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A Phase I clinical trial of spicamycin derivative KRN5500 (NSC 650426) using a Phase I accelerated titration "2B" design

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Summary

The spicamycin derivative KRN5500 was considered as a potential anti-cancer agent based on *in vitro* and preclinical studies. A Phase I study involving 24 cancer patients in whom tumors were refractory to all other conventional therapies was conducted to determine the dose limiting toxicity, maximum tolerated dose, effectiveness, and pharmacokinetic parameters of this drug administered by 1-h IV infusion daily for five consecutive days every 3 weeks. Using an accelerated dose titration strategy, 8.4 mg/m^2/d $\times$5 days was the maximum administered dose. Severe gastrointestinal and hepatic toxicities were observed at doses at or above 4.3 mg/m^2/d $\times$5. The recommended Phase II dose is 4.3 mg/m^2/d $\times$5. The distribution of KRN5500 followed a two-compartment model, and clearance did not decrease significantly over the dose range 0.8–8.4 mg/m^2/d $\times$5. No significant correlation was observed between plasma levels and toxicity. No tumor responses were observed among the 14 patients evaluable for response.

Introduction

Spicamycin, a nucleoside antibiotic derived from *streptomyces alanosinicus*, generated interest as a cancer drug due to the potent differentiation it induced in HL-60 and M1 myeloid cells [1,2]. A series of spicamycin derivatives was synthesized and screened for antitumor activity, and among these, KRN5500 (NSC 650426) demonstrated the greatest activity and the highest therapeutic index in tumor models. KRN5500 has a unique structure with an unusually long acyl tail (Figure 1). Preclinical studies have suggested that the long acyl tail is important in the uptake and function of the agent [3–5].

KRN5500 has shown good activity in *in vitro* studies against gastric cancer, colon cancer, lung cancer, and leukemia cell lines [6–9]. In human tumor xenograft models of gastric cancer, colon cancer and esophageal cancer, KRN5500 had response rates comparable or superior to mitomycin-C [10]. In SCID mice, KRN5500 was effective against experimental hepatic metastasis from human colon cancer COL-1 strain [3]. In addition, the agent showed antitumor activity in cancer cell strains resistant to various antitumor agents in both *in vitro* and *in vivo* models [10,11]. This activity is possibly due to inhibition of protein synthesis and/or protein glycosylation [10,11].

Effect of administration schedule was evaluated by using a human colon cancer xenograft model [9]. Repeated dose treatment regimens (daily treatment for 5 days or intermittent treatment) were more effective than single dose treatment regimens.

The unique chemical structure of KRN5500 and its encouraging preclinical antitumor activity, including in tumor models resistant to various chemotherapeutic drugs, provided the rationale for clinical development. We conducted a Phase I trial with an administration schedule of day 1–5 every 21 days to evaluate the standard objectives of maximum

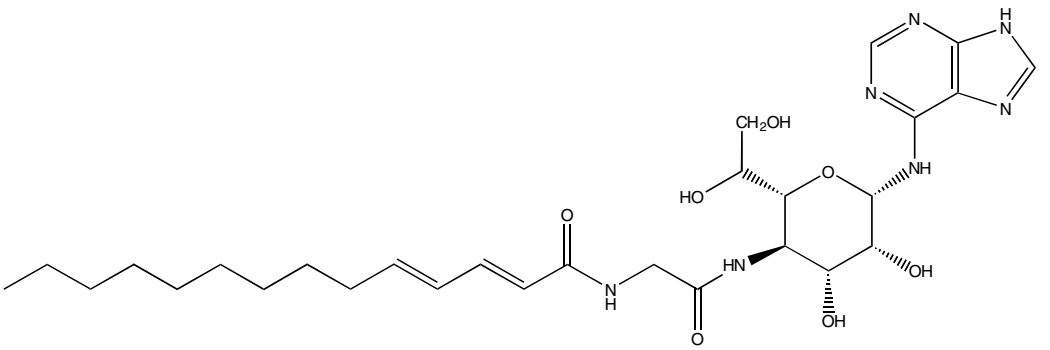

Figure 1. Chemical structure of KRN5500.

tolerated dose (MTD) and toxicity, and to characterize the drug pharmacokinetics.

Methods

Patients

Patients with histologically confirmed solid tumors refractory to conventional therapy or for which no effective therapy existed were eligible for this study. Eligibility criteria included: age >15 years; Eastern Cooperative Oncology Group (ECOG) performance status of ≤2; a projected survival time of at least 12 weeks; no chemotherapy or radiation therapy for 4 weeks prior to initiation of therapy (6 weeks for mitomycin C or nitrosourea); recovery from any adverse effects of prior therapy; adequate bone marrow function (white cell count $\geq 4000/mm^3$, platelets $>100{,}000/mm^3$); adequate hepatic function (total bilirubin ≤1.5 mg%, SGOT and SGPT ≤2 times the upper limit of normal); adequate renal function (creatinine ≤1.5 mg% and 24 h creatinine clearance ≥60 ml/min). Patients were excluded for history of myocardial infarction within 6 months of therapy, congestive heart failure > NYHC II, clinically significant arrhythmias, HIV positive status, pregnancy or lactation. All patients provided a signed informed consent as per the institutional guidelines and the IRB approved protocol.

