Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.9567-17
Intern Med 57: 939-946, 2018
http://internmed.jp

【 ORIGINAL ARTICLE 】

A Phase I/II Study for Dose-finding, and to Investigate the Safety, Pharmacokinetics and Preliminary Efficacy of NK012, an SN-38-Incorporating Macromolecular Polymeric Micelle, in Patients with Multiple Myeloma

Masaki Ri[1], Kenshi Suzuki[2], Shinsuke Iida[1], Kiyohiko Hatake[3], Takaaki Chou[4], Masafumi Taniwaki[5], Noriko Watanabe[6] and Tetsuji Tsukamoto[6]

Abstract:

Objective Multiple myeloma (MM) is the second most common hematological cancer. An attempt to treat MM using a topoisomerase I inhibitor was made based on our previous non-clinical studies suggesting the usefulness of an SN-38 derivative. Our aim was to conduct a phase I/II study of NK012, a micelle-forming SN-38 conjugate, in patients with relapsed/refractory multiple myeloma (RRMM).

Methods NK012 was administered at doses of 12-24 mg/m^2 and the safety, pharmacokinetics and preliminary efficacy were evaluated.

Results Neutropenia was the most common grade 3 or 4 adverse drug reaction. Grade 4 neutropenia accounted for the majority of dose-limiting toxicities and only appeared at a dose of 24 mg/m^2. The maximum concentrations and the area under the concentration-time curves from time zero to infinity for both NK012 and its active metabolite SN-38 increased in a dose-dependent manner. The best overall response was stable disease, which was achieved in 12 out of 16 patients.

Conclusion The recommended dose of NK012 monotherapy for RRMM patients was concluded to be 20 mg/m^2. However, this phase I/II study was terminated at the end of the phase I stage because no patients showed an objective response. Additional clinical studies of combination therapy with NK012 and other agents are warranted.

Key words: multiple myeloma, micelle, SN-38, bone marrow, extramedullary lesions

(Intern Med 57: 939-946, 2018)
(DOI: 10.2169/internalmedicine.9567-17)

Introduction

Multiple myeloma (MM), which is the second most common form of hematological cancer, is formed by malignant plasma cells. Nearly 230,000 people have this condition worldwide. In 2012, approximately 114,000 new cases were diagnosed and 80,000 patients died (1). MM is characterized by the overproduction of monoclonal proteins, with unique clinical symptoms. During disease progression, it is often accompanied by several unfavorable complications (2), including extramedullary lesions and leukemic changes. The main treatment regimens that are preferred for relapsed or refractory multiple myeloma (RRMM) include proteasome inhibitors (PIs) (3-5) and/or immunomodulatory drugs (IMiDs) (5-7). However, most patients with MM develop resistance to PIs and/or IMiDs during treatment, which makes RRMM incurable. In the search for better treatments, several novel agents are under investigation (7), including new types of PIs, IMiDs, histone deacetylase (HDAC) inhibitors, and

[1]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Japan, [2]Department of Hematology, Japanese Red Cross Medical Center, Japan, [3]Department of Hematology and Oncology, The Cancer Institute Hospital of Japanese Foundation for Cancer Research, Japan, [4]Department of Internal Medicine, Niigata Cancer Center Hospital, Japan, [5]Center for Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Japan and [6]Nippon Kayaku Co., Ltd., Japan
Received: May 24, 2017; Accepted: June 18, 2017; Advance Publication by J-STAGE: December 8, 2017
Correspondence to Dr. Masaki Ri, rrmasaki@med.nagoya-cu.ac.jp

monoclonal antibody drugs. Nevertheless, the development of newer therapies as additional treatment options to overcome such drug resistance is crucial for improving the outcomes of treatment.

The constitutive expression of topoisomerase I (Topo I), a target molecule of SN-38, and high expression levels of carboxylesterase-2, which metabolizes CPT-11 to SN-38, were previously reported in human MM cells (8). CPT-11 monotherapy significantly reduced the tumor volume in a mouse xenograft model of subcutaneously transplanted human MM cells, and its combination with bortezomib completely eradicated the tumor masses in all mice (n=5) (8). These results suggested that CPT-11 or SN-38 could be candidate drugs that can be used in the treatment of MM.

