A Phase I and Pharmacological Study with Imidazolium-*trans*-DMSO-imidazole-tetrachlororuthenate, a Novel Ruthenium Anticancer Agent

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ABSTRACT

Purpose: **NAMI-A {H_2Im[*trans*-$RuCl_4$(DMSO)HIm] or imidazolium-*trans*-DMSO-imidazole-tetrachlororuthenate} is a novel ruthenium-containing compound that has demonstrated antimetastatic activity in preclinical studies. This Phase I study was designed to determine the maximum-tolerated dose (MTD), profile of adverse events, and dose-limiting toxicity of NAMI-A in patients with solid tumors. Furthermore, the ruthenium pharmacokinetics (PK) after NAMI-A administration and preliminary antitumor activity were evaluated.**

Patients and Methods: **Adult patients with solid tumors received NAMI-A as an i.v. infusion over 3 h daily for 5 days every 3 weeks. PK of total and unbound ruthenium was determined during the first and second treatment using noncompartmental pharmacokinetic analysis. The total accumulation of ruthenium in WBCs was also quantified.**

Results: **Twenty-four patients were treated at 12 dose levels (2.4–500 mg/m^2/day). At 400 mg/m^2/day, blisters developed on the hands, fingers, and toes. At 500 mg/m^2/day, blisters persisted from weeks to months and slowly regressed. Although no formal common toxicity criteria (CTC) grade 3 developed, painful blister formation was considered dose limiting. Because the first signs developed at 400 mg/m^2/day, the advised dose for further testing of NAMI-A was determined to be 300 mg/m^2/day on this schedule. PK analysis revealed a linear relationship between dose and area under the concentration-time curve (AUC) of total and unbound ruthenium (R^2 = 0.75 and 0.96, respectively) over the whole dose range. Plasma clearance of total ruthenium was 0.17 ± 0.09 liter/h, and terminal half-life was 50 ± 19 h. The volume of distribution at steady state of total ruthenium was 10.1 ± 2.8 liters. The accumulation of ruthenium in WBC was not directly proportional to the increasing total exposure to ruthenium. One patient with pretreated and progressive nonsmall cell lung cancer had stable disease for 21 weeks.**

Conclusion: **NAMI-A can be administered safely as a 3-h i.v. infusion at a dose of 300 mg/m^2/day for 5 days, every 3 weeks.**

INTRODUCTION

The success of cisplatin [*cis*-diamminedichloroplatinum (II)] as an anticancer agent has stimulated the search for other organometallic cytotoxic compounds with more acceptable toxicity profiles and, if possible, an increase of antitumor activity. Although some inorganic elements have proven to be very useful as drugs to cure or diagnose diseases (1), in general heavy metals and their complexes are well known for their toxic effects. Acute heavy metal poisoning results in severe gastrointestinal symptoms, particularly nausea, vomiting, diarrhea, and abdominal pain. The kidney is frequently affected by heavy metals, because they accumulate in and damage renal tubular cells resulting in disturbance of renal metabolic processes. Chronic low-level exposure to heavy metals can cause neuromuscular injury (2).

In the last three decades, a wide range of ruthenium agents has been synthesized and tested for antitumor properties. Despite their low cytotoxic potential *in vitro*, many ruthenium complexes increase the lifetime expectancy of tumor-bearing hosts (3). Ruthenium, being a group 8 transition metal (the so-called platinum group), was studied to ameliorate cisplatin toxicity and potency. It was expected that ruthenium complexes reduce tumor growth by a mechanism of interaction with cellular DNA, similar to that shown by cisplatin (4). However, a closer examination of ruthenium compounds revealed that these drugs show a number of differences compared with cisplatin. First, ruthenium complexes tend to accumulate preferentially in neoplastic masses in comparison with normal tissue (5, 6). Second, ruthenium complexes probably use transferrin, for its similarities with iron, to accumulate in the tumor. A transferrin-ruthenium complex can be actively transported into tumor tissues that have high transferrin-receptor densities. Once bound to the transferrin receptor, the complex liberates ruthenium that can be easily internalized in the tumor (3, 7). Next, ruthenium(III) complexes likely remain in their relatively inactive ruthenium(III) oxidation state until they reach the tumor site. In this environment, with its lower oxygen content and pH than normal tissue, reduction to the more reactive ruthenium(II) oxidation state takes place. This reaction, named "activation by reduction" would provide not only a selective toxicity but also an efficacy toward hypoxic tumors known to be resistant to

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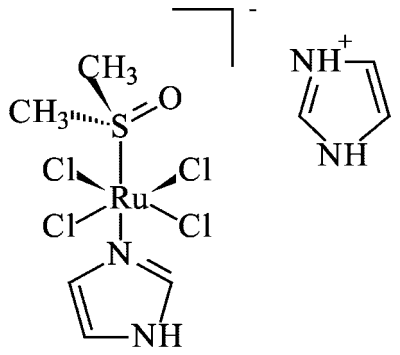

Fig. 1 Chemical structure of NAMI-A. Molecular weight = 458.18 g/mol. Molecular formula, $C_8H_{15}Cl_4N_4ORu(III)S$.

chemotherapy and/or radiotherapy (3, 8). Finally, some complexes are more effective against the tumor metastases than against the primary tumor (9). In view of this, ruthenium compounds may have a pattern of cytotoxicity and antitumor activity that is different from that of cisplatin.

NAMI-A, H_2Im[*trans*-$RuCl_4$(DMSO)HIm] or imidazolium-*trans*-DMSO-imidazole-tetrachlororuthenate (Fig. 1) is a novel and promising ruthenium anticancer agent with selective antitumor activity. The action of NAMI-A seems to be independent of the type of primary tumor and stage of growth of the metastasis (10–12). The precise mechanism of action, however, has yet to be proven, although recent evidence suggests an interaction with cell cycle regulation resulting in a transient accumulation of cells in the G_2-M phase (12–15). Furthermore, NAMI-A increases the capsule thickness around the primary tumor and the extracellular matrix around tumor blood vessels, thereby preventing tumor cells from invading surrounding tissue and blood vessels (14, 16). More recently, NAMI-A was shown to inhibit matrix metalloproteinases and to induce antiangiogenic effects (17). Furthermore, NAMI-A binds covalently to DNA, and it cannot be excluded that its cytotoxicity is associated with a direct effect on DNA.[4]

NAMI-A has characteristics comparable with its parent compound NAMI. NAMI-A is a ruthenium compound that replaces Na^+ with ImH^+ in the molecule of NAMI. More specifically, NAMI and NAMI-A turn out to be capable both of preventing the formation of metastasis and of inhibiting their growth, which makes NAMI-A an ideal candidate for clinical testing. Furthermore, NAMI-A had a better chemical stability than NAMI, and NAMI-A reduced lung metastasis with an effect superior to that of NAMI (9, 16). The antimetastatic activity of NAMI-A is dependent on both dose and treatment schedule and is higher after daily repeated low doses than after administration of high doses combined with long drug-free intervals, as indicated in preclinical studies (18, 19).

