the present study (25). On the other hand, CPT-11 disappeared from the tumors before exerting sufficient antitumor activity. For both drugs, however, the concentrations of SN-38 in orthotopic gastric tumors were higher than those in peritoneal nodules. This is consistent with previous reports stating the poor delivery of anticancer drugs to peritoneal metastatic cells probably because of some obstacles such as abundant interstitium or high interstitial pressure. To date, we reported that NK012 can

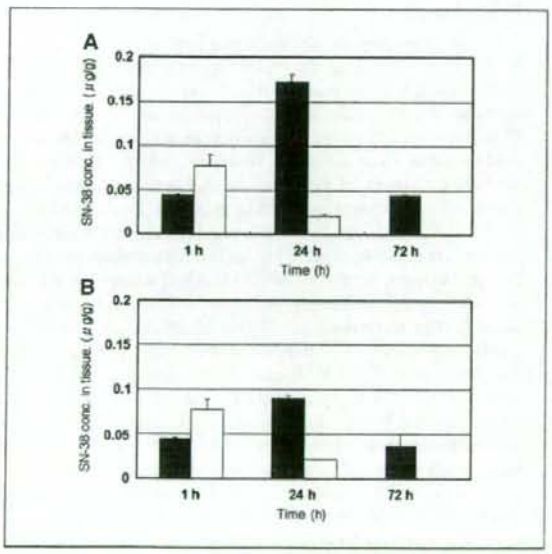

Figure 3. Concentration-time profile of free SN-38. NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was injected 26 d after implantation of 44As3Luc gastric cancer cells (*columns*, mean; *bars*, SD). *A*, concentration (*conc.*) of free SN-38 in orthotopic gastric tumor tissue of 44As3Luc mouse model after 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*).

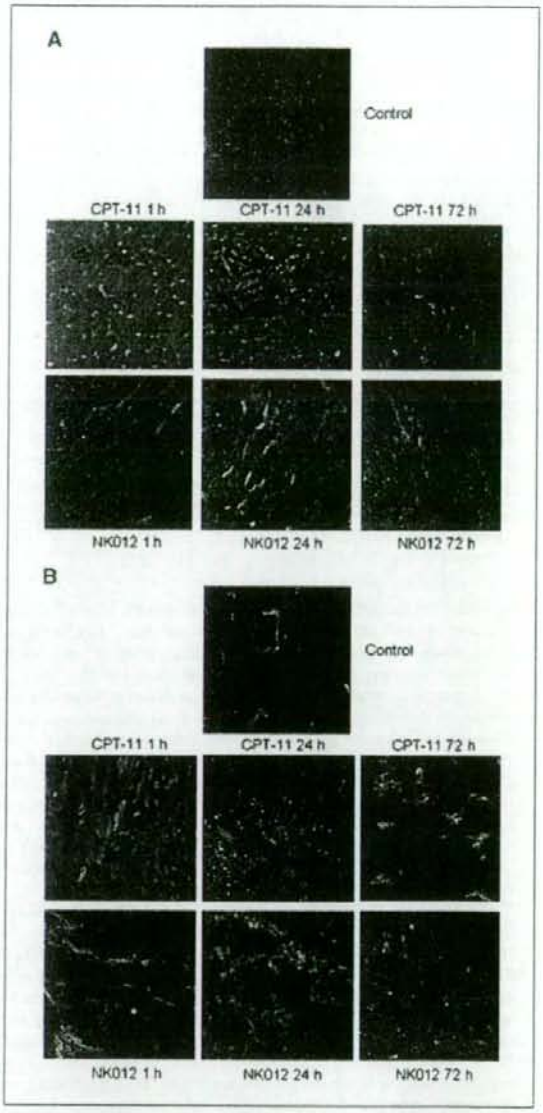

Figure 4. Tissue distribution of NK012 and CPT-11 as determined by fluorescence microscopy. Orthotopic gastric tumors or peritoneal nodules of 44As3Luc mouse model were excised 1, 24, and 72 h after i.v. injection of NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg). Each mouse was i.v. administered with fluorescein-labeled *Lycopersicon esculentum* lectin just before being sacrificed 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 447 nm. The same fluorescence condition can be applied for visualizing NK012 and CPT-11 fluorescence. Free SN-38 cannot be detected under this fluorescence condition. *A*, distribution of NK012 or CPT-11 in orthotopic gastric tumors (×100). *B*, distribution of NK012 or CPT-11 in peritoneal nodules (×100).