NK012 is a micelle-forming macromolecular prodrug that is prepared by the conjugation of SN-38 (7-ethyl-10-hydroxycamptothecin) with amphiphilic block copolymers (9-11). Unlike CPT-11, the release of SN-38 from NK012 is non-enzymatic, and is therefore expected to exhibit a stable therapeutic effect, independent of the carboxylesterase enzymatic activity, which varies among patients. In addition, the frequency and the severity of diarrhea in association with NK012 are expected to be lower in comparison the frequency and severity associated with CPT-11 because the cholinergic mechanism that was suggested to be associated with CPT-11 was not found in NK012. The antitumor activity of NK012 was examined in a mouse model of orthotopic MM using CD138-positive U2661B1 cells. The intravenous administration of NK012 was found to suppress the plasma M protein concentration and the proliferation of infiltrating myeloma cells in the bone marrow in a dose-dependent manner (11).

The results from these CPT-11 and NK012 studies indicated there was sufficient value in the further clinical evaluation of NK012. Phase I studies of NK012 in patients with solid tumors have already been conducted in Japan (12) and the US (13), and the recommended dose is 28 mg/m^2. The main dose-limiting toxicity of NK012 was neutropenia. However, it was strongly anticipated that the myelosuppression would be more severe in patients with MM in comparison to patients with solid tumors, due to the different characteristics of the primary disease. Thus, we concluded that a separate clinical study was necessary to identify the recommended dose of NK012 in MM patients.

This phase I/II study was planned to evaluate the safety, pharmacokinetics and efficacy of NK012. In the phase I stage, we aimed to determine the recommended dose for patients with RRMM. In the phase II stage, we aimed to evaluate the efficacy at the recommended dose.

Materials and Methods

Study design

This was an open-label, multi-center, phase I/II study (JapicCTI-111652). This study consisted of two stages. In the phase I stage, we planned to evaluate the safety, pharmacokinetics, and efficacy of NK012 in RRMM, and to determine the recommended dose for the phase II stage. In the phase II stage, we planned to investigate the efficacy and safety of NK012 at the recommended dose.

Dose escalation was carried out in accordance with the 3+3 design (14). The dose was increased until the level at which at least two of three to six patients experienced dose-limiting toxicities (DLTs). The recommended dose of the phase II stage was defined conventionally, as the dose just below the level of this toxic dose; however, the final decision was made, with reference to the recommendations of an Independent Efficacy/Safety Data Review Committee. In phase I, the starting dose (level 1) of NK012 was set at 12 mg/m^2. The dose was selected because grade 3 and 2 neutropenia first occurred at this dose in phase I studies in Japan (12) and the US (13), respectively. Conversely, a patient continued NK012 treatment for more than 10 cycles at 9 mg/m^2 in the phase I study in the US (13). Thus, the dose escalation plan was set as 12, 16, 20, 24, and 28 mg/m^2 (levels 1, 2, 3, 4, and 5, respectively).

Patients

Patients with symptomatic RRMM and a history of treatment with at least two standard regimens, at least one of which contained bortezomib, lenalidomide, or thalidomide, were eligible. The further inclusion criteria were as follows: 20-79 years of age; the estimation or measurement of the disease state with serum M-protein values of IgG ≥1.0 g/dL, IgA ≥0.5 g/dL, or IgD ≥0.2 g/dL, and a urine M-protein value of ≥0.2 g/24 hours; in patients in whom the functions of the main organs were well maintained (shown by a neutrophil count of ≥1,500/μL, a platelet count of ≥7.5×10^4/μL, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of ≤3 times the upper limit of normal, a serum creatinine level of ≤2.0 mg/dL; a performance status (PS) of 0-2 on the East Cooperative Oncology Group (ECOG)/Zubrod scale. Additionally, patients with a PS of 3 due to an osteolytic lesion were eligible for inclusion. Patients with homozygous *UGT1A1*28* or *UGT1A1*6* or both heterozygous *UGT1A1*28* and *UGT1A1*6*, in a UGT1A1 genetic polymorphism assay were excluded to improve the precision of the study.

The administration of the study drug

NK012 was intravenously administered over 30 min on the first day of a 4-week cycle. Dexamethasone (6.6 mg/body) was administered intravenously prior to the administration of NK012 as a prophylactic measure against nausea and vomiting. The maximum number of cycles was defined as eight. However, the continuation of treatment beyond the eighth cycle was permitted based on the patient's benefit and was determined by the study sponsor and the investigator in charge.

Assessment

Safety assessments, including the observation of adverse events, vital signs, and laboratory tests, were initially performed every week. However, beyond the third cycle, investigators were permitted to reduce the frequency of observation to every other week if the patient's safety status was considered stable based on the observation of past cycles. Adverse events were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. DLT was defined as any of the following adverse events (AEs) in the first cycle: grade 4 neutropenia persisting for at least 8 days, grade 4 thrombocytopenia requiring blood transfusion, and grade 3 or 4 non-hematologic adverse events (blood chemistry, urinalysis, signs and symptoms). If the severity of nausea, vomiting, and diarrhea decreased to grade ≤2 with appropriate treatment, they were not recognized as DLTs. Additionally, grade 3 hypersensitivity, grade 3 decreased appetite, and a grade ≥3 increase in alkaline phosphatase (ALP) were likely to be associated with new bone formation, grade ≥3 electrolyte level abnormalities or abnormal chemistry parameters associated with tumor lysis syndrome were not considered to be DLTs.