Preclinical pharmacological studies with NAMI-A showed selective activity against lung metastases of murine tumors (10, 11, 16, 20) and a relatively low toxicity in mice and dogs (13, 21, 22). Preclinical toxicological studies revealed primarily alterations of kidney, liver, and gastrointestinal function (22).

A Phase I dose-escalating study of NAMI-A was performed in our institute. Each cohort received NAMI-A daily for 5 days, every 3 weeks. The starting dose for this study was established at 2.4 mg/m^2/day (1/30th of the LD10 in mice), which was equivalent to a total dose of 12 mg/m^2. The objectives of this study were as follows: (*a*) to determine the MTD and dose-limiting toxicity (DLT) of NAMI-A when given to patients with advanced malignancies not amenable to other cancer treatment; (*b*) to determine the incidence of toxicity of NAMI-A when given at this schedule; (*c*) to determine the ruthenium pharmacokinetics (PK) of NAMI-A; and (*d*) to determine any responses that may result from treatment with NAMI-A.

PATIENTS AND METHODS

Patient Eligibility Criteria. Patients were eligible if they had a histologically or cytologically confirmed diagnosis of advanced malignancies, for which no conventional therapy exists. Other eligibility criteria included a WHO performance status of 0–2, anticipated life expectancy of at least 3 months, and age ≥18 years. Previous anticancer chemotherapy had to be discontinued for at least 4 weeks before entry into the study or 6 weeks before entry in case of pretreatment with nitrosourea or mitomycin C. Radiation therapy should have ended at least 3 weeks before study entry and any investigational anticancer therapy at least 4 weeks.

All patients had to have acceptable bone marrow function, total bilirubin ≤25 mM, and normal serum creatinine. Aspartate aminotransferase and alanine aminotransferase had to be less than or equal to twice the normal upper limit, but in case of liver metastases these values had to be ≤5 times the normal upper limit. Patients were excluded if they had clinical evidence of hearing loss (≥CTC grade 2), clinical signs of symptomatic brain metastases or carcinomatous leptomeningitis, concomitant acute infection, or when they were unwilling and unable to undergo sampling for PK. Further exclusion criteria were pregnancy, inadequate use of contraception, and prior treatment with ruthenium complexes. The Medical Ethics Committee of the hospital approved the study protocol, and all patients had to give written informed consent.

Treatment Plan and Study Design. NAMI-A was administered daily for 5 days every 3 weeks, until disease progression or until unacceptable toxicity, whichever occurred first. The starting dose was 2.4 mg/m^2/day, which was equivalent to a total dose of 12 mg/m^2 and was given as an i.v. infusion over 3 h. Doses were escalated in decreasing rates and depended on the clinical judgement of the investigators. Dose escalation was scheduled as follows: 2.4; 4.8; 9.6; 19.2; 38.4; 76.8; 115; 172.5; 230; 300; 400, and 500 mg/m^2/day. At nontoxic dose levels, doses were escalated by 100%. When CTC grade 1 and 2 toxicity developed (except alopecia and untreated nausea and vomiting), doses were escalated by 50% steps. When more than grade 2 toxicity developed, dose escalations of 33% maximum were applied. Initially, one patient per dose level was treated. When a dose that produced a grade 1 nonhematological or grade 2 hematological toxicity was reached, three patients per dose

[4] D. Pluim, R. C. van Waardenburg, J. H. Beijnen, *et al.*, manuscript accepted.

level were treated. If a DLT occurred in one of the three patients within one cohort during the first cycle of treatment, then up to three additional patients were treated at that level. If two or more than two of six patients experienced a DLT at a dose level, dose escalation was terminated and up to six patients were included at the previous dose level. Each cohort received NAMI-A until disease progression and/or unacceptable toxicity occurred. The maximum-tolerated dose was defined as the dose below the dose at which two of six patients experienced DLT.

To increase the safety of the administration of NAMI-A and to minimize nephrotoxicity, a pre- and post-hydration schedule was used during the 5 days of NAMI-A administration at the higher dose levels. This consisted of 1000 ml of 0.45% NaCl /2.5% glucose over 4 h before treatment and 2000 ml of 0.45% NaCl /2.5% glucose over 16 h after NAMI-A infusion. During the posthydration, 20 mmol KCl, 500 mg of magnesium sulfate, and 2.6 ml of calcium gluconate were added.

Drug Formulation and Administration. NAMI-A (10 mg/vial) was provided by SIGEA Srl (Trieste, Italy). NAMI-A aseptically prepared, lyophilized products were manufactured in-house (Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, the Netherlands). It consists of a yellow cake that is contained in a 10-ml vial. It was freeze-dried from 5 mM citric acid (Merck, Darmstadt, Germany) and 2.5% mannitol (BUVA BV, Uitgeest, the Netherlands) formulation solution. Reconstitution occurred with 5.0 ml of distilled water (B. Braun Medical, Melsungen, Germany), and 0.9% NaCl (B. Braun Medical) was used for further dilution to infusion concentrations. The reconstituted and diluted products were protected from light and were administered as soon as possible after reconstitution. The infusion fluid was stable for 3.5 h at room temperature when protected from light. NAMI-A was infused i.v. in 3 h dissolved in physiological saline. Pharmaceutical development has been described previously (23).