more selectively accumulate and retain longer in various tumor xenografts transplanted s.c. compared with CPT-11 (15–17). In the present study, we succeeded in demonstrating higher accumulation and longer retention of NK012 compared with CPT-11 in orthotopic and peritoneal disseminated gastric cancer model that is closer to human gastric cancer in clinics.

Peritoneal dissemination sometimes causes intestinal obstruction, which enhances the enterohepatic circulation of SN-38 after direct damage to the small intestine, and makes the use of CPT-11 difficult (26, 27). In the present study, no mouse in the NK012 group developed diarrhea. The dose-limiting toxic effects of CPT-11 seem to be neutropenia and diarrhea. In our previous data, however, there was no significant difference in the level of SN-38 in the small intestine between mice treated with NK012 and mice treated with CPT-11 despite the higher plasma area under the concentration of NK012 than CPT-11 (15). Moreover, no serious diarrhea has been reported even at the MTD dose in two phase I clinical trials against advanced solid tumors in Japan and the US (28, 29).

In conclusion, we showed that NK012 exerts significantly more potent antitumor activity against peritoneal dissemination of scirrhous gastric cancer cells than CPT-11, indicating the possibility of the clinical evaluation of this drug in patients with disseminated grastic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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SN-38内包高分子ミセルNK012

日米独立 phase Ⅰ試験

特集　臨床開発中のDDS製剤

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Two independent phase I trials of NK012, SN-38 incorporating micellar nanoparticle for patients with advanced cancer, opened in Japan and United States of America

NK012 is a micellar drug that incorporates the CPT-11 active metabolite SN-38. Currently, CPT-11 is used for the treatment of a wide variety of cancer ; however, it is expected that NK012 may show stronger antitumor effects of SN-38 than CPT-11. A phase I trial of NK012 was performed separately in the US and Japan, and the result of each trial showed that NK012 is well tolerated, suggesting its clinical usefulness.

It is anticipated that the subsequent phase Ⅱ and Ⅲ trials will demonstrate the advantages of NK012 as a drug delivery system.

NK012は，CPT-11の活性体のSN-38を内包した高分子ミセル化製剤である．現在CPT-11は，幅広いがん腫に用いられているが，NK012はそのCPT-11と比較しSN-38の薬効を効率よく発現できると考えられている．日本とアメリカでそれぞれ独立したNK012の臨床第Ⅰ相試験が行われ，その結果が報告された．

これらの結果から，NK012の忍容性が確認され有用性が示唆された．次相からの試験において，そのDDS製剤としての特徴をはっきり証明することが期待されている．

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key words : NK012, micellar nanoparticle

固形腫瘍に対する抗がん剤治療におけるDDS製剤の役割は，より選択的に薬剤をがん罹患部に到達させることで治療効果を高め，かつ同時に抗がん剤に伴う有害反応の軽減を図ることにある．その理論的背景となるのが，EPR(enhanced permeability and retention)効果である．固形腫瘍においては，一般的に新生腫瘍血管が増生している一方で，それに見合うリンパ回収系の増生がなく，腫瘍局所においては著しい血管透過性の亢進が起きていることによって，正常血管では血管外に漏出しにくい高分子物質も腫瘍血管からは漏出しやすく，かつ，腫瘍局所でいったん漏出した高分子物質はその場に停滞しやすいといった理論をEPR効果とよぶ[1~3]．このEPR効果を利用して，リポソーム製剤やミセル製剤が開発され，いくつもの臨床試験が行われ，一部は臨床実用化されている．