The QT/QTc interval was assessed by electrocardiography (ECG) in the first cycle of the phase I stage according to the following schedule and procedure: 1) the baseline was measured at three time points on the day before treatment (the planned pretreatment time, 1 hour, and 6 hours); 2) on the first day of treatment, at the same time points as on the previous day; and 3) at 24 hours and 4 weeks after the first treatment.

The blood samples for the pharmacokinetics (PK) analysis were obtained from each patient in the first cycle at the following time points: before, just after the start of the infusion, and a further 1, 6, 24, and 72 hours, and 7, 14, and 28 days after the end of the NK012 infusion. The drug concentration in the bone marrow aspirate was measured at 7 days after the administration in patients who gave their consent. In parallel, a smear was prepared to investigate the percentage of plasma cells.

The efficacy of NK012 was evaluated according to the International Myeloma Working Group (IMWG) Uniform Response Criteria and Bladé criteria/European Group for Blood and Marrow Transplant (EBMT) criteria. The serum M-protein levels were measured at 2 and 4 weeks after each cycle of NK012 administration, at the central laboratory (SRL, Tokyo, Japan).

The PK analysis

Blood samples (2 mL) were collected with heparin as an anticoagulant, and were cooled immediately on ice. The samples were centrifuged and plasma was obtained. Bone marrow aspirate (0.5 mL to 1 mL) was collected and part of the fluid was used to prepare the smear. The remaining fluid was transferred into a heparin-containing tube placed on ice. Ice-cold 0.1 mol/L hydrochloric acid was added all biological samples to prevent the hydrolysis of NK012.

The concentrations of free SN-38 (polymer-unbound SN-38) and SN-38-glucuronide were measured by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection (15). The total concentration of SN-38 (polymer-bound and unbound) was measured by analyzing hydrolyzed samples (16).

The pharmacokinetic parameters were calculated using the WinNonlin software program (version 6.1, Pharsight Corporation, Mountain View, USA) with a non-compartmental analysis.

Statistical analysis

The sample size in the phase II stage was set at 41 patients, based on the number that was required to meet the threshold and expected overall response rate (10% and 25%, respectively) with 80% power and a type I error of 0.05.

A two-sided confidence interval with a confidence level of 95%, and a two-tailed test with a significance level of 0.05 were used for the statistical analyses. The safety analysis was conducted in the safety analysis cohort, which included patients who received at least one dose of the study drug. The efficacy endpoints of this study were the overall response rate according to the IMWG criteria [stringent complete response (sCR), complete response (CR), very good partial response (VGPR) and partial response (PR)], progression-free survival (PFS) and overall survival (OS). The median values and 95% confidence intervals for the PFS, and OS curves were estimated using the Kaplan-Meier method.

Results

Phase I stage

Patient characteristics

This trial, which was sponsored by Nippon Kayaku (Tokyo, Japan), started in November 2011, and was terminated in February 2014 at the completion of the eighth cycle of treatment by the last enrolled patient. All 16 patients enrolled received NK012 treatment. The patient' characteristics are shown in Table 1. All of the 16 patients had previously received bortezomib, and 11 patients (69%) had previously received IMiDs, including lenalidomide and thalidomide. High-risk cytogenetic abnormalities, t(4;14), t(14;16), and/or del(17p), were found in five patients (31%). Three patients enrolled in the 24 mg/m^2 cohort had extramedullary lesions.

Dose escalation

Three patients each were enrolled in the level 1 (12 mg/m^2), 2 (16 mg/m^2), and 3 (20 mg/m^2) cohorts. No DLTs were observed at these levels. In the level 4 (24 mg/m^2) cohort, one of the first three patients experienced grade 4 neutropenia (which persisted for at least 8 days), and grade 3 anemia, as DLTs. We therefore planned to add three patients to the cohort. Actually, four patients were enrolled because the fourth patient gave their consent prior to the administra-

Table 1. Demographics and Other Baseline Characteristics.