Patient Evaluation. A complete medical history and physical examination were completed before registration and at the end of the treatment, including documentation of measurable and evaluable disease. Prior tumor therapy had to be recorded. All other medications that patients received were also recorded. Before each cycle, the physical examination was repeated, and hematology and serum chemistry were checked. A urine analysis was performed, and the 24 h creatinine clearance was measured. Hematology and serum chemistry were checked weekly. Tumor evaluations and an audiogram were performed every 2 cycles. Patients were considered evaluable for response if they had measurable disease that meets the RECIST criteria (24), received at least one complete cycle (*i.e.*, 5 days) of drug administration, and had repeat measurements of tumor. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (25). The following toxicities were considered DLT: any grade 3 neutropenia complicated by fever or infection or grade 4 neutropenia lasting longer than 5 days; any nonreversible grade 2 neurological toxicity; any ≥grade 3 renal toxicity despite adequate pre- and post-hydration; and ≥grade 3 nonhematological toxicity (with the exception of alopecia and untreated nausea and vomiting).

Pharmacokinetic Studies. Pharmacokinetic studies were performed in all patients. Pharmacokinetics of total ruthenium in plasma and ultrafiltrable ruthenium in plasma ultrafiltrate were determined during cycle 1 and 2. Ruthenium-DNA adducts and the accumulation of ruthenium in peripheral WBC were also determined during these cycles. During the first NAMI-A course 5-ml blood sample were taken on day 1 at 0, 1, 2, 3, 3.25, 3.5, 4, 5, 7, 10, 13, and 24 h after start of the 3 h infusion. On day 5, blood samples were taken at 0, 1, 3, 3.5, 4, 9, and 24 h after start of the infusion. During the second NAMI-A cycle, 5-ml blood samples were taken on day 1 at 0, 3, 4, 9, and 24 h and on day 5 at 0, 3, 4, and 24 h after start of the infusion. In the last six patients of this clinical trial, additional blood samples were taken before start of the NAMI-A infusion on days 3 and 4, and on days 7 and 14 after start of the first infusion of course 1 as well as of course 2. The extra blood samples were taken to obtain additional information about ruthenium concentrations and accumulations in plasma at late time points. The blood samples were collected in heparinized tubes from the arm contralateral to that receiving the drug infusion. The sample was immediately centrifuged at 4°C for 10 min and approximately 2000 × *g*. The resulting plasma layer was then removed and two 1.0-ml aliquots were transferred to two Amicon Centrifree ultrafiltration devices equipped with 30 kDa cutoff filters. The loaded filter systems were immediately centrifuged at room temperature at approximately 1500 × *g* for 10 min using a fixed angle rotor. Per filter system, this yields approximately 80 μl, thus a total of 160 μl of plasma ultrafiltrate for the analysis of any unbound ruthenium. The plasma ultrafiltrate was immediately stored at −20°C until analysis. The remaining plasma in the heparinized tube was transferred to plastic vials and was also stored at −20°C until analysis of total ruthenium.

During the first and second cycle, all urine was collected up to 24 h after the last NAMI-A administration in portions of 24 h. The urine collected during each time interval was thoroughly mixed, the total volume was recorded, and an aliquot of 10 ml was transferred to a clean polypropylene tube and stored at −20°C until analysis. A validated method using graphite furnace atomic absorption spectrometry was applied to determine the total and ultrafiltrable ruthenium plasma concentrations and urine ruthenium concentrations (26). Total ruthenium concentrations, urine samples, and ruthenium ultrafiltrates were determined in diluted plasma samples (26).

Prior to the infusion and at 4 and 24 h after start of the first and 5th infusion during the 1st and the 2nd cycle, whole blood samples of 15 ml were collected in heparinized tubes for the determination of ruthenium-DNA adducts in WBC and processed as described previously (27, 28). For determination of ruthenium-DNA adduct levels in WBC, the assay as described for determination of cisplatin GG- and AG-intrastrand adducts was applied (27, 28). This assay was optimized in our laboratory to apply it for ruthenium-DNA-adducts. DNA was isolated from these WBC using a high salt extraction method (29). Briefly, after thawing of the frozen WBC sample, ammonium bicarbonate was added up to a concentration of 0.1 M. The cell lysate was digested overnight at 42°C with 0.8% SDS and 0.5 mg/ml proteinase K. After digestion, 3 ml of saturated NaCl (6 M) was added to each tube. Tubes were shaken vigorously and centrifuged at 2500 × *g* for 15 min. The precipitated protein was left at the bottom of the tube, and the DNA containing supernatant was transferred to another tube. Two volumes of absolute eth-

anol at room temperature were added for DNA precipitation. DNA was washed twice with 70% ethanol and subsequently dissolved in Tris-EDTA buffer. DNA was quantitated spectrophotometrically, and a sample of 50 mg was digested, using Nuclease P1, DNase I, and alkaline phosphatase, as described previously (27, 28). Following digestion, adducts were separated from nucleosides by strong cation exchange chromatography, using strong cation-exchange solid-phase extraction cartridges and a vacuum manifold. To the purified adducts, the internal standard thymidinyl-thymidine was added. Next, samples were dried using a Speedvac, and ruthenium was removed from the DNA using 0.2 M NaCN at 65°C for 2 h. After this treatment, products were labeled using polynucleotide kinase and γ-^{32}P-ATP. The labeled products were separated by high-performance liquid chromatography, using a C_{18} (Inertsil ODS-80A; 150 $\times$ 4.6 mm, 5 mm) column, and eluent consisting of 4.5% methanol/0.1 M KH_2PO_4 (pH 4.0). Products were detected using a Canberra Packard 525TR flow scintillation analyser and Cerenkov radiation.

Pharmacokinetic Analysis. Noncompartmental pharmacokinetic parameters were estimated by the computer program WinNonlin (version 3.0; Pharsight Corp., CA). The maximal drug concentration (C_{max}) was derived directly from the experimental data. The area under the concentration-time curve (AUC) from time point 0 to 24 h (AUC_{0-24}; *i.e.*, day 1) after start of the i.v. infusion, NAMI-A was calculated by the linear trapezoidal method, as well as for the AUC from time point 96–120 h (AUC_{96-120}; *i.e.*, day 5). The terminal half-life, total body clearance (Cl) and volume of distribution of steady state (V_{ss}) were estimated by extrapolation of the terminal elimination phase by the last 3–4 data points of the plasma ruthenium concentration-time curves. Pearson correlation coefficients were calculated between kinetic parameters. The PK parameters were reported as mean $\pm$ SD.