今回，SN-38内包の高分子ミセル化製剤であるNK012の第Ⅰ相試験が日本とアメリカで独立して行われ，その結果が報告されたので，その内容を概説したい．

NK012の概要

NK012は，イリノテカン(CPT-11)の活性体のSN-38を内包した高分子ミセル化製剤である．難溶性であるSN-38をポリエチレングリコールとポリグルタミン酸のブロックコポリマーに化学的に結合させると，外核に親水性鎖，内核に疎水性鎖を有するナノパーティクルを形成する(図1)．もともとSN-38は，植物アルカロイドであるcamptothecinから，その腫瘍効果を高め，毒性を軽減した化合物として合成された[4]．しかしながら，SN-38が水難溶性であったため，SN-38の誘導体であるCPT-11が開発され，実臨床に展開され，現在，大腸がん，

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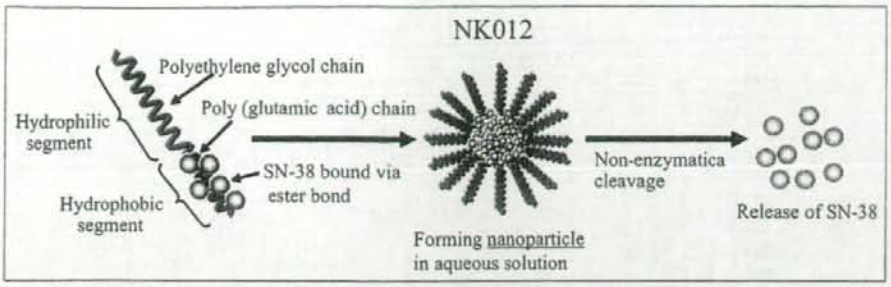


図1 NK012(SN-38内包ミセル体)の構造 (Doi T et al., 2008)[10]

肺がん，卵巣がんなど幅広いがん腫において用いられている．

CPT-11の抗腫瘍活性は，体内でcarboxylesterase(CE)によりSN-38に変換され効果を発揮すると考えられているが，その変換効率が10%以下であること[5,6]とCE活性には個人差があること[7]が知られており，より効率的で有効な抗腫瘍効果を得るためには，SN-38を直接利用することが有用であると考えられていた．そこで，水難溶性のSN-38をミセル化することで，静脈内投与を可能としたのがNK012である．

NK012の平均粒径は約20 nmであり，リポソーム製剤や他のミセル体抗がん剤と比較するとやや小さめである．37℃のPBS(phosphate buffered saline)内でのNK012からSN38のリリース速度は，24時間で57%，48時間で74%である一方，37℃の5%ブドウ糖液下でのリリースは，24時間で1%，48時間で3%と低値を示しており，このことからもNK012は投与前の環境では安定であり，かつ投与後，生体内では比較的速やかにSN38をリリースする性質が示された[8]．

各種細胞株における *in vitro* での細胞増殖抑制効果(IC_{50})は，CPT-11の約43～340倍と強い抗腫瘍効果を示し[8]，また，ヌードマウスの皮下に移植したヒト腫瘍6系(結腸がん3系，胃がん2系，小細胞がん1系)に対する抗腫瘍効果試験においても，NK012は検討したすべてのヒト腫瘍系に対してCPT-11に比較し高い有効性を示した[9]．ここでのCPT-11と同等の効果を示すNK012の投与量はSN-38換算量としてCPT-11の1/5～1/10であり，NK012はミセル形成型高分子プロドラッグにすることによってSN-38の薬効を効率よく発現できることが示唆された．

投与量 (mg/m²)	症例数	
2	1	AT法による100%増量
4	1	
8	1	増量幅をFibonacci変法に切替え
12	3	
16	3	
20	3	増量幅を4mg/m²増に変更
24	3	
28	3	DLT 1/3 → 3例追加
28	3	DLT 2/6(1例確定できず) → 3例追加
28	3	
計	24	(Doi T et al., 2008)[10]