Factor	Category	12 mg/m^2 n=3, (%)	16 mg/m^2 n=3, (%)	20 mg/m^2 n=3, (%)	24 mg/m^2 n=7, (%)	Total n=16, (%)
Gender	Male	2 (66.7)	2 (66.7)	2 (66.7)	6 (85.7)	12 (75.0)
	Female	1 (33.3)	1 (33.3)	1 (33.3)	1 (14.3)	4 (25.0)
Age	Median	59.0	62.0	62.0	67.0	62.0
	range	46-71	51-77	51-65	56-72	46-77
Performance status	0	3 (100)	1 (33.3)	2 (66.7)	3 (42.9)	9 (56.3)
	1		2 (66.7)	1 (33.3)	2 (28.6)	5 (31.3)
	≥2				2 (28.6)	2 (12.5)
Cytogenetic abnormalities	t (4;14)		3 (100)		1 (14.3)	4 (25.0)
	17p		1 (33.3)		1 (14.3)	1 (6.3)
	t (14;16)					1 (6.3)
	13q	1 (33.3)	1 (33.3)		2 (28.6)	4 (25.0)
Serum	IgG	2 (66.7)	2 (100)	3 (100)	4 (66.7)	11 (78.6)
M-protein	IgA	1 (33.3)			2 (33.3)	3 (21.4)
Urine	κ		1 (100)		2 (50.0)	3 (50.0)
M-protein	Λ	1 (100)			2 (50.0)	3 (50.0)
Prior therapy	Radiation therapy	1 (33.3)	1 (33.3)	2 (66.7)	2 (28.6)	6 (37.5)
	ASCT		1 (33.3)	2 (66.7)	4 (57.1)	7 (43.8)
	Bortezomib alone	1 (33.3)	1 (33.3)	1 (33.3)	2 (28.6)	5 (31.3)
	Bortezomib and IMiDs	2 (66.7)	2 (66.7)	2 (66.7)	5 (71.4)	11 (68.8)

ASCT: autologous stem cell transplantation, IMiDs: immunomodulatory drugs

Table 2. Adverse Drug Reactions Following NK012 Administration (more than 10% and at Least of Grade 3 Severity in One Patient).

Adverse drug reactions	Number of patients (n=16)	
	Any grade (%)	≥Grade 3 (%)
Hematologic toxicities		
Neutropenia	15 (93.8)	13 (81.3)
Leukopenia	15 (93.8)	12 (75.0)
Hb decrease	15 (93.8)	4 (25.0)
Lymphopenia	14 (87.5)	8 (50.0)
Red blood cell decrease	10 (62.5)	4 (25.0)
Ht decrease	10 (62.5)	4 (25.0)
Thrombocytopenia	7 (43.8)	2 (12.5)
Non-hematologic toxicities		
Anorexia	10 (62.5)	3 (18.8)
Potassium decrease	4 (25.0)	2 (12.5)
CRP increase	4 (25.0)	1 (6.3)
Anemia	2 (12.5)	2 (12.5)
Sodium decrease	2 (12.5)	1 (6.3)

Hb: hemoglobin, Ht: hematocrit, CRP: C-reactive protein

tion to the third patient. Eventually, four of the seven patients in the in the level 4 group experienced six DLTs (grade 4 neutropenia persisting for at least 8 days, n=3; grade 3 anemia, n=1; grade 3 hyponatremia, n=1; and grade 4 thrombocytopenia requiring blood transfusion, n=1). Thus, the recommended dose for the phase II stage was determined to be 20 mg/m^2 every 4 weeks.

Safety

All 16 patients who were treated with NK012 were included in the safety analysis. Hematologic or non-hematologic adverse drug reactions (ADRs) occurred in more than 10% of the patients; the grade ≥3 ADRs that occurred in at least one patient are shown in Table 2. All patients experienced at least one ADR. The most commonly observed ADRs were hematologic toxicities; neutropenia was the most common grade ≥3 ADR. Grade 4 neutropenia was observed in 1 of the 3 patients (33.3%) in the 20 mg/m^2 group and in 6 of the 7 patients (85.7%) in the 24 mg/m^2 group.

A total of seven serious adverse events (SAEs) occurred in three patients: one patient in the 20 mg/m^2 group and two patients in the 24 mg/m^2 group. A causal relationship with NK012 could not be excluded for six SAEs in three patients, including two cases of decreased appetite and one case each of leukopenia, anemia, pneumonia, and tremor. No patients died during the study period.

The evaluation of QT/QTc prolongation following NK012 treatment showed slight prolongation in comparison to the serum polymer-unbound SN-38 level (data not shown).