The total accumulation of ruthenium in WBC was reported as the concentration of ruthenium in nanomoles (measured by atomic absorption spectrophotometry) per mg protein (measured with the Bradford method) and was quantified in 23 patients.

RESULTS

Patients Characteristics. A total of 24 patients were included in the study. Patient characteristics are presented in Table 1. Median age of the patients was 56 years (range 35–70), and most patients were in good general condition. As anticipated for a Phase I trial, this population of patients had received extensive prior therapy. All patients had had prior systemic chemotherapy, and the patients had various tumor types.

A total of 49 courses of NAMI-A were administered. The number of patients treated at each dose level and the number of cycles administered are summarized in Table 2. One patient did not receive NAMI-A, because of clinical deterioration before the start of the first infusion. One patient refused to continue with NAMI-A after one course at dose level 172.5 mg/m^2/day because he suffered from adverse events, although these did not reach DLT. Drug related toxicities in this patient were CTC grade 2 nausea and CTC grade 2 anorexia.

Adverse Events. All patients who had received NAMI-A were evaluable for toxicity. The main treatment-related hematological adverse events per patient as a function of dose are presented in Table 3. Overall, hematological toxicity was negligible, and none of the hematological adverse events were clearly dose-related. CTC grade 1 anemia occurred on only one patient. CTC grade 2 anemia and lymphocytopenia occurred on only 10 patients (40%), and there were no grade 3 or 4 adverse events.

Table 1 Patient characteristics

	No. of patients	% of patients
Total number	24	
Male/female	14/10	58/42
Median age (range)	56 (35–70)	
WHO performance status		
0	2	8
1	17	71
2	5	21
Previous therapy		
Radiotherapy, systemic therapy and surgery	7	29
Surgery and systemic therapy	8	33
Radiotherapy and systemic therapy	3	13
Systemic therapy	6	25
Tumor type		
Colon	5	
Colorectal	2	
Nonsmall cell lung carcinoma	2	
Melanoma	2	
Ovary	2	
Mesothelioma	2	
Pancreas	2	
Synovia sarcoma	1	
Bileduct carcinoma	1	
Gastric	1	
Broncho Alveolar	1	
Oesophageal	1	
Liposarcoma	1	
Leiomyosarcoma	1	

The main treatment-related nonhematological adverse events per patient as a function of dose are presented in Table 4. Eleven patients (46%) suffered a grade 3 adverse event of some type, and 1 patient (4%) experienced CTC grade 4 diarrhea. Other toxicities encountered were tinnitus, edema, anorexia, alopecia, and dyspnea, which usually occurred at the higher dose levels. The CTC grade never exceeded grade 2.

One patient per dose level was treated with NAMI-A at 2.4, 4.8, 9.6, 19.2, and 38.4 mg/m^2/day. No serious drug-related toxicities were observed at these dose levels. From 76.8 mg/m^2/day, the dose was escalated 50% to 115 mg/m^2/day, because of drug-related CTC grade 1 diarrhea, CTC grade 2 phlebitis, and CTC grade 2 fatigue. The next dose levels were 172.5 mg/m^2/day (+50%) and 230 mg/m^2/day (+33%). At dose level 19.2, 38.4, 76.8, and 172.5 mg/m^2/day, phlebitis at the site of infusion was observed in 4 patients (16%). Therefore, it was decided to administer NAMI-A via a central venous access in the next patients.

One patient (4%) experienced a CTC grade 2 hypersensitivity reaction, and one patient (4%) experienced a CTC grade 3 hypersensitivity reaction at dose level 300 mg/m^2/day during the first course. The symptoms were definitely related to the study

Table 2 Dose escalation

Dose (mg/m^2/day)	2.4	4.8	9.6	19.2	38.4	76.8	115	172.5	230	300	400	500
N	1	1	1	1	1	1	1	3	3	6	3	2
No. of cycles	8	1	1	2	6	2	2	5	6	9	5	2

medication and were observed 30 min after the end of the first NAMI-A administration in the first patient and 20 min before the end of the first NAMI-A administration in the second patient. This adverse event was considered a DLT in the second patient, because he experienced tachycardia, shortness of breath, and a reduced blood pressure. The first patient had similar symptoms but of less severity. Granisetron was already given as premedication because of nausea and vomiting experienced at the lower dose levels. Dexamethasone was added as premedication because of the hypersensitivity reactions observed at 300 mg/m^2/day. The other patients at this dose level had no infusion-related reactions. At 400 mg/m^2/day blisters filled with clear serous type fluid developed on the hands and fingers and toes. At 500 mg/m^2/day, two patients were included. Blisters persisted from weeks to months and slowly regressed. Although no formal CTC grade 3 developed, painful blister formation was considered dose limiting. Because the first signs developed at 400 mg/m^2/day, the advised dose for further testing of NAMI-A was determined to be 300 mg/m^2/day on this schedule.

Other clinical abnormalities per patient as a function of dose are presented in Table 5. CTC grade 2 creatinine increase occurred at dose level 230, 300, 400, and 500 mg/m^2/day and CTC grade 4 bilirubin occurred at dose level 172.5 mg/m^2/day.