Pretreatment evaluation

Before entry, a complete history and physical examination with documentation of the height and weight was required. Pretreatment laboratory studies included complete blood count with differential and platelet count, prothrombin time, partial thromboplastin time, thrombin time, fibrinogen, protein C, protein S, antithrombin III, bilirubin, alkaline phosphatase, electrolytes, creatinine, SGPT, SGOT, urinalysis, and 24 h urine creatinine clearance. Chest X-ray, EKG, and an HIV test were also required prior to registration. Patients were required to have all pretreatment laboratory studies within 8 days and radiology studies utilized for tumor measurements within 28 days of registration.

Drug preparation and administration

KRN5500 was manufactured by Ben Venue laboratories and was supplied by DCTDC/NCI. The drug was supplied in two kits: Kit A and Kit B. Kit A was one vial of 1.5 ml which consisted of KRN5500 – 5.0 mg, *N*,*N*-dimethylacetamide – 0.05 mg, propylene glycol – 0.4 mg, polysorbate 80–0.3 mg and ethanol – 0.6 mg. Kit B consisted of one ampule of 1.0 ml containing monoethanolamine – 0.10 mg, and water for injection – 0.9 mg. The contents of Kit B were mixed with Kit A, giving a solution containing 2 mg/ml of KRN5500 at a pH of 11 which is stable for at least 8 h at room temperature. This solution was further diluted with 0.9% normal saline for injection to provide a final drug concentration in the range of 4–50 mcg/ml for intravenous infusion over 1 h. This solution is compatible with PVC intravenous infusion bags, and is stable at room temperature for at least 4 h.

Study design

This Phase I study was planned and conducted according to the accelerated titration 2B design

described by Simon et al. [12]. Toxicity was graded using the NCI's Common Toxicity Criteria (CTC). The starting dose of KRN5500 was 0.8 mg/m^2/day, days 1–5, repeated every 21 days, infused over 1 h through a central venous catheter. Initial cohorts contained only one patient until the first instance of first course dose limiting toxicity (DLT) or a second instance of first course CTC grade 2 toxicity, except for nausea, vomiting, fatigue, anorexia, anemia, alopecia, fever, local reactions, and alkaline phosphatase. Subsequent cohorts were expanded to three patients. DLT was defined as grade 4 neutropenia lasting $\geq$3 days, grade 4 thrombocytopenia, or $\geq$grade 3 nonhematologic toxicity except for untreated nausea, vomiting, fatigue, anorexia, anemia, alopecia, fever, local reactions, and alkaline phosphatase.

Dose levels were increased by a fixed 40% increment between cohorts. Patients were escalated to the next higher dose level if, in the prior course, the patient did not experience any toxicity worse than CTC grade 1, with the exception of above-mentioned toxicities. Otherwise, intra-patient escalation did not occur. If one patient experienced DLT in the first course, intra-patient dose escalation and single patient cohorts were terminated. No patient was escalated to a dose level at which two patients had experienced DLT. Patients were to be de-escalated to the next lower dose level if they experienced DLT with current course or if two previous patients experienced DLT at the dose level at which the patient was being treated. Six patients were to be studied at a dose level below the dose level that resulted in ≥ 2 patients with DLTs in the first course. If $\leq$1 patient developed DLT at this dose level, it was designated the MTD. The dose at which two patients developed first course DLT was designated the maximum administered dose (MAD).

Response assessment and continuation of therapy

Patients were required to receive at least two courses to be considered evaluable for response assessment. Complete response was defined as the complete disappearance of all lesions and normalization of any elevated markers with no new lesions for at least 4 weeks. Partial response required a decrease of $\geq$50% in the sum of the products of the perpendicular diameters of all measured lesions and no new lesions for at least 4 weeks. Progression of disease was defined as an increase of $\geq$25% in the sum of the products of the perpendicular diameters of all measured lesions, or appearance of new lesions. Patients with stable disease, partial or complete remission while being treated continued receiving the drug. Prior to re-treatment, blood counts must have recovered to white blood cell count $\geq$4000 mm^3 and platelets $\geq$100,000 mm^3, and chemistry parameters reflecting liver and renal function must have returned to normal or pretreatment values.

Pharmacokinetic and pharmacodynamic studies

On day 1 and 5 of a cycle, a 12 cc blood sample was collected into a heparin-sodium vacutube at predose, 30 min into the infusion, and 0 min (end of infusion), 5, 15, 30, 60, 90, 120, 240, 480, 720, and 1440 min post-infusion. Additionally, pre- and postdose blood samples were collected on days 2, 3, and 4 of each cycle. Plasma was isolated by centrifugation and stored at –20 °C until assay for KRN5500. A 24-h urine sample was collected from eight patients on day 1 of first cycle and aliquots frozen at –20 °C until assay.

KRN5500 assay. KRN5500 in plasma and urine samples was measured using a modified reverse phase HPLC method with Spicamycin-65 (SPK-65) as internal standard following solid phase extraction [15]. To 1 ml plasma, 2 ml of chilled methanol was added and vortexed for 10 s. The sample was kept on ice for 5 min and then centrifuged for 5 min. Clear supernatant was transferred into 4 ml of 50 mM sodium phosphate buffer (pH = 6) and mixed well. A 1 cc Waters μ Bondapak C18 SPE column was treated with 2 ml methanol followed by 2 ml sodium phosphate buffer (pH = 6). The diluted supernatant from the protein precipitated plasma sample was then loaded onto the SPE column. The column was washed with 2 ml deionized water, followed by 3 ml of diethyl ether. KRN5500 was eluted using 1 ml methanol. To this methanolic extract in a Sarstedt microfuge tube, 50 μl of SPK-65 (at 4 μg/ml in methanol) was added, mixed, and evaporated to dryness in the Turbovap at 40 °C under 10 psi of N_2 for 20 min. The sample was reconstituted in 125 μl of 90% methanol, vortexed for 10 s and then centrifuged for 5 min. Clear supernatant was transferred to a low volume insert in a HPLC vial and 100 μl were injected for HPLC.