図2 増量経過および症例数
AT法：accelerated titration method

本剤の毒性については，前臨床試験においてリンパ・造血器，消化管，生殖器，および皮膚への殺細胞作用に基づく毒性が認められた．DDS製剤に期待される効果の一つとして，正常組織への分布の減少による有害事象の軽減がある．

CPT-11の用量制限毒性の一つである下痢は，CPT-11とSN-38の腸管への直接障害が原因の一つと考えられている．NK012とCPT-11投与後の小腸におけるfree SN-38の濃度分布の検討では，両者に差は認められなかった[8]．しかし，NK012はCPT-11自体としての腸管障害がないぶん，下痢症状を減弱させる可能性が示唆され，恩田らは，ラットのモデルを用いてNK012がCPT-11に比較し強い抗腫瘍効果を発揮すると同時に，下痢症状を軽減させると報告している[9]．

表1 血液毒性

Dose (mg/m²)	n	白血球減少				好中球減少				血小板減少			
		Grade				Grade				Grade			
		1	2	3	4	1	2	3	4	1	2	3	4
2~8	3	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

(Doi T et al., 2008)[10]

表2 非血液毒性

	2~20 mg/m² (n=12)				24 mg/m² (n=3)				28 mg/m² (n=9)			
	Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4
悪　心	8	2	0	0	3	0	0	0	3	3	1	0
食欲不振	6	3	0	0	3	0	0	0	3	2	2	0
嘔　吐	3	1	0	0	0	0	0	0	2	3	0	0
下　痢	3	0	0	0	0	1	0	0	4	4	0	0
疲　労	7	0	0	0	2	1	0	0	4	2	0	0
発熱性好中球減少	0	0	0	0	0	0	0	0	0	0	1	0
感　染	0	1	0	0	0	0	0	0	0	0	1	0
心房粗動	0	0	0	0	0	0	0	0	0	0	1	0
脱　毛	7	1	–	–	1	2	–	–	5	3	–	–
γGTP 増加	0	1	1	0	1	0	0	0	2	1	0	0

(Doi T et al., 2008)[10]

NK012 第Ⅰ相試験：日本

筆者らは，2008年10月21～24日までスイスのジュネーヴで開催されたEORTC-NCI-AACRにおいて，日本におけるNK012第Ⅰ相試験の報告を行った[11]．その報告の概要を以下にあげていく．

1. 試験の目的

NK012の用量制限毒性(DLT)，最大耐量(MTD)，有害事象などを評価項目とした安全性や薬物動態(PK)の検討を行い，第Ⅱ相試験での推奨用量(RD)を決定することであり，また可能な症例で抗腫瘍効果を確認することであった．

2. 方　法

対象は，組織学的に悪性腫瘍と診断され，標準治療に無効もしくは適切な治療がない症例で，主要臓器機能が充分保持され，PS(performance status)が0-2，20歳以上74歳未満などの条件にて選択した．

開始投与量は，前臨床試験においてマウスLD_{10}の1/10が17.3 mg/m²，イヌのTDL(toxic dose low)の1/3が2.13 mg/m²であったことから，2 mg/m²と設定し，規定に沿って投与量を増量していった(**図2**)．用法・用量は5%ブドウ糖液250 mLに溶解し，30分で点滴静注を行い，これを3週間ごとに実施した．毒性の評価はCTCAE(Ver.3.0)で行い，抗腫瘍効果の判定はRECISTを用いて行った．

また，血液・尿をサンプルとし，PK解析も実施した．DLTの規準は，① 5日以上継続するgrade 4の好中球減少(<500/μL)，② grade 4の血小板減少(<25,000/μL)，③ grade 3以上の非血液毒性(grade 3までの食欲不振，悪心，嘔吐，過敏症は除く)とした．