Pharmacokinetics

The time profiles of the mean plasma concentration of polymer-bound SN-38 and polymer-unbound SN-38 in patients who received 12-24 mg/m^2 of NK012 are shown in Fig. 1. The pharmacokinetic parameters of polymer-bound SN-38, polymer-unbound SN-38, and SN-38-glucuronide are shown in Table 3. The pharmacokinetic profiles of NK012 in MM patients were similar to those in patients with solid

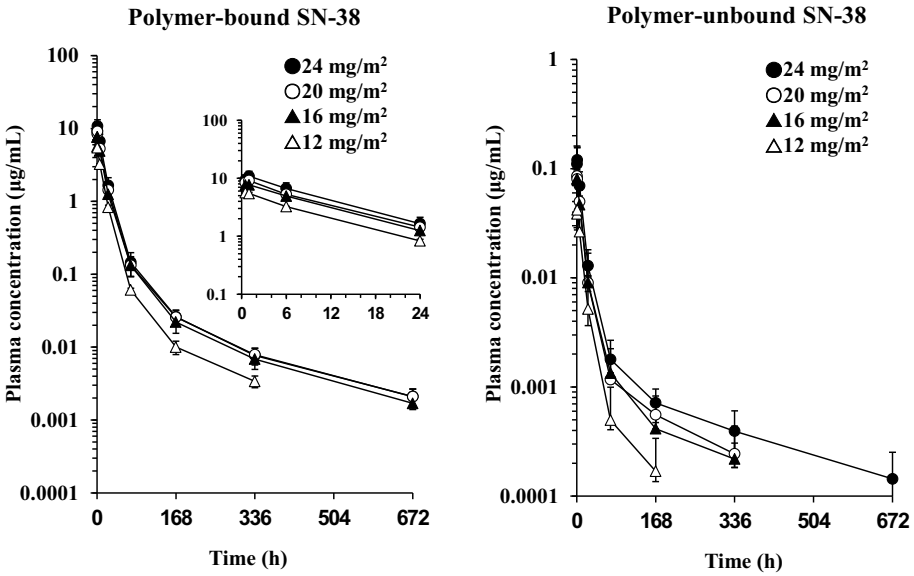

Figure 1. **The plasma concentration-time profiles of polymer-bound (left) and polymer-unbound (right) SN-38 in patients with MM after an intravenous infusion of NK012 at 20 mg/m². Error bars show the standard deviation.**

Table 3. **Pharmacokinetic Parameters of Polymer-bound SN-38, Polymer-unbound SN-38, and SN-38-glucuronide.**

Dose (mg/m²)		Polymer-bound SN-38			Polymer-unbound SN-38			SN-38-G		
		C_{max} (μg/mL)	$T_{1/2z}$ (h)	$AUC_{inf.}$ (μg·h/mL)	C_{max} (μg/mL)	$T_{1/2z}$ (h)	$AUC_{inf.}$ (μg·h/mL)	C_{max} (μg/mL)	$T_{1/2z}$ (h)	$AUC_{inf.}$ (μg·h/mL)
12	Mean	5.69	124	88.6	0.0425	89.9	0.677	0.0281	138	1.04
	SD	0.23	27	1.9	0.0143	47.7	0.095	0.0132	53	0.54
16	Mean	7.82	142	139	0.0829	83.1	1.27	0.0588	127	2.30
	SD	-	-	-	-	-	-	-	-	-
20	Mean	9.15	143	152	0.0859	121	1.35	0.0461	195	2.05
	SD	1.01	9	17	0.0093	23	0.17	0.0254	63	0.97
24	Mean	11.0	148	185	0.137	222	2.01	0.0746	210	3.32
	SD	2.6	10	39	0.045	101	0.43	0.0328	86	1.41

C_{max}: maximum concentration, $T_{1/2z}$: terminal-phase half-life, $AUC_{inf.}$: area under the concentration-time curve from time zero to infinity

tumors (12, 13). The mean maximum concentrations (C_{max}) and area under the concentration-time curves from zero to infinity ($AUC_{inf.}$) of polymer-unbound SN-38, polymer-bound SN-38, and SN-38-glucuronide increased in a dose-dependent manner at doses of 12-24 mg/m².

One patient in each of the 12, 16, 20, and 24 mg/m² cohorts consented to the donation of their bone marrow aspirate. For the patient in the 24 mg/m² cohort, the plasma concentration at 7 days after the start of treatment was excluded from the analysis because it could not be determined due to an insufficient sample volume. There was a dose-dependent increase in the concentrations of polymer-unbound and polymer-bound SN-38 in the bone marrow aspirate (Supplementary material). The concentration of polymer-unbound SN-38 in the bone marrow aspirate was generally higher than that in plasma collected at the same time.