HPLC conditions. KRN5500 plasma and urine concentrations were analyzed using a YMC ODS-A, C18, 4.6 $\times$ 150 mm, 5 μ column with a Waters μ Bondapak C18 GuardPak precolumn. The mobile phase consisted of methanol : water (74 : 26) that was filtered and degassed prior to use. Analysis of a 100 μl

injected sample was performed at 1.0 ml/min for 30 min, and KRN5500 and internal standard were detected at 264 nm.

The retention times of KRN5500 and SPK-65 (internal standard) were 16.8 and 22.5 min, respectively. The recovery of the drug by SPE extraction was almost complete and the method was highly reproducible with an intra/inter day variation of less than 6%. The minimum detectable amount was 1 ng and the limit of quantification was 10 ng/ml. A set of standards consisting of 10, 50, 100, 500, and 1000 ng/ml KRN5500 spiked into plasma (expired blood bank plasma) were run on each day of analysis, and the method was linear ($r = 0.99+$) in the concentration range of 10–1000 ng/ml. The concentration of KRN5500 in the patient samples was determined from the linear relationship between the peak area ratio of KRN5500 to internal standard and the concentration.

Reference standards of both KRN5500 and SPK-65 (lot # SK 020) from Kirin Brewery Company Ltd. were provided by Dr Thomas Corbett at Wayne State University. A stock standard of 200 μg/ml of KRN5500 was prepared in 5% dimethylacetamide in methanol and stored at 4 °C. Run standards of 400 ng/ml were prepared from stock by diluting with methanol.

Data analysis

Different PK parameters of KRN5500 were computed using a two-compartment model and WinNonlin software (Pharsight Inc.). Plasma PK parameters were examined as possible determinants of drug toxicity. Nonparametric statistical methods were used for analysis of the drug clearance data. The relationship of KRN5500 dose level to drug clearance in cycle 1 was assessed using the Jonckheere-Terpstra k-sample rank sum test for ordered alternatives [13]. That test is more sensitive (i.e., powerful) for specific patterns of association, e.g., trends, than a general test for any departure from equality. For this test, a one-sided significance level was used, since there was a directional hypothesis regarding the drug safety issue of decreasing clearance with rising dose. The difference in drug clearance levels from day 1 to day 5 focused on a clearance difference calculated as day 1 clearance minus day 5 clearance. The mean clearance difference was tested versus zero with the Wilcoxon signed-rank test for paired data [13]. This paired comparison used a two-sided significance level. Since the sample sizes were small, both analyses were conducted using exact inference methods based on permutation testing [14].

Results

Patient characteristics are shown in Table 1. Twenty-four patients were registered for the daily $\times 5$ regimen from February 1997 to March 1998. Twenty-three patients had received prior chemotherapy, nine of whom had received more than two regimens prior to enrollment. The majority of patients ($n = 8$) had colo-rectal cancer.

Two patients started the first course but did not complete it due to detection of new brain metastasis (after one dose) in one patient and sepsis (after one dose) unrelated to the drug in another patient. These two patients participated in the pharmacokinetic study on day 1 but not day 5. One patient decided not to receive any treatment after registering for the protocol. These three patients were not evaluable for the determination of maximum tolerated dose and were replaced by subsequent patients at the same dose level.

Three patients received only the first three dosages of the planned five dosages during the first cycle. One patient at the 4.3 mg/m^2 dose level required epidural catheter placement due to worsening tumor pain. One patient at the 8.4 mg/m^2 dose

Table 1. Patient demographics

Number	24
Tumor types	
Colo-rectal	8
Lung cancer	3
Renal	3
Melanoma	3
Others	7
Age	
Range (median)	32–74 (60.5) years
Gender	
Males : females	15 : 9
Performance status (ECOG)	
Range (median)	0–2 (1)
0.1	23
2	1
Prior chemotherapy regimens	
0–2	15
>2	9
Prior radiation therapy	14

level developed dehydration and elevation of creatinine that corrected promptly on hydration with intravenous fluids. This patient went on to receive all five dosages of the second cycle at the same dose level without any evidence of renal dysfunction. The third patient, who received only three dosages at the 6.0 mg/m^2 dose level, developed grade 3 emesis associated with mild elevation of creatinine and, therefore, did not receive the last two planned days of treatment. Therefore, these three patients participated in the pharmacokinetic study on day 1 but not day 5. Pharmacokinetic samples from two other patients were not evaluable on day 5 of cycle 1 because of hemolysis.

In summary, twenty-three patients were treated with KRN5500. Pharmacokinetics were evaluable on cycle 1, day 1 in all 23 patients and on cycle 1, day 1, and day 5 in 16 patients.