Table 3 Occurrence of possibly, probably, or definitely drug-related hematological toxicities at all dose levels per patient

Item	Dose level (mg/m^2)	Grades 1	Grades 2	Total no. of patients (%)
Hemoglobin				
	2.4	0	0	0
	4.8	0	0	0
	9.6	0	0	0
	19.2	0	1 (4%)	1 (4%)
	38.4	0	0	0
	76.8	0	0	0
	115	0	0	0
	172.5	1 (4%)	0	1 (4%)
	230	0	1 (4%)	1 (4%)
	300	0	2 (8%)	2 (8%)
	400	0	1 (4%)	1 (4%)
	500	0	0	0
Lymphocytes				
	2.4	0	0	0
	4.8	0	0	0
	9.6	0	0	0
	19.2	0	1 (4%)	1 (4%)
	38.4	0	0	0
	76.8	0	0	0
	115	0	1 (4%)	1 (4%)
	172.5	0	1 (4%)	1 (4%)
	230	0	0	0
	300	0	2 (8%)	2 (8%)
	400	0	0	0
	500	0	0	0

Pharmacokinetics. Blood samples for the measurement of total and ultrafiltrable ruthenium were obtained from all 24 subjects during the 1st and 2nd cycle of treatment. Complete plasma concentration-time curves were obtained for total and ultrafiltrable ruthenium on day 1 and day 5. Fig. 2 presents plasma concentration-time curves of total and unbound ruthenium in plasma during course 1 in patients receiving 230, 300, and 400 mg/m^2/day. The concentrations of total ruthenium were generally much higher than the concentrations of ultrafiltrable ruthenium at all doses and time points. The lower limit of quantitation was 0.50 μg/ml for total ruthenium in plasma and urine and 0.1 μg/ml for ultrafiltrable ruthenium. As the ultrafiltrable ruthenium concentrations of the dose levels 2.4, 4.8, 9.6, and 19.2 mg/m^2/day were below the lower limit of quantitation, the PK parameters of these dose levels could not be estimated. Mean PK parameters on days 1 and 5 of the first NAMI-A cycle of total and unbound drug at each dose level are shown in Table 6. The total plasma ruthenium concentrations were higher at day 5 compared with day 1. Moreover, AUC_{96-120} (day 5) was approximately 3 times higher than AUC_{0-24} (day 1) for total plasma ruthenium. The values of the AUC_{0-24} in total plasma were 110–290 times higher compared with the values in ultrafiltrable plasma. The values of the AUC_{96-120} were 159–269 times higher for total plasma than for ultrafiltrable plasma. Noncompartmental analysis and regression analysis of the plasma concentration of total and ultrafiltrable ruthenium showed that AUC_{0-24} was proportional to dose. A plot of the total and ultrafiltrable ruthenium plasma AUC_{0-24} *versus* the dose of NAMI-A in mg/m^2 is presented in Fig. 3 and revealed a weak correlation between dose and AUC of total ruthenium ($R^2 = 0.32$). At dose level 300 mg/m^2, one exploiter was observed with an AUC_{0-24} of 4230 μg*h/ml. If this exploiter was deleted form the graph, a more linear relationship appeared with $R^2 = 0.67$. There also was a weak correlation between dose and AUC of ultrafiltrable ruthenium ($R^2 = 0.43$). Noncompartmental analysis and regression analysis of the plasma concentration of total and ultrafiltrable ruthenium showed that AUC_{96-120} was also proportional to dose (data not shown). Mean plasma clearance (Cl) of total ruthenium was 0.17 ± 0.09 liter/h, and mean terminal half-life was 50 ± 19 h. Mean V_{ss} of total ruthenium was 10 ± 2.8 liters. Mean Cl of unbound ruthenium levels in plasma ultrafiltrate samples was 75 ± 24 liter/h, and mean terminal half-life was 6 ± 3 h. Mean V_{ss} of unbound ruthenium levels was 510 ± 440 liters.

Urine was collected over the 5 days of administration during cycle 1 and 2 in 21 of the 24 patients. At dose level 2.4 mg/m^2/day, urine concentrations of ruthenium were below the lower limit of quantitation. The mean total cumulative urinary excretion for all dose groups combined was 16% ± 7%. There was no apparent dose dependency.

The ruthenium concentration has been determined in ascitic fluid in two patients. In one patient, dosed at 9.6 mg/m^2/

Table 4 Occurrence of possibly, probably, or definitely drug-related non-hematological toxicities at all dose levels

Toxicity	Dose level (mg/m²)	Grades 1	2	3	4	Total no. of patients (%)
Gastrointestinal toxicity						
Nausea						
	2.4	1 (4%)	0	0	0	1 (4%)
	4.8	0	1 (4%)	0	0	1 (4%)
	9.6	0	1 (4%)	0	0	1 (4%)
	19.2	1 (4%)	0	0	0	1 (4%)
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	1 (4%)	0	0	0	1 (4%)
	172.5	1 (4%)	1 (4%)	0	0	2 (8%)
	230	2 (8%)	0	1(4%)	0	3 (12%)
	300	2 (8%)	0	1(4%)	0	3 (12%)
	400	1 (4%)	0	0	0	1 (4%)
	500	0	0	0	0	0
Vomiting [a]						
	2.4	1 (4%)	0	0	0	1 (4%)
	4.8	0	0	0	0	0
	9.6	0	1 (4%)	0	0	1 (4%)
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	1 (4%)	0	0	0	1 (4%)
	172.5	1 (4%)	0	0	0	1 (4%)
	230	1 (4%)	0	1(4%)	0	2 (8%)
	300	1 (4%)	1 (4%)	1(4%)	0	3 (12%)
	400	1 (4%)	1 (4%)	0	0	2 (8%)
	500	0	0	0	0	0
Diarrhea						
	2.4	1 (4%)	0	0	0	1 (4%)
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	1 (4%)	0	0	0	1 (4%)
	115	0	0	0	0	0
	172.5	1 (4%)	0	0	0	1 (4%)
	230	2 (8%)	0	0	0	2 (8%)
	300	2 (8%)	0	0	1 (4%)	3 (12%)
	400	0	0	0	0	0
	500	0	0	0	0	0
Main other toxicities						
Fatigue						
	2.4	1 (4%)	0	0	0	1 (4%)
	4.8	0	1 (4%)	0	0	1 (4%)
	9.6	0	0	1(4%)	0	1 (4%)
	19.2	1 (4%)	0	0	0	1 (4%)
	38.4	0	0	0	0	0
	76.8	0	1 (4%)	0	0	1 (4%)
	115	0	0	0	0	0
	172.5	1 (4%)	0	1(4%)	0	2 (8%)
	230	0	1 (4%)	0	0	1 (4%)
	300	1 (4%)	2 (8%)	0	0	3 (12%)
	400	1 (4%)	0	0	0	1 (4%)
	500	0	0	0	0	0
Phlebitis						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	1 (4%)	0	0	1 (4%)
	38.4	1 (4%)	0	0	0	1 (4%)
	76.8	0	1 (4%)	0	0	1 (4%)
	115	0	0	0	0	0
	172.5	0	1 (4%)	0	0	1 (4%)
	230	0	0	0	0	0
	300	0	0	0	0	0
	400	0	0	0	0	0
	500	0	0	0	0	0