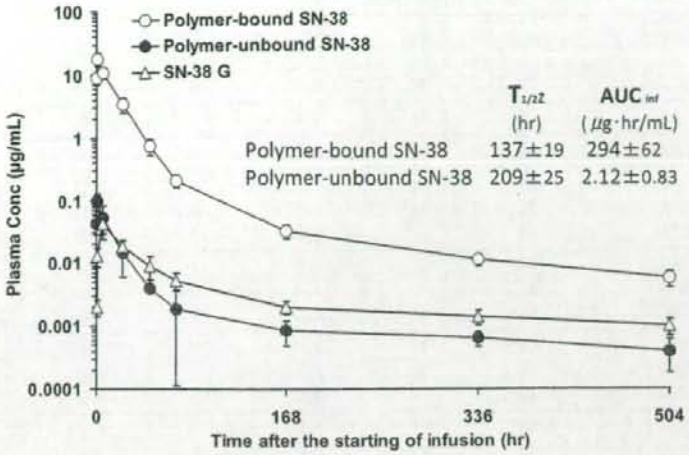


図3
NK012 (28 mg/m²) 投与後の各 PK パラメーター血中濃度 (n=9)

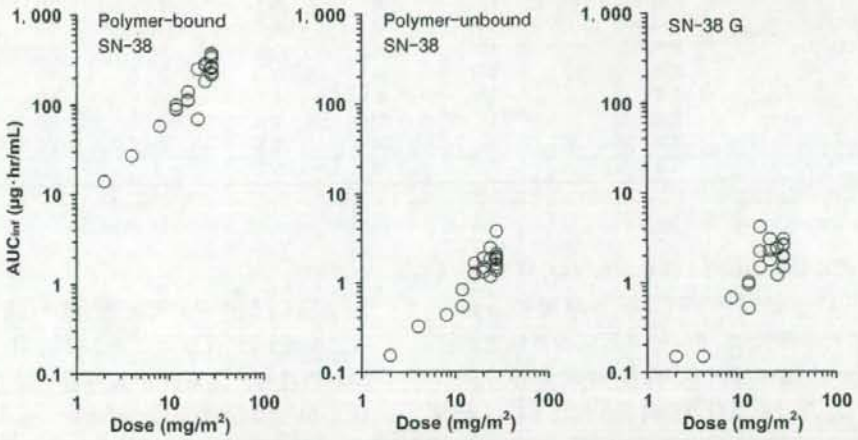


図4 投与量と AUC の関係 (Doi T et al. 2008)[10]

3. 結 果

症例の内訳は全24例中，大腸がんが最多で12例，ついで膵臓がん4例，小細胞肺がん3例，食道がん3例，非小細胞肺がん1例，肺カルチノイド1例であった．増量法は，開始用量を2 mg/m²とし accelerated titration method にて1例ごとに100%増量した．8 mg/m²投与群において，好中球数が投与前値にくらべて約50%減少したため，効果安全性評価委員会に諮り，安全性を考慮し，以降は Fibonacci 変法に変更し，増量した．その後，20 mg/m²投与群において，grade 4 の好中球減少が出現したため，増量幅を以降4 mg/m²に変更した．28 mg/m²投与群において，DLT である grade 3 の発熱性好中球減少が出現したため，3例追加検討した．その追加3例中1例に grade 4 の好中球減少が発現したが，その時点では DLT の評価が困難であったため，効果安全性評価委員会の答申を受け，さらに3例再追加し，28 mg/m²投与群は計9例での評価となった．