Toxicity and response

The major toxicity profile of KRN5500 administered daily for 5 days every 3 weeks is presented in Table 2. Other toxicities, that did not appear dose-dependent and were grade 1 in severity, were weight loss, cirrhosis, weakness, and decreased appetite. Deep venous thrombosis also occurred in one patient. It was not felt to be drug-related. A total of 37 courses were administered amongst twenty-three treated patients. Significant myelotoxicity was not observed. Gastrointestinal toxicities such as nausea, emesis, diarrhea, and hepatic toxicity were the most common adverse effects. The onset of hepatic toxicity, either in the form of elevation of hepatic enzymes or bilirubin, occurred within a week of starting KRN5500 and the toxicity resolved after 7–10 days.

DLT occurred during cycle 1 in two of six patients at the MAD of 8.4 mg/m^2/d $\times$5 and two of three patients at 6.0 mg/m^2/d $\times$5. One of these patients developed grade 3 hyperbilirubinemia with elevation of liver enzymes which resolved in 8 days. A second patient died on the sixth day of the first course. This patient had a history of metastatic rectal carcinoma involving the chest wall with rib involvement and had a pretreatment performance status of two. The patient did have elevated alkaline phosphatase (749, normal = 189) prior to treatment but had normal bilirubin and AST/ALT and was not known to have hepatic metastasis. On day 5 of the first course, the patient complained of extreme fatigue and was found to have elevated AST (169) and alkaline phosphatase (1444) with a normal bilirubin. Early next morning, the patient was found unresponsive at home and was pronounced dead after being taken to the emergency room. Autopsy studies revealed pulmonary congestion, subacute pancreatitis, and evidence of acute and chronic pericholangitis. A third patient, with evidence of hepatic metastases, developed severe hepatic toxicity after the second course at 8.4 mg/m^2/d.

During the initial escalation phase, pt #11, the first patient treated at the 6.0 mg/m^2/d $\times$5 dose experienced maximum grade 1 toxicity. As a result, escalation proceeded to 8.4 mg/m^2/d, daily $\times$5. At this dose, two of six patients experienced $\geq$ grade 3 non-hematologic toxicity. When expanding the

Table 2. Toxicities by KRN5500 dose level

Dose (mg/m^2)	#Patients[a] in cohort	#Cycles	Grade of toxicity experienced															
			Nausea/emesis			Diarrhea			Liver				Fatigue			Other[b]		
			1	2	3	1	2	3	2	3	4	5	1	2	3	1	2	3
0.8	1	1											1				1	
1.12	2	3	1			1								1				
1.56	1	1		1			1											
2.18	4	4	1	3		1	1	1					2			1		
3.00	3	3	1	1									2			1		
4.30	6	9	3	1		1	1	1	1		1		4	1	1		1	
6.00	5	5	1	1	2	2					1		2			2		1
8.40	6	11	2	4		4			1	2		1	3	1		2	1	1

[a]The number of patients per dose sums to 28 (more than the 23 treated patients) due to intra-patient dose-escalation in 5 patients.
[b]Other toxicities included fatigue, weight loss, rash, loss of appetite, and instance of increased creatinine and DVT.

6.0 mg/m^2/d $\times$5 dose level, as a result of de-escalation from 8.4 mg/m^2/d, the next two patients developed $\geq$ grade 3 non-hematologic toxicity. Additional de-escalation to 4.3 mg/m^2/d $\times$5 occurred. This dose was expanded to include a total of six patients. While on study, maximum toxicity at this dose (4.3) during first cycle was grade 2 in five of six patients. At the 4.3 mg/m^2/d dose level, only one patient experienced DLT. The patient's bilirubin at baseline was 0.6 mg/dl and increased to 4.0 mg/dl day 11 post treatment with resolution by day 25. The SGOT and SGPT also increased with bilirubin (maximum grade 2), with resolution to normal within 5 days.

As a result of one of six patients experiencing DLT, this dose (4.3 mg/m^2/d) was identified as the MTD and recommended Phase II starting dose of KRN5500 via a 1-h infusion with daily $\times$5 administration. Of the nine courses administered at this dose, there was one instance of grade 4 hepatic toxicity, diarrhea, and confusion. The MTD for KRN5500 was 4.3 mg/m^2/d administered for five consecutive days every 3 weeks.

Only one patient received more than two cycles of KRN5500. This patient developed elevation of pancreatic enzymes associated with nausea and emesis after the third day of the fourth cycle. Elevation of pancreatic enzymes was not observed in any other patient.

None of the fourteen patients who received two cycles and were evaluable for tumor response demonstrated any evidence of response with KRN5500.

Pharmacokinetics

The time course of KRN5500 concentration in plasma was determined at every dose level on days 1 and 5 (Figure 2A and B). Across all dose levels and in every