Efficacy

The efficacy of NK012 was evaluated in all 16 patients. According to the IMWG criteria, no patients achieved a PR or better. Twelve of the 16 patients were considered to have stable disease (SD), while the remaining four patients were considered to have progressive disease (PD). Similar results were obtained in the assessment by Bladé criteria: one patient was considered to have a minimal response, and 11 patients were considered to have no change. The maximum reduction in the serum M-protein level was 43.2%, and was observed in the 20 mg/m² group. The maximum reduction in the urine M-protein level was 49.8%, and was observed in the 12 mg/m² group. The maximum numbers of treatment cycles at each dose were as follows: 29 cycles (median: 8) in the 12 mg/m² group, 23 cycles (median: 3) in the 16 mg/m² group, 19 cycles (median: 14) in the 20 mg/m² group. The maximum number of treatment cycles in the 24 mg/m²

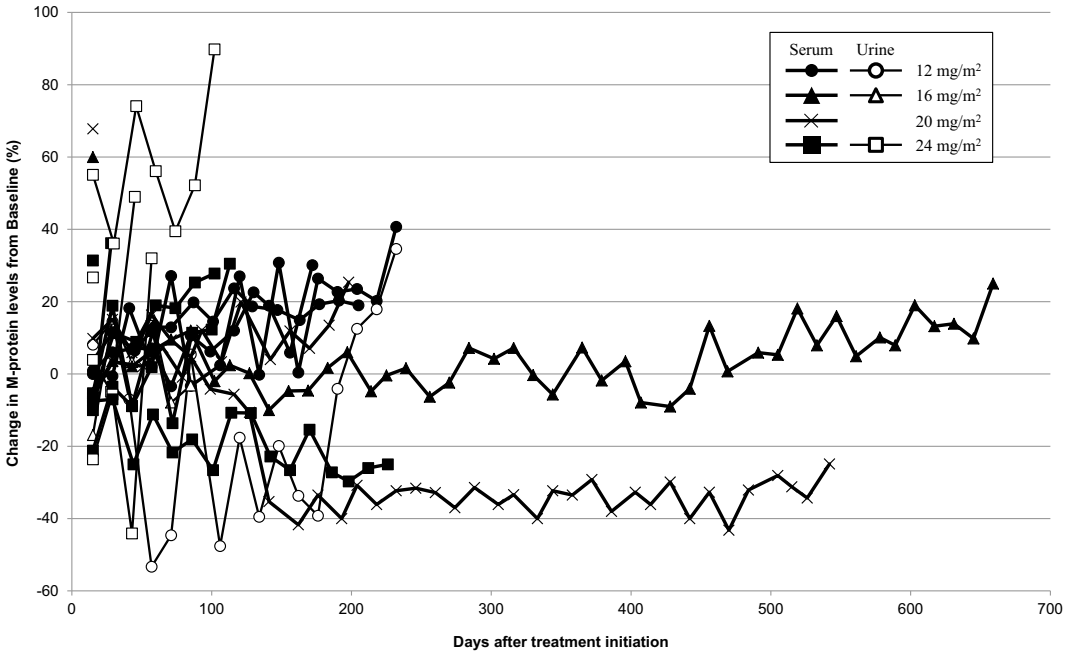

Figure 2. **The time course of the serum and urine M-protein levels until the confirmation of disease progression in 16 individual patients. The serum (closed circle) or urine (open circle) M-protein levels were measured at the baseline and every two weeks, after the administration of the study drug in each cycle.**

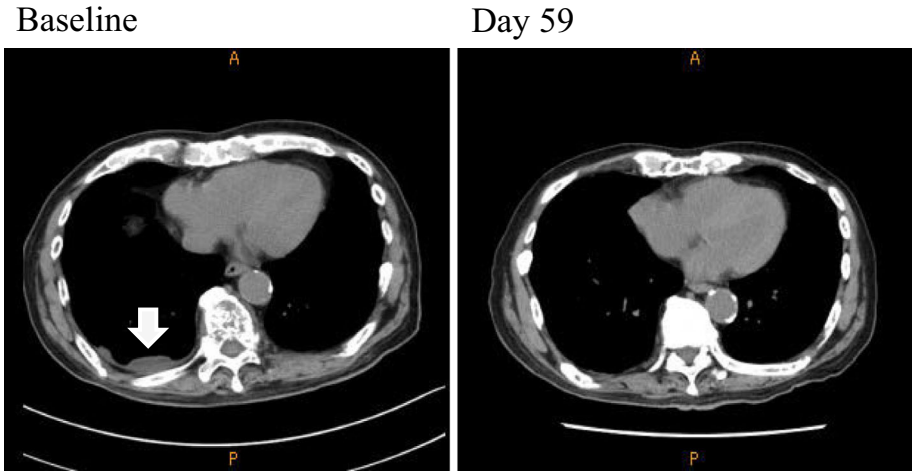

Figure 3. **The disappearance of an extramedullary lesion following treatment with NK012. A 70-year-old man with an extramedullary lesion in the basal part of the right lung (indicated by the arrow in the left CT image) was treated with NK012 at a dose of 24 mg/m^2. The lesion was observed to have disappeared on the CT image obtained 59 days after the initiation of NK012 treatment (right).**

group was 8 (median: 3).