また，UGT1A1*28/*28 のホモ接合体を持つ症例に対する増量も計画されていたが，該当する症例はいなかった．

有害事象に関して DLT は，16 mg/m²投与群に1例，24 mg/m²投与群に1例，28 mg/m²投与群に5

表3 NK012の効果

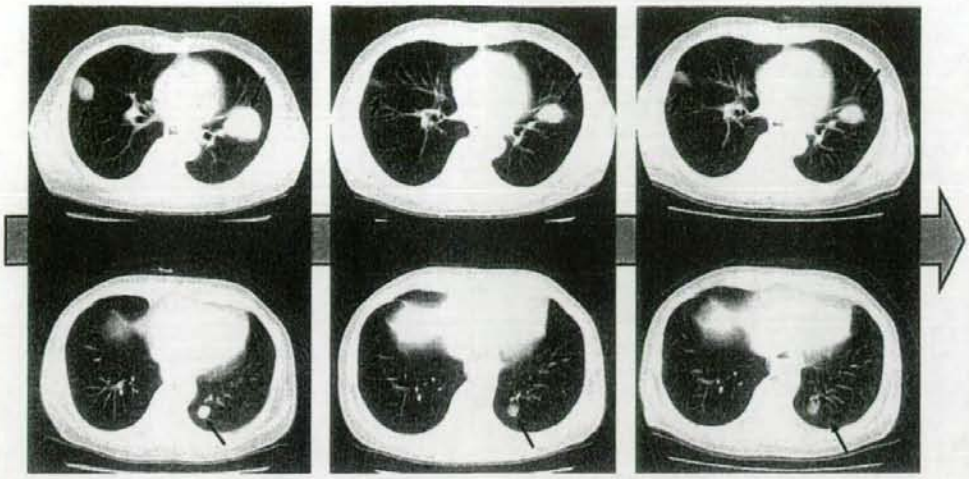
投与量 (mg/m²)	原疾患	年齢	性別	前治療 CPT-11	投与コース	効果
2	大腸がん	74	M	あり	2	PD
4	大腸がん	69	M	あり	1	PD
8	膵臓がん	65	M	なし	2	PD
12	膵臓がん	55	M	なし	4	SD
	大腸がん	71	M	あり	10	SD
	大腸がん	41	F	あり	2	PD
16	大腸がん	60	F	あり	4	SD
	大腸がん	61	M	あり	6	SD
	大腸がん	63	M	あり	2	PD
20	大腸がん	59	M	あり	6	SD
	小細胞肺がん	66	M	あり	7	SD
	大腸がん	49	M	あり	2	PD
24	小細胞肺がん	70	M	あり	10	(SD)
	膵臓がん	60	M	なし	3	PD
	非小細胞肺がん	68	F	なし	4	SD
28	小細胞肺がん	62	F	あり	1	NE
	大腸がん	67	M	あり	10	SD
	大腸がん	66	M	あり	3	PD
	膵臓がん	58	F	なし	2	PD
	食道がん	57	M	なし	7	PR
	大腸がん	56	F	あり	2	PD
	食道がん	51	M	なし	2	PD
	肺カルチノイド	45	M	なし	7+	PR
	食道がん	73	M	なし	4	SD

例の計7例に認められた．16 mg/m² 投与群では，4コース目にgrade 3のγGTP増加が認められ，24 mg/m² 投与群では，3コース目に5日以上継続するgrade 4の好中球減少が認められた．28 mg/m² 投与群では，1コース目の9例中2例に5日以上継続するgrade 4の好中球減少およびgrade 3の発熱性好中球減少がそれぞれ1例ずつ認められ，2コース目以降に5日以上継続するgrade 4の好中球減少，好中球減少を伴う感染(grade 3)，およびgrade 3の心房粗動をそれぞれ1例ずつ認められた．

増量規定では，28 mg/m² 投与群9例中1コース目のDLT発現が2例であったことから，さらなる増量は可能であったが，2コース目以降に発現したDLTなども含め，効果安全性評価委員会で検討した結果，本試験ではこれ以上の増量は行わないこととし，MTDを28 mg/m² と判断し，また，主なDLTがG-CSFなどでコントロール可能な好中球減少であったことから，推奨投与量を28 mg/m² とした．

つぎに，副作用発現状況を**表1**に示す．血液毒性は，20 mg/m² 投与群では2例grade 4の好中球減少が出現し，28 mg/m² 投与群では全例がgrade 3以上の好中球減少を認めていた．血小板減少は，28 mg/m² 投与群ではgrade 2が3例であった．非血液毒性については，主な事象は消化管毒性であったが，多くは一過性で，問題となると考えられた下痢については，28 mg/m² 投与群ではgrade 3以上はなく，grade 2が4例であった(**表2**)．