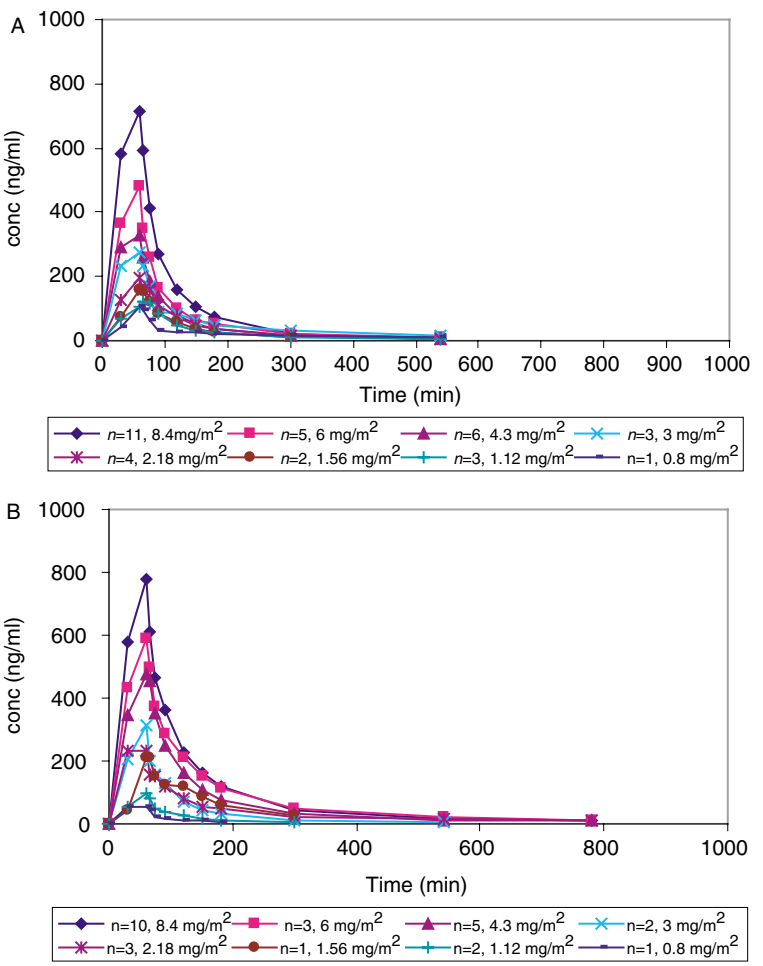

Figure 2. Mean plasma concentration-time profiles of KRN5500 as a function of dose after (A) Day 1 and (B) Day 5 treatment.

patient but one, KRN5500 disposition exhibited a bi-exponential decay, indicating that the drug distributes according to a two compartmental model. Less than 1% of the administered dose was recovered in a 24-h urine collection (data not shown). The pharmacokinetic parameters of KRN5500 on days 1 and 5 are given in Table 3. A linear relationship was obtained between dose and average Cmax or AUC (Figures 3 and 4). Dose did not affect half-life (Figure 4A), although there appeared to be a dose effect on clearance (Figure 4B). Mean Vss ranged from 5.56 to 20.1 L/m^2 and dose administered appeared to have no effect on it. Hence, minor changes in volumes of the drug solution infused would not have changed the volume of distribution or other PK data of KRN5500.

For the 23 patients who received KRN5500 on day 1 of cycle 1, there was a statistically significant trend toward increasing drug clearance with increasing dose level ($p = 0.0076$ via the exact Jonckheere-Terpstra test, one-sided) (Figure 5). Among the 16 patients with evaluable pharmacokinetics on both days 1 and 5 of cycle 1, sample size was sufficiently large to compare days 1 and 5 clearance within a dose level only at 4.3 and 8.4 $mg/m^2/d$ $\times 5$. At the 8.4 $mg/m^2/d$ dose level ($n = 4$ patients), day 1 clearance is not significantly different from day 5 clearance because the mean clearance difference ($20.15\ ml/min * m^2$) is not significantly different from zero ($p = 0.63$ via the exact Wilcoxon signed-rank test, two-sided). Again, at the 4.3 $mg/m^2/d$ dose level ($N = 4$ patients), the mean clearance difference

Table 3. Mean ± SD pharmacokinetic parameters of KRN 5500, all cycles combined

Dose mg/m^2	Day	*n*		C_{max} (ng/ml)	$T_{1/2}$ alpha (min)	$T_{1/2}$ beta (h)	AUC (ng · min/ml)	Clearance ($L/h \cdot m^2$)	MRT (h)	Vss (L/m^2)
0.8	1	1		93.48	19.98	6.81	18,647	2.59	7.74	20.14
	5	1		60.84	6.99	1.55	5434	8.90	1.23	10.89
1.12	1	3	Mean	124.57	48.99	1.92	12,827	5.69	1.55	8.64
			SD	36.75	39.55	0.73	4722	1.95	0.74	5.44
	5	2	Mean	89.60	7.24	1.52	8789	7.64	1.47	11.05
			SD	3.40	7.79	1.13	449	0.40	0.73	4.99
1.56	1	2	Mean	152.01	15.91	1.50	15,057	6.22	1.38	8.60
			SD	4.62	8.89	1.26	339	0.13	0.50	3.28
	5	1		180.81	4.88	1.19	26,139	3.60	1.79	6.42
2.18	1	4	Mean	196.39	10.98	0.96	21,021	6.26	1.27	7.78
			SD	35.62	7.70	0.39	1881	0.60	0.42	2.12
	5	3	Mean	262.22	2.53	1.29	28,859	5.17	1.41	6.26
			SD	60.17	2.39	0.54	13,659	2.09	0.73	1.10
3	1	3	Mean	291.93	9.39	3.14	39,068	4.63	3.38	12.97
			SD	85.08	7.42	2.06	19,061	1.64	2.40	4.14
	5	2	Mean	299.72	1.58	0.68	25,093	7.25	0.77	5.56
			SD	42.07	0.87	0.05	4402	1.33	0.07	0.52
4.3	1	7	Mean	424.29	7.94	1.09	35,145	8.60	0.94	7.94
			SD	232.15	6.61	0.33	16,889	3.64	0.18	3.66
	5	5	Mean	498.80	19.07	1.47	51,056	5.50	1.41	7.53
			SD	182.85	9.55	0.59	18,563	2.27	0.35	2.49
6	1	5	Mean	478.83	7.65	1.26	42,542	9.68	1.17	11.72
			SD	206.80	4.98	0.69	21,144	3.48	0.72	10.60
	5	3	Mean	599.58	5.96	1.18	67,019	6.15	1.43	7.10
			SD	103.96	3.04	0.71	29,711	3.15	0.87	1.18
8.4	1	11	Mean	753.18	9.72	1.09	64,045	8.20	0.95	7.64
			SD	142.84	4.50	0.25	13,479	1.89	0.22	1.79
	5	10	Mean	785.95	8.06	1.26	79,873	6.95	1.32	8.21
			SD	157.32	8.23	0.63	28,522	2.20	0.69	2.22