Table 4 Continued

Toxicity	Dose level (mg/m²)	Grades 1	2	3	4	Total no. of patients (%)
Fever						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	1 (4%)	0	0	0	1 (4%)
	38.4	1 (4%)	0	0	0	1 (4%)
	76.8	0	0	0	0	0
	115	0	1 (4%)	0	0	1 (4%)
	172.5	0	0	0	0	0
	230	0	2 (8%)	0	0	2 (8%)
	300	1 (4%)	1 (4%)	2 (8%)	0	4 (16%)
	400	0	1 (4%)	0	0	1 (4%)
	500	0	0	0	0	0
Stomatitis						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	0	0	0	0	0
	172.5	1 (4%)	0	0	0	1 (4%)
	230	1 (4%)	0	0	0	1 (4%)
	300	1 (4%)	0	0	0	1 (4%)
	400	1 (4%)	2 (8%)	0	0	3 (12%)
	500	0	2 (8%)	0	0	2 (8%)
Hyper-Sensitivity						
	300	0	1 (4%)	1 (4%)	0	2 (8%)
Thrombosis						
	2.4	0	0	1 (4%)	0	1 (4%)
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	0	1 (4%)	0	0	1 (4%)
	172.5	0	0	0	0	0
	230	0	0	0	0	0
	300	0	1 (4%)	0	0	1 (4%)
	400	0	0	0	0	0
	500	0	0	0	0	0
Blisters						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	0	0	0	0	0
	172.5	0	0	0	0	0
	230	0	0	0	0	0
	300	0	0	0	0	0
	400	0	1 (4%)	0	0	1 (4%)
	500	0	2 (8%)	0	0	2 (8%)

[a] Severe vomiting in patients at dose levels 172.5; 230, 300, and 400 mg/m² was adequately controlled with granisetron

day, ruthenium could not be determined in a sample at 17 days after the first NAMI-A administration. In the second patient, who received 172.5 mg/m^2/day, ruthenium was measured at 7 days after the first NAMI-A administration. The amount in the released ascites fluid was as high as 33% of the administered dose.

DNA Adducts. Ruthenium-GG and ruthenium-AG adduct concentrations were below the quantification level at all dose levels. Therefore, it was decided to analyze the total accumulation of ruthenium in WBC. No relationship was found between the ruthenium concentration (in nmol/mg DNA) and AUC of unbound ruthenium. This indicates that the accumula-

Table 5 Occurrence of other non-hematological laboratory abnormalities at all dose levels per patient

Item	Dose level (mg/m²)	Grades 1	2	3	4	Total no. of patients (%)
Creatinine increase						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	0	0	0	0	0
	172.5	0	0	0	0	0
	230	0	1 (4%)	0	0	1 (4%)
	300	1 (4%)	2 (8%)	0	0	3 (12%)
	400	0	1 (4%)	0	0	1 (4%)
	500	1 (4%)	0	0	1 (4%)	
Bilirubin Increase						
	2.4	0	0	0	0	0
	4.8	0	0	0	0	0
	9.6	0	0	0	0	0
	19.2	0	0	0	0	0
	38.4	0	0	0	0	0
	76.8	0	0	0	0	0
	115	0	0	0	0	0
	172.5	0	0	0	1 (4%)	1 (4%)
	230	0	0	0	0	0
	300	1 (4%)	0	0	0	1 (4%)
	400	0	0	0	0	0
	500	0	0	0	0	0
Hyperkalemia						
	172.5	0	0	0	1 (4%)	1 (4%)

tion of ruthenium in WBC was not directly proportional to the increasing total exposure to ruthenium.

Response. Twenty patients (83%) were evaluable for response. Four patients (17%) were not considered evaluable for response, because they did not receive at least 1 cycle of drug, or because they did not have measurable tumor. One patient (4%) with nonsmall cell lung carcinoma (NSCLC) had stable disease for 21 weeks. The duration of stable disease was measured from the start of the treatment until the criteria for progression were met. Nineteen patients (79%) showed disease progression. There were no documented partial or complete responses.

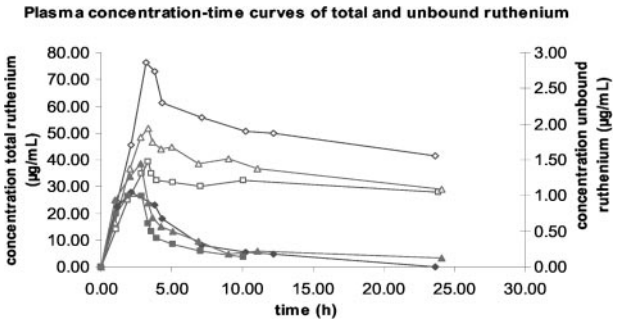

Fig. 2 Plasma concentration-time curves of total (*open symbols*) and unbound ruthenium (*closed symbols*) during course one in patients receiving 230 mg/m^2/day (□), 300 mg/m^2/day (△) and 400 mg/m^2/day (◇).

DISCUSSION

In this clinical study NAMI-A was administered as an i.v. infusion over 3 hours daily for 5 days every 3 weeks in 24 patients with solid tumors. The patients had a median age of 56 years (range 35–70), and they had a variety of primary tumors (Table 1). The patients were treated at 12 dose levels (2.4–500 mg/m^2/day; Table 2). On the basis of the preclinical pharmacological and toxicological findings, 2.4 mg/m^2/day was chosen as the starting level. The daily times 5 schedule every 3 weeks was chosen based on the preclinical activity and toxicity data and on the experience that organometallic complexes may show toxicity correlated with the maximum plasma concentration of the drug.