PK結果を**図3**に示す．これは28 mg/m² 投与群におけるPK結果で，ポリマー結合SN-38，非結合SN-38，SN-38Gともに長時間にわたって血中濃度を保っていることがわかる．既報告[11]のCPT-11のPKと比較し，$T_{1/2}$ではポリマー非結合SN-38はSN-38の15倍，ポリマー結合SN-38はCPT-11の30倍，AUCはそれぞれ2倍，10倍と，$T_{1/2}$・AUCともに高値を示しており，NK012の血中滞留性の高さが確認された．**図4**は投与量とAUCについて



治療前　　1コース後　　2コース後

図5　食道がん患者(NK012：28 mg/m^2 投与群) PR 例
(Doi T et al, 2008)[10]

関連をみたものであるが，ポリマー結合，非結合 SN-38，SN-38G ともに用量依存的に AUC が増加していた．

各投与量における抗腫瘍効果は，28 mg/m^2 投与群において，食道がんおよび肺カルチノイドの1例に部分奏効(PR)が認められた．大腸がんでは PR 例は認められていないが，12例中全例が CPT-11 既治療例であるにもかかわらず12例中5例に安定(SD)が認められ，4例が6コース以上の投与が可能であった(表3)．

食道がんの PR 症例を図5に提示する．肺両葉の転移が縮小していることがわかる．

以上をまとめると，NK012の国内第Ⅰ相試験での主な DLT は好中球減少であり，第Ⅱ相試験における推奨投与量は 28 mg/m^2 である．PK 解析では NK012 の血中滞留性は良好であり，ポリマー結合型 SN-38 ならびにポリマー非結合型 SN-38 の AUC は，用量依存的に増加していることが認められた．

NK012 第Ⅰ相試験：アメリカ

2008年5月30日～6月3日まで米シカゴで開催されたアメリカ臨床腫瘍学会(ASCO)において，アメリカにおける NK012 の第Ⅰ相試験の結果が報告された[12]．

表4　患者背景(アメリカ第Ⅰ相試験)

N=32：(M/F=9/23)
Median age：60.5 years(40-78)
Tumor type：
NSCLC(non small cell lung carcinoma)：9
Breast：6
Colorectal：4
Esophageal：2, Gastric：2, Ovarian：2, SCLC：2
Neuroendcrine：1, Pancreatic：1, Testicular：1
Uterine：1, Primary unknown tumor：1

(Burris HA Ⅲ et al, 2008)[12]

この第Ⅰ相試験における目的は，日本での試験と同様に NK012 の DLT と MTD を決定し，第Ⅱ相試験での RD を決めること，また PK および抗腫瘍効果を検討することであった．

投与方法も日本での試験と同様に，3週ごとに30分かけて静脈内投与が行われた．また，CPT-11 の副作用の発現に関与しているといわれる UGT1A1 遺伝子多型のスクリーニングを患者登録前に行い，UGT1A1*28/*28 ホモ接合体を持つ患者に対しては，安全性を考慮して減量投与された．

対象は，すでに他の治療を受けた進行性固形がんで臓器機能が許容できる患者32例(男性9例女性23例)で，年齢の中央値は60.5歳(40～78歳)で

表5　増量経過と症例(Non-*28/*28：アメリカ第Ⅰ相試験)

Level	Dose	#Pts	#Cycles	DLT (cycle 1 only)
1	9 mg/m²	3	14	0
2	12 mg/m²	3	13	0
3	16 mg/m²	3	27+	0
4	21 mg/m²	3	18+	0
5	28 mg/m²	6	20+	1 (G4 neutropenia>7days)
6	37 mg/m²	5	8+	2*1)
Total		23	100+	

*1) Grade 3 neutropenia with pneumonia
Inability to start 2nd cycle due to prolonged neutropenia

表6　増量経過と症例(*28/*28：アメリカ第Ⅰ相試験)