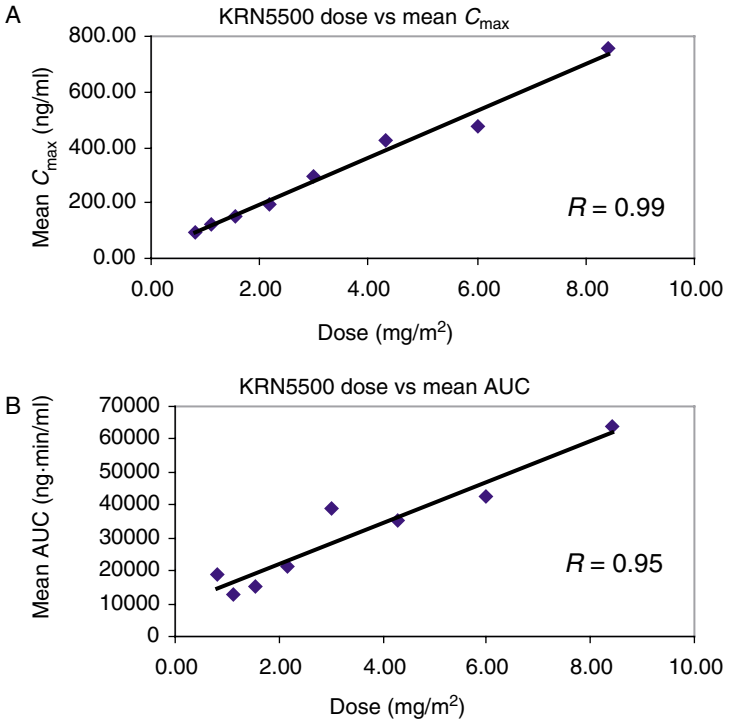

Figure 3. Dose versus (A) mean C_{max} and (B) AUC. R is the Pearson product-moment correlation coefficient.

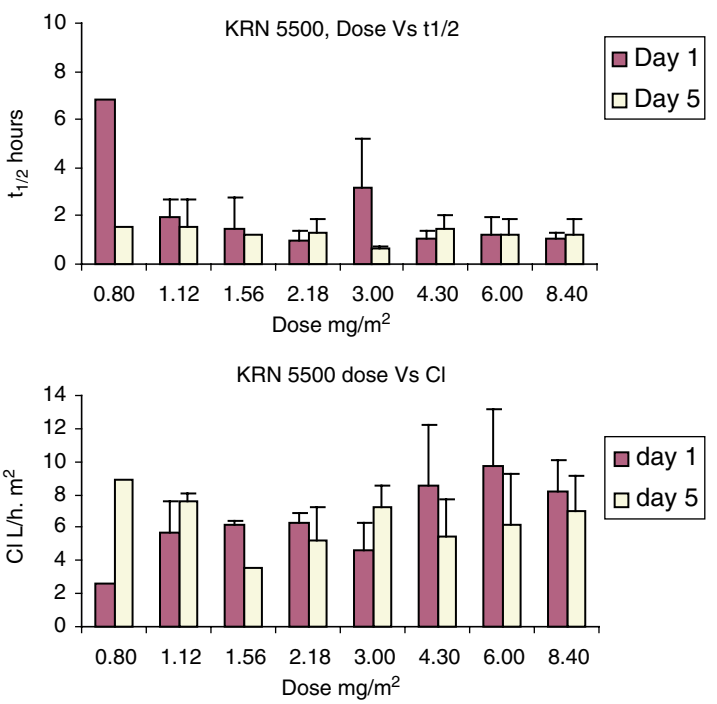

Figure 4. Dose versus (A) halflife and (B) clearance on day 1 and day 5. Error bars represent one standard deviation.