The median PFS (min-max) of all enrolled patients, according to IMWG criteria, was 135 days (14-812 days). One patient each in the 16 mg/m^2 20 mg/m^2 groups displayed no disease progression for more than 1 year. The time course of the serum or urine M-protein levels from the initiation of treatment with the study drug to the confirmation of disease progression in each patient is shown in Fig. 2. The median OS (min-max) of all of the enrolled patients was 443 days (140-843 days).

Three in the 24 mg/m^2 group had extramedullary lesions (soft tissue masses). None of the extramedullary lesions progressed during the study period. In particular, the extramedullary lesion of one patient was found to have completely disappeared by the end of the second cycle (Fig. 3).

Phase II stage

After the completion of the phase I stage, the initiation of the phase II stage was evaluated by the Independent Efficacy/Safety Data Review Committee. The committee did not recommend the initiation of the phase II stage because the results of the phase I stage suggested that it was unlikely

that an objective response would be achieved at the initially expected level. The study sponsor accepted the recommendation, and terminated the study without initiating the phase II stage.

Discussion

This study was conducted to evaluate the safety and tolerability, as well as pharmacokinetics and preliminary efficacy, of NK012 in patients with RRMM. The safety analysis revealed that neutropenia was the most common DLT to occur in association with the administration of NK012. This was similar to observations in previous phase I studies involving patients with solid tumors (12, 13). Six DLTs were observed in four of the seven patients in the 24 mg/m^2 group, but no DLTs were observed in the 20 mg/m^2 group. Thus, it was concluded that the recommended dose for patients with MM should be 20 mg/m^2. The recommended dose determined in this study was lower than that in previous phase I studies involving solid tumors (28 mg/m^2) (12, 13). The suspected reason for this was that the hematopoietic functions in patients with MM differed from those in patients with solid tumors.

The major grade ≥3 drug reactions included myelosuppression [neutropenia (13/16; 81.3%), leukopenia (12/16; 75.0%), and lymphopenia (8/16; 50%)]. However, these toxicities were manageable with adequate supportive care, such as the prophylactic or therapeutic administration of G-CSF and/or antibiotics. The careful monitoring of myelosuppression would be important during NK012 treatment. Among the non-hematological toxicities, the severe diarrhea that was a clinical problem with CPT-11, was not observed. This result was consistent with the results of previous phase I studies involving patients with solid tumors (12, 13).

The plasma concentrations of polymer-bound SN-38 and polymer-unbound SN-38 showed a decline within one week and accumulation was not considered to be significant in the weekly dosing schedule. The plasma concentrations increased in a dose-dependent manner no differences were evident among the 16, 20 and 24 mg/m^2 groups, which reflected the small increment in the dosage.

As NK012 was observed to be highly distributed in the bone marrow of rats (data not shown), the concentration of polymer-bound or unbound SN-38 in bone marrow, one of the target tissues of nanoparticle distribution, was measured for exploratory purposes. The existence of NK012 (polymer-bound SN-38) and its active form (polymer-unbound SN-38) in bone marrow was confirmed (Supplementary material). As the NK012 concentration in bone marrow was not less than the SN-38 concentration at 168 hours after administration, it is possible that the NK012 was distributed to the bone marrow and released SN-38 there. Further investigations are necessary to understand the dynamics of NK012 in plasma and bone marrow.

None of the patients in the present study showed a PR following NK012 treatment. The best response - SD - was achieved in 12 of the 16 patients. These results indicated that the objective tumor response in patients receiving NK012 monotherapy - measured according to the serum or urine M-protein level - was insufficient. With regard to the patients who showed a limited objective tumor response, three patients continued to receive NK012 treatment without progression for more than 1 year; in some cases, long-term disease control was observed at a lower dose. Three patients with extramedullary lesions were enrolled in this study. Interestingly, in two cases, the extramedullary lesions showed no progression during the study period; in the third case, the lesion rapidly disappeared. Varettoni et al. reported that the prevalence of extramedullary involvement had increased in recent years (2000-2007) in comparison to previous reports (1971-1999), and that the overall survival and progression-free survival times in patients with extramedullary disease were shorter in comparison to patients without extramedullary disease (17). An effective treatment strategy for extramedullary disease is desired, and this remarkable response of the extramedullary lesions represents another area in which NK012 may be clinically effective.