Level	Dose	Number of Pts	Pts able to dose escalate (% of current dose)
1a	4.5 mg/m²	1	1(100%)
3a	8.0 mg/m²	1	1(100%)
4a	10.5 mg/m²	2	2(75%)
6a	18.5 mg/m²	5*1)	1(75%)

*1) Includes three wt/*28 variant patients

あった．32例のがん腫の内訳は，非小細胞肺がん9例，乳がん6例，大腸がん4例，食道がん，胃がん，卵巣がん，小細胞肺がんがそれぞれ2例，その他は1例ずつのがん腫であった(**表4**)．NK012投与の前にCPT-11による治療を受けていたのは3例で，すべて大腸がんの患者であった．発表時点で，23人の患者は9～37 mg/m²まで6段階の用量レベルで，トータル100サイクルの治療を受けていた(**表5**)．6人の*28/*28ホモ接合体の患者は4.5～18.5 mg/m²までの4段階の用量レベルで治療を受けていた(**表6**)．

DLTは21 mg/m²投与群まではみられなかったが，28 mg/m²投与群では，6例のうち1例の患者に7日間以上つづくgrade 4の好中球減少を認めた．さらに，37 mg/m²投与群では，5例のうち2例にDLT(好中球減少を伴った肺炎および1週間以上の治療延期が生じた好中球減少)がみられた．

治療による毒性は血液学的なものが多く，grade 3/4の好中球減少が全32例中15例にみられ，grade 3/4の血小板減少が3例にみられた．長引く好中球減少によって，つぎのサイクルを延期することになった患者は6例であった．また，重篤な消化管毒性はみられなかった．

表7　治療効果(アメリカ第Ⅰ相試験)

Partial response (PR)
4 patients
Breast：3 (triple negative：3)
SCLC：1
Stable disease (SD)
16 patients
NSCLC, Gastric, Unknown primary
Ovarian, Pancreas, Esophageal
Progressive disease (PD)
9 patients
Inevaluable-2 patients, Too early-1patients

抗腫瘍効果は，PRが4例，SDが16例(7例が4サイクル以上)，病状の進行(PD)がみられたのが9例，2例は評価不能で，1例は発表時点では未評価であった．PRが得られた4例の内訳は，トリプルネガティブ乳がん3例，および小細胞肺がん1例であった(**表7**)．

これらの結果から，NK012は充分な忍容性があることが確認され，さらに，第Ⅰ相試験の段階でトリプルネガティブ乳がんなどの難治性がんに抗腫瘍効果を示したことで，臨床的な面からも高い効果が期待される結果であった．

つぎの第Ⅱ相試験におけるNK012のRDは28 mg/m²となり，CPT-11への感受性を持つ腫瘍タイプの患者を対象とした試験を考えている．

今後の方向性

2008年の日米双方におけるNK012の第Ⅰ相試験の結果から，その忍容性が確認され，有用性が示唆

されたことで，現在，第Ⅱ相試験に向けての準備が進められている．対象としては，第Ⅰ相試験の結果から乳がんや，現在CPT-11が標準治療の選択肢となっている大腸がんや肺がんがあがると考えられる．

また，以前よりNK012がVEGF高発現した腫瘍血管増生により，その効果を高めることが考えられてきたが[8]，最近の報告では血管豊富な腫瘍である腎細胞がんのマウスモデルにおいて，CPT-11と比較しNK012がより高い抗腫瘍効果を示した報告がなされており[13]，第Ⅰ相試験で検討された以外の腫瘍も，今後は開発の候補となりうると考える．

NK012は，既存の臨床応用されている抗がん剤をミセル化したものであることから，つぎの第Ⅱ相試験以降において，そのDDS製剤としての特徴をはっきり証明することが求められており，また期待されるところでもある．

また最近，大腸がんのマウスモデルにおいて，NK012の5-FUとの併用がCPT-11と5-FUを併用した場合と比較し，より高い効果を示した報告もあり[14]，単剤のみならず他の薬剤との併用療法の開発も，第Ⅱ相試験後のつぎのステップとして視野に入ってくると考えられる．

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