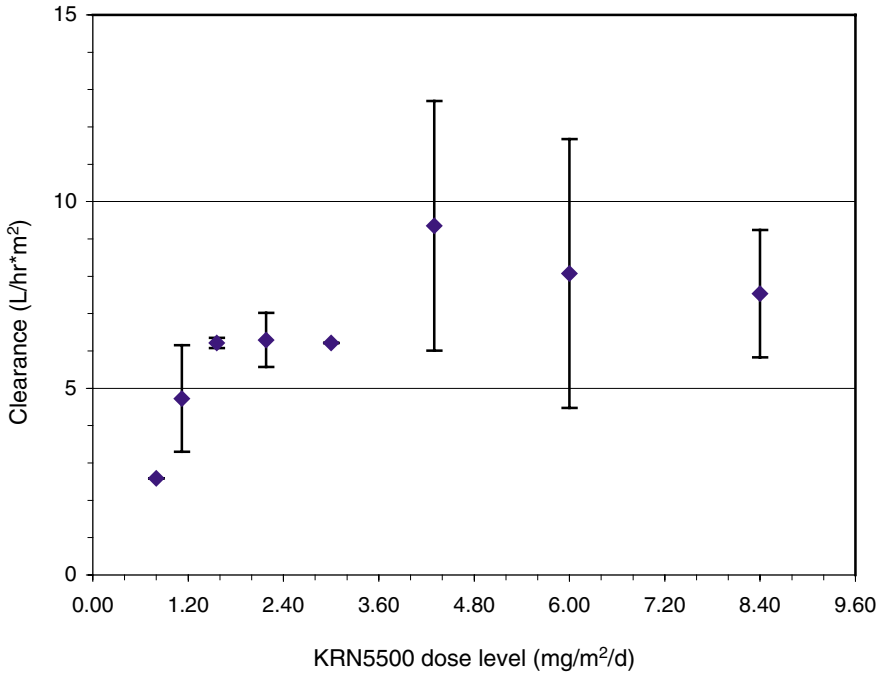

Figure 5. Mean clearance of KRN5500 versus dose level on Day 1, cycle 1. Error bars represent one standard deviation.

(53.12 ml/min $*$ m^2) is not significantly different from zero ($p = 0.13$ via the exact Wilcoxon signed-rank test, two-sided).

Three patients treated at 8.4 mg/m^2/d developed grade 3+ hepatotoxicity, as did one patient treated at 6.0 mg/m^2/d and one at 4.3 mg/m^2/d (Table 2). However, no significant correlation ($p > 0.05$) was observed between either Cmax or AUC and either the percentage change in SGOT or total bilirubin levels from baseline. With the exception of one patient at 6.0 mg/m^2/d, there were no important differences in plasma concentration–time profiles between these patients who experienced grade 2+ hepatotoxicity and those who did not (Figure 6).

Discussion

The interest in the spicamycin derivative KRN5500 was generated due to its apoptotic effect in leukemia cells as well as the significant cytotoxic effects in tumor models including in tumor models resistant to other chemotherapy drugs [6–8]. KRN5500 also showed significant activity in an animal model of hepatic metastasis from a human colon cancer COL-1 strain [3]. The exact mechanism of action is unclear. The drug may produce cytotoxic effects in tumor cells through more than one mechanism, including inhibition of protein synthesis. Single dose studies in rats demonstrated evidence of acute hepatic toxicity shortly after administration, while in dogs and monkeys, nausea, emesis and intestinal bleeding were prominent. The toxicity was reversible within approximately 2 weeks (Personal Communication, Toxicity and Pharmacology Branch, NCI). Myelotoxicity was not observed. Hepatic dysfunction including impaired protein synthesis and a disorder of lipid metabolism were the main toxicities in rats treated for 28 days.

Similar to the animal studies, gastrointestinal toxicities were the most common adverse events observed in patients, with hepatic toxicity being the DLT. Most patients who developed nausea and emesis were premedicated with ondansetron. A more aggressive antiemetic regimen including the use of dexamethasone may be required to adequately suppress this adverse effect. Hepatic toxicity was observed at 4.30 mg/m^2 or higher dosages and all the patients except one had hepatic metastasis.

It is noteworthy that the recommended Phase II dose of 4.3 mg/m^2/d $\times 5$ is 35-fold below the dose level of 150 mg/m^2/d $\times 5$ that shows maximum