The drug was in general well tolerated. Main toxicities, possibly or probably related to study medication, were phlebitis, hypersensitivity reactions, and the formation of blisters. Nausea and vomiting were significantly disabling. These gastrointestinal toxicities were observed at almost all dose levels, but with increasing severity at the higher doses (Table 4). Severe vomiting could adequately be controlled with granisetron. Dexamethasone (8 mg i.v.) was added as premedication because of the hypersensitivity reactions observed at the dose of 300 mg/m^2/day. At dose level 230, 300, 400, and 500 mg/m^2/day, mild renal dysfunction (CTC grade 1 and 2) was observed, which was reversible. Renal toxicity was completely reversed at 3 weeks after the end of drug administration and did not result in treatment delays. To increase the safety of the administration of NAMI-A and to minimize nephrotoxicity a pre- and post-hydration schedule was used.

Table 6 Summary of noncompartmental pharmacokinetic parameters for total and ultrafiltrable ruthenium

SDs were not calculated for $n < 3$.

	Total ruthenium concentrations					Unbound ruthenium concentrations				
Dose level (mg/m^2/day)	C_{max} (μg/ml)	$T_{1/2}$[a] (h)	AUC_{0-24} (μg·h/ml)	Cl (liter/h)	V_{ss} (liter)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC_{0-24} (μg·h/ml)	Cl (liter/h)	V_{ss} (liter)
Day 1 course 1										
2.4	0.5	NE	8.8	0.02	8.6	NE	NE	NE	NE	NE
4.8	0.7	59	11.9	0.13	11.2	NE	NE	NE	NE	NE
9.6	2.2	73	35.6	0.08	8.3	NE	NE	NE	NE	NE
19.2	3.4	28	41.7	0.34	13.6	NE	NE	NE	NE	NE
38.4	10.5	56	156	0.12	9.4	0.23	3.0	0.9	55.2	214
76.8	14.0	86	257	0.08	9.9	0.64	3.0	2.1	47.6	164
115	25.5	49	459	0.15	10.8	0.69	2.0	2.2	92.3	230.1
172.5	37.4 ± 6.2	37 ± 18.6	579 ± 92	0.20 ± 0.03	10.1 ± 3.6	0.9 ± 0.2	6.0 ± 3.0	3.2 ± 2.7	71.5 ± 36.1	349 ± 29.3
230	55.8 ± 18.7	60 ± 36.5	895 ± 228	0.14 ± 0.06	10.7 ± 4.1	1.2 ± 0.3	9.0 ± 6.2	6.2 ± 2.1	64.1 ± 29.0	489 ± 164
300	72.3 ± 22.3	49 ± 23.9	1789 ± 1249	0.13 ± 0.06	7.9 ± 1.6	1.3 ± 0.3	8.3 ± 3.7	6.2 ± 3.5	66.8 ± 44.2	436 ± 64.5
400	77.5 ± 22.7	25 ± 6.7	1218 ± 321	0.29 ± 0.05	10.9 ± 3.0	1.1 ± 0.7	9.9 ± 6.3	7.3 ± 4.6	124 ± 80.1	1516 ± 742
500	66.70	32	1093	0.31	14.2	1.79	8.7	10.0	79.0	690
Dose level (mg/m^2/day)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC_{96-120} (μg·h/ml)	Cl (liter/h)	V_{ss} (liter)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC_{96-120} (μg·h/ml)	Cl (liter/h)	V_{ss} (liter)
Day 5 course 1										
2.4	1.7	127	38.4	0.01	2.3	NE	NE	NE	NE	NE
4.8	1.9	89.1	41.2	0.03	3.8	NE	NE	NE	NE	NE
9.6	4.5	65.5	101	0.03	3.2	NE	NE	NE	NE	NE
19.2	10.8	67.5	231	0.04	3.8	NE	NE	NE	NE	NE
38.4	25.3	48.6	502	0.04	3.0	0.2	10.6	2.1	25.5	364.2
76.8	43.8	43.8	946	0.04	2.4	0.4	4.1	3.6	37.2	182.5
115	71.6	54.7	1504	0.05	3.6	0.8	5.8	6.3	41.7	278.4
172.5	96.5 ± 2.1	55.6 ± 25.5	3003 ± 1750	0.04 ± 0.01	3.1 ± 0.7	1.2 ± 0.3	26.9 ± 22.4	16.2 ± 10.5	19.7 ± 13.6	438 ± 67.2
230	150 ± 33.2	184 ± 90.4	3237 ± 640	0.01 ± 0.005	3.4 ± 0.9	1.4 ± 0.2	11.7 ± 4.9	12.0 ± 3.9	31.8 ± 11.4	418 ± 169
300	183 ± 17.3	69.9 ± 27.3	3737 ± 304	0.04 ± 0.01	4.2 ± 0.6	1.9 ± 0.3	15.1 ± 1.2	19.0 ± 3.3	19.5 ± 5.3	391 ± 108
400	229 ± 65.9	30.4 ± 20.3	3829 ± 458	0.10 ± 0.04	3.3 ± 1.1	1.6 ± 0.2	13.2 ± 9.0	17.5 ± 3.5	29.7 ± 8.2	615 ± 119
500	144	41.0	2519	0.13	6.4	1.1	37.4	15.8	46.2	964

[a] $T_{1/2}$, terminal half-life; AUC, area under the concentration-time curve; Cl, mean plasma clearance; V_{ss}, volume of distribution of steady state; NE, not evaluable.

The maximum-tolerated dose, which is the advised dose for future testing, was established at 300 mg/m^2/day on this schedule. The main and dose-limiting toxicity was blister formation especially on the hands and feet. Painful eruptions developed on the fingers and toes. Blisters were poorly reversible and could last for several weeks. This toxicity is unusual for currently applied anticancer agents. The clinical picture was different from the hand-foot syndrome that can be seen with capecitabine. The toxicity was also different from the typical nail and skin toxicity that can be induced by docetaxel. Attempts were undertaken to measure concentrations of ruthenium in the blister fluid but levels were below lower limit of quantitation. At this level, toxicity was mild to moderate and quite different from the classical platinum anticancer drugs. General malaise and mild nausea and vomiting were the main toxicities. Renal dysfunction was rare, as were myelosuppression and tinnitus.