In conclusion, the recommended dose of NK012 monotherapy for UGT1A1 *wt/wt and wt/*28* genotype patients with RRMM was concluded to be 20 mg/m^2. This phase I/II study was terminated at the end of the phase I stage because no patients showed an objective response. The further clinical development of NK012 as a monotherapy for RRMM was not recommended because of its low efficacy. In the future, NK012 should be considered for the treatment of RRMM as a combination therapy with other agents, including PIs. This is supported by a mice model of orthotopic MM, in which bortezomib/NK012 prolonged the median survival time in comparison to bortezomib alone (11).

Nippon Kayaku Co., Ltd, the study sponsor, was responsible of the study design and funded the study. NK012 was provided by Nippon Kayaku. Nippon Kayaku collected and analyzed the data and contributed to the interpretation of the results of the study.

Author's disclosure of potential Conflicts of Interest (COI).
Masaki Ri: Honoraria, Celgene; Research funding, Celgene. Shinsuke Iida: Honoraria, Janssen, Celgene, Ono Pharmaceutical, Takeda, Bristol-Myers Squibb and Novartis. Takaaki Chou: Honoraria, Celgene, Ono Pharmaceutical, Bristol-Myers Squibb and Novartis. Noriko Watanabe: Employment, Nippon Kayaku. Tetsuji Tsukamoto: Employment, Nippon Kayaku.

Acknowledgement
We thank the patients who participated in this trial and express our appreciation of the assistance and understanding of their families.

References

1. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer **132**: 1133-1145, 2013.

2. Albarracin F, Fonseca R. Plasma cell leukemia. Blood Rev **25**: 107-112, 2011.

3. Richardson PG, Sonneveld P, Schuster M, Irwin D, Stadtmauer E, Facon T. Extended follow-up of a phase 3 trial in relapsed multiple myeloma: final time-to-event results of the APEX trial. Blood **110**: 3557-3560, 2007.

4. Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med **352**: 2487-2498, 2005.

5. Dimopoulos M, Spencer A, Attal M, Prince HM, Harousseau JL, Dmoszynska A. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med **357**: 2123-2132, 2007.

6. Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med **357**: 2133-2142, 2007.

7. Nooka AK, Kastritis E, Dimopoulos MA, Lonial S. Treatment options for relapsed and refractory multiple myeloma. Blood **125**: 3085-3099, 2015.

8. Yano H, Kayukawa S, Iida S, et al. Overexpression of carboxylesterase-2 results in enhanced efficacy of topoisomerase I inhibitor, irinotecan (CPT-11), for multiple myeloma. Cancer Sci **99**: 2309-2314, 2008.

9. Matsumura Y. Preclinical and clinical studies of NK012, an SN-38-incorporating polymeric micelles, which is designed based on EPR effect. Adv Drug Deliv Rev **63**: 184-192, 2011.

10. Oerlemans C, Bult W, Bos M, Storm G, Nijsen JF, Hennink WE. Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. Pharm Res **27**: 2569-2589, 2010.

11. Miyazaki O, Sekine K, Nakajima N, et al. Antimyeloma activity of NK012, a micelle-forming macromolecular prodrug of SN-38, in an orthotopic model. Int J Cancer **134**: 218-223, 2014.

12. Hamaguchi T, Doi T, Eguchi-Nakajima T, et al. Phase I study of NK012, a novel SN-38-incorporating micellar nanoparticle, in adult patients with solid tumors. Clin Cancer Res **16**: 5058-5066, 2010.

13. Burris HA, Infante JR, Anthony Greco F, et al. A phase I dose escalation study of NK012, an SN-38 incorporating macromolecular polymeric micelle. Cancer Chemother Pharmacol **77**: 1079-1086, 2016.

14. Le Tourneau C, Lee JJ, Siu LL. Dose escalation methods in Phase I cancer clinical trial. J Natl Cancer Inst **101**: 708-720, 2009.

15. Takahashi A, Ohkohchi N, Yasunaga M, et al. Detailed distribution of NK012, an SN-38-incorporating micelle, in the liver and its potent antitumor effects in mice bearing liver metastases. Clin Cancer Res **16**: 4822-4831, 2010.

16. 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 **66**: 10048-10056, 2006.

17. Varettoni M, Corso A, Pica G, Mangiacavalli S, Pascutto C, Lazzarino M. Incidence, presenting features and outcome of extramedullary disease in multiple myeloma: a longitudinal study on 1003 consecutive patients. Ann Oncol **21**: 325-330, 2010.