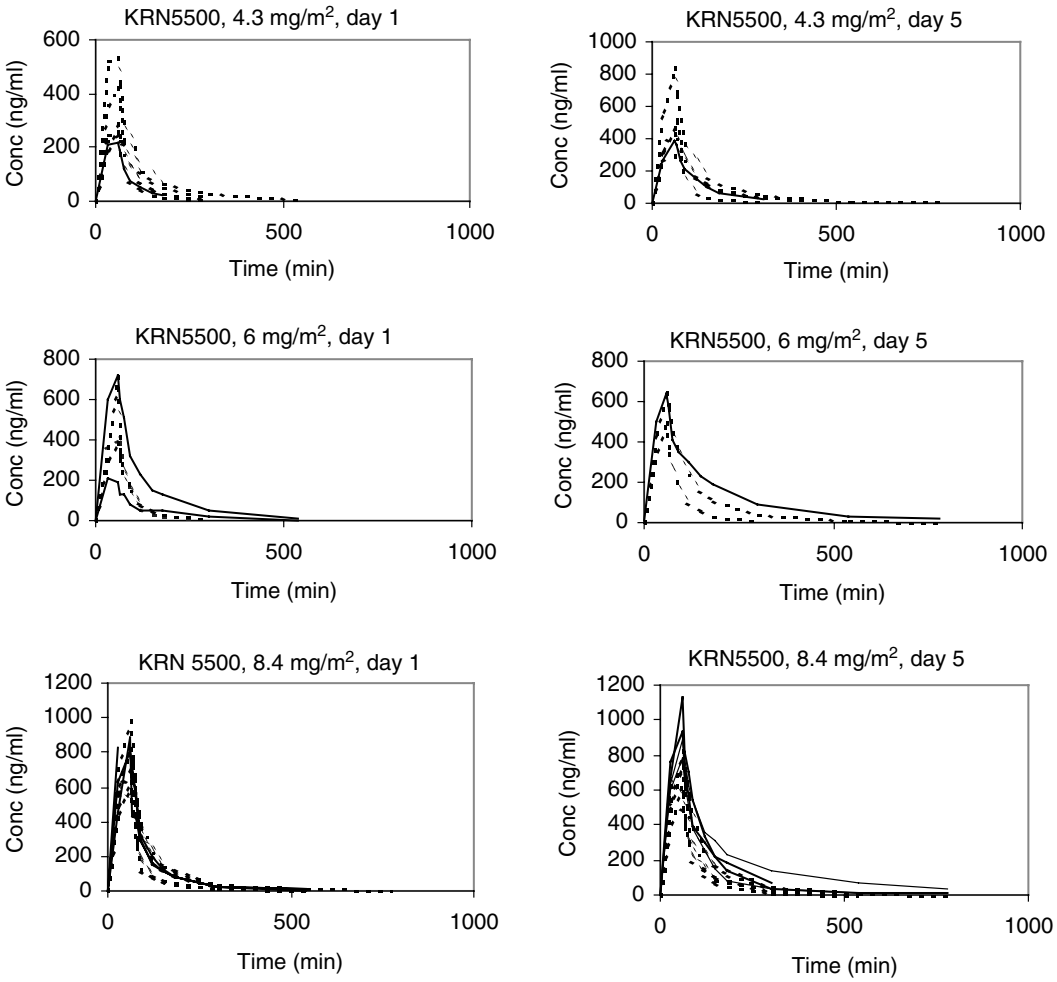

Figure 6. Comparison of plasma concentration-time profiles in patients who experienced grade 2+ hepatotoxicity (solid lines) and those who did not (dashed lines).

efficacy in the COLO 205 xenograft model [9]. In addition, antitumor activity was lost when the dose was reduced in this preclinical model to 22.5 mg/m^2/d $\times$5. Unless there are substantial differences in the pharmacokinetics of KRN5500 between mice and humans, the recommended Phase II dose of 4.3 mg/m^2/d $\times$5 will achieve exposure levels well below those associated with efficacy against human tumor xenografts. The lack of efficacy in this Phase I clinical trial is consistent with the preclinical models. Modifications in dosing schedules and/or dosage forms has reduced the toxicity and improved the efficacy of several anticancer agents [16–19]. Optimized drug delivery is as important as drug discovery itself, and drug delivery may play a very important role in realizing the therapeutic potential of KRN5500. A recent report by Matsumura et al. [17] indicates that incorporation of KRN5500 into polymeric micelles reduces its toxicity in mice. Thus, targeting of KRN5500 to tumor tissues may reduce toxicity and facilitate further dose escalation to levels that are efficacious in preclinical models.

Prior to the Phase I trial, KRN5500 pharmacokinetics were investigated in rats, mice, dogs, and monkeys (Personal Communication, Toxicity and Pharmacology Branch, NCI). In rats, the drug exhibited linear PK in the dose range of 1.2–30 mg/m^2 with a biphasic decay in plasma concentrations. Elimination of KRN5500 was prolonged in rats as well as monkeys following repeated administration. Clearance appeared to decrease in monkeys upon repeated dosing. However, such a decrease was not

statistically significant (Personal Communication, Toxicity and Pharmacology Branch, NCI).

In the present study in cancer patients, KRN5500 exhibited linear PK in the dose range of 0.8–8.4 mg/m^2 as was observed in rats in the dose range of 1.2–30 mg/m^2 (Figures 3 and 4). Distribution of KRN5500 followed a two-compartment model as reported by Takama et al. [20]. Although, the pattern of the clearance difference is quite inconsistent over increasing dose levels, there were sufficient sample sizes at the 8.4 and 6.0 dose levels for analysis that showed that clearance on days 1 and 5 are not significantly different. Almost superimposable plasma concentration versus time profiles on days 1 and 5 in different patients, together with the conclusion that clearance does not decrease significantly as dose level increases, indicate that the drug is not accumulated in the body.

Interest in novel designs of Phase I trials was generated by concerns about too many patients being treated at biologically sub-optimal doses, as well as prolonged study durations to complete Phase I trials based on conventional modified Fibonacci designs. An accelerated titration 2B design was implemented in this Phase I study as described by Simon et al. [12]. This design has two dose escalation phases. In the first phase, the escalation is conducted on an accelerated basis in one patient cohorts. The accelerated phase is terminated if a patient develops first course DLT or two patients develop first course grade 2 toxicity. In the second phase, the cohorts are expanded to include three patients. The 2B design permits intra-patient dose escalation, thus reducing the number of patients treated at low dosages. A second trial of daily $\times 5$ was conducted with KRN5500 at the Dana Farber Cancer Institute. In that trial, a modified Fibonacci design was used with a minimum cohort size of three patients. That required enrollment of 26 patients to define the same recommended Phase II dose (Personal Communication, Paul Eder). The duration of the time to complete the trial was also, at a minimum, 9 months longer. Therefore, the accelerated titration design achieved the same result with significantly fewer patients treated and more rapid completion of the Phase I trial.

Thus, we conclude that the MTD of KRN5500 is 4.3 mg/m^2 when administered for 5 days every 3 weeks. The drug demonstrated approximately linear pharmacokinetics with no significant correlation of plasma levels to toxicity. Clearance did not decrease significantly with increasing dose. The MTD determined by this study is well below the therapeutic dose in preclinical models. Further evaluation of this drug may require different schedules as well as different delivery systems to reduce the toxicity and improve the efficacy.

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