Bioanalytical, pharmacokinetic, and dynamic monitoring were performed in all patients. In blood, the following two different forms of ruthenium were present after drug administration: protein-bound and ultrafiltrable ruthenium. After administration of NAMI-A at doses of 2.4, 4.8, 9.6, and 19.2 mg/m^2/day, ultrafiltrable ruthenium levels were below the quantification level. Administration of NAMI-A at higher doses resulted in ultrafiltrable ruthenium levels varying between 0.2 and 1.8 μg/ml. Total ruthenium (*i.e.*, ultrafiltrable plus protein-bound ruthenium) was measured in plasma. PK of total ruthenium revealed a weak linear relationship between the dose and AUC. AUC_{96-120} (day 5) was approximately 3 times higher than AUC_{0-24} (day 1). This finding indicates that there was accumulation of protein-bound ruthenium in plasma. Total ruthenium concentrations were higher compared with the ultrafiltrable ruthenium concentrations (Fig. 2). Analysis of the ultrafiltrate samples showed that NAMI-A is highly bound to proteins. Moreover, the values of AUC_{0-24} and AUC_{96-120} of total plasma were higher compared with the values of ultrafiltrable ruthenium. Ruthenium showed linear elimination based on the linear relationship between the NAMI-A dose and the AUC and C_{max} of total and unbound drug. The Cl of total ruthenium was very low compared with the Cl of ultrafiltrable ruthenium and shows significant variability. The same was observed for the V_{ss}. The terminal half-life of total ruthenium on day 1 was approximately 50 ± 19 h. On day 5, the terminal half-life was approximately 73 ± 43 h. Also, the AUC measured on day 5 was always three times greater than that of day 1. Thus, the terminal half-life appeared to be shorter after the first dose than after the fifth dose. In comparison, the terminal half-life of ultrafiltrable ruthenium was 6.5 h. In view of the long terminal

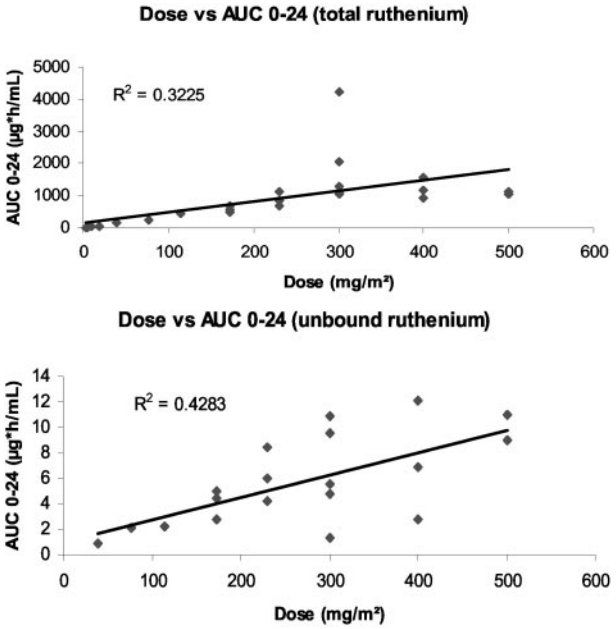

Fig. 3 Area under the concentration-time curve from time point 0 to 24 h (AUC_{0-24}) of total and ultrafiltrable ruthenium plotted against the dose of imidazolium-*trans*-DMSO-imidazole-tetrachlororuthenate (NAMI-A; in mg/m^2).

half-life and daily times 5 administration, no precise estimate could be obtained of the half-life on day 1.

The PK in plasma of NAMI-A following repeated i.v. treatment in mice indicated a terminal half-life of approximately 18 h. In this clinical study, we found a V_{ss} of approximately 10 liters and terminal half-life longer than in mice, which indicates that in humans the total body retention of ruthenium was longer than expected based on the preclinical *in vivo* study (30).

The mean total cumulative urinary excretion for all dose groups combined was 16% $\pm$ 7% measured up to 120 h after the first infusion. Because of the long retention of NAMI-A, a significant portion of the ruthenium dose had not been excreted at the end of the urine collection time.

At dose level 230, 300, 400, and 500 mg/m^2/day, CTC grade 1 and 2 reversible creatinine increase was observed, which indicates that the circulating ruthenium is toxicologically active. The main toxicity of NAMI-A in preclinical studies was renal toxicity (12, 30), which seemed at least in part reversible (12, 30). However, renal toxicity was not dose limiting. It is of interest to evaluate whether a single weekly dose shows a more favorable toxicity profile than that of the 5-day treatment used.

Intrastrand ruthenium-GG and -AG adducts could not be determined in DNA of WBCs, because the levels were below the quantification level. Therefore, the total accumulation of ruthenium in WBC was analyzed. Results indicate that no linear relationship existed between the ruthenium concentration and the AUC of ultrafiltrable ruthenium. Binding of ruthenium complexes to DNA has been proven *in vitro* using calf thymus DNA and human tumor cell lines (31–34).[4] At equimolar exposure of calf thymus DNA the number of ruthenium-DNA complexes from NAMI-A is the same as that of platinum-DNA complexes from cisplatin.[4] It is of interest that NAMI-A binds in a unique way to DNA as the number of ruthenium-GG and -AG adducts is much lower than that of platinum-GG and -AG adducts at equimolar concentrations. In cell lines, the number of DNA complexes of NAMI-A is far lower than that of cisplatin.[4] These results indicate that ruthenium-metal complexes represent a novel class of metal-based antitumor compounds acting by a mechanism different from classical GG and AG DNA intrastrand cross-linking (31, 32, 33, 34).[4]

Disease stabilization was observed in heavily pretreated patients with advanced NSCLC. One patient (4%) with NSCLC had stable disease for 21 weeks. Partial or complete responses were not observed and 19 patients (79%) showed disease progression. Because of extensive pretreatment, no tumor responses could be expected.

In conclusion, the recommended dose for NAMI-A when given as a daily i.v. infusion for 5 days every 3 weeks is 300 mg/m^2/day. Slowly reversible blister formation was considered DLT. To test the anticancer activity of NAMI-A, a Phase II study is advised as the next step in the clinical development. It is of interest to explore different schedules of NAMI-A, especially the weekly i.v. administration. This may result in a better therapeutic index. Furthermore, the long disease stabilization in NSCLC, clearly progressive prior to the start of NAMI-A, may have relevant clinical meaning. At the advised dose, it is of interest to explore activity of NAMI-A in selected solid tumors including NSCLC.

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