Phase I Clinical Trial and Pharmacokinetic Study of the Spicamycin Analog KRN5500 Administered as a 1-Hour Intravenous Infusion for Five Consecutive Days to Patients with Refractory Solid Tumors

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ABSTRACT

Purpose: The spicamycin analogue KRN5500 is a nucleoside-like antibiotic with broad spectrum activity against human solid tumor models. It appears to possess a novel mechanism of action directed against the endoplasmic reticulum and Golgi apparatus with effects on protein processing. A Phase I trial was undertaken to determine the maximum tolerated dose (MTD), dose-limiting toxicities, and pharmacokinetic behavior of KRN5500 given as a 1-h i.v. infusion for 5 consecutive days every 3 weeks.

Experimental Design: Adult patients with refractory solid tumors, good performance status, and normal renal, hepatic, and hematological function were eligible for the study. At least three patients were evaluated at each dose level, and a modified Fibonacci algorithm was used for dose escalation. The MTD was based on the occurrence of severe toxicity during the first cycle of therapy. The plasma pharmacokinetics of KRN5500 was characterized during the first week of dosing.

Results: Characteristics of the 26 patients entered into the study were as follows: 13 males and 13 females; median age, 54.5 years (range, 40–70 years); and Eastern Cooperative Oncology Group performance status 0–1. A majority had refractory colorectal carcinoma (17 of 26 patients) with at least two prior regimens of therapy. The dose of KRN5500 was escalated from 0.8 to 4.9 mg/m²/day in five dose levels, and the MTD was 2.9 mg/m²/day. All dose-limiting toxicities were nonhematological and included pulmonary toxicities, hyperglycemia, fatigue, hepatotoxicity, and ataxia, with one fatality due to interstitial pneumonitis. Clinically significant toxicities occurring in multiple patients that were not dose-limiting included nausea/vomiting, diarrhea, fatigue, neurological symptoms, hyperbilirubinemia, hyperglycemia, lymphopenia, and thrombocytopenia. There were no objective responses, although 3 of 17 evaluable patients exhibited disease stabilization for 5–6 cycles. The pharmacokinetics for the first dose of KRN5500 was biexponential and linear across all five dose levels. Mean values of pharmacokinetic parameters were as follows: total plasma clearance, 6.15 ± 2.37 liters/h/m²; apparent volume of distribution at steady state, 6.56 ± 1.98 liters/m²; biological half-life, 1.29 ± 0.37 h; and mean residence time, 1.07 ± 0.31 h. Clearance was significantly lower (P = 0.011) in the eight patients who were at least 65 years old (4.6 ± 1.6 liters/h/m²) as compared with the 18 younger patients (7.1 ± 2.3 liters/h/m²). Peak plasma concentrations of KRN5500 in the cohort receiving the MTD ranged from 350 to 400 ng/ml.

Conclusions: The MTD of KRN5500, when given as a 1-h i.v. infusion for 5 consecutive days, was 2.9 mg/m²/day. The only suggestion of therapeutic activity observed in this study was disease stabilization in three patients with chemorefractory colorectal cancer. Administering KRN5500 as a continuous i.v. infusion with the objective of prolonging systemic exposure to potentially cytotoxic concentrations of the drug should be considered.

INTRODUCTION

The spicamycins are a mixture of unique nucleoside-like antitumor antibiotics produced by Streptomyces alanosinicus that were discovered in a screening effort to identify new agents that induced differentiation of myeloid leukemia cells (1, 2). They are closely related to the septacidin antitumor and antifungal antibiotics, being composed of an adenine moiety linked through an aminoheptose group to a glycine residue N-acylated with a saturated fatty acid of variable chain length (Fig. 1). The nature of the fatty acid side chain significantly influences the potency and efficacy of the antiproliferative effects of the compound as well as its acute toxicity toward mice (3). Among the various semisynthetic derivatives of spicamycin that have been prepared, the compound afforded by replacing the naturally occurring fatty acids with a tetradecadiene moiety, KRN5500, exhibited the most favorable balance of pharmacological effects. KRN5500 inhibits the in vitro proliferation of cell lines...
derived from a wide variety of human cancers, including leukemias and solid tumors (4–7). Activity has been established against xenografts of human breast, colon, esophageal, gastric, and lung tumors in nude mice when the drug was given by multiple i.v. or i.p. injections (3, 5, 8–10). Daily i.v. injection for 5 days was one of the most effective administration schedules (8). Treatment with KRN5500 also effectively inhibited the growth of hepatic metastases induced by intrasplenic injection of the COL-1 human colon cancer cell line in severe combined immunodeficient mice (11).

The mechanism responsible for the antiproliferative activity of KRN5500 has not been unequivocally established. KRN5500 preferentially inhibits protein synthesis, rather than the synthesis of DNA or RNA, at concentrations that are severalfold higher than those required for growth inhibition (3, 5, 9). The pattern of activity of KRN5500 in the NCI2 in vitro antitumor drug screen is different from that of any anticancer drug approved for clinical use or investigational agent previously tested, suggestive of a novel mechanism of action (5). Recent findings indicate that cytotoxicity of the drug is mediated by the endoplasmic reticulum-Golgi secretory apparatus (5, 12). Distinct changes in the morphological appearance of the Golgi apparatus are induced by continuously exposing cancer cells to KRN5500. These changes are associated with an alteration in the normal processing of glycoproteins that appears to result from an inhibition of the removal of mannose from precursor glycoproteins and the subsequent incorporation of other saccharides.

As shown in Fig. 1, KRN5500 is very likely a prodrug, which undergoes intracellular deacylation of the fatty acid moiety to afford the active species, SAN-Gly (4, 9). Consistent with this behavior, protein synthesis is inhibited when intact P388 leukemia cells, but not rabbit reticulocyte lysates, are exposed to KRN5500 (10). Furthermore, whereas SAN-Gly effectively inhibits protein synthesis in rabbit reticulocyte lysates, it is inactive against intact neoplastic cells because the metabolite is not readily taken up by the cells. Evidently, the lipophilic fatty acid tail of KRN5500 is necessary for transport into cells (4). The observation of decreased intracellular concentrations of SAN-Gly in sublines of human tumor cells with varying degrees of resistance to KRN5500 is particularly relevant to its potential clinical effectiveness (4).

A Phase I trial was undertaken to evaluate the administration of KRN5500 as a 1-h i.v. infusion for 5 consecutive days, repeated at an interval of 3 weeks, in adult cancer patients with refractory solid tumors. The primary objectives of the study were to determine the MTD, identify the DLTs, and characterize the plasma pharmacokinetics of KRN5500 when given according to this schedule.

**MATERIALS AND METHODS**

**Patient Selection**

The study was restricted to patients with a histologically confirmed solid tumor that was either refractory to conventional therapy or for which no standard treatment existed. Patients had to be at least 18 years old with a minimum life expectancy of 2 months. The mandated time between prior treatment of the malignancy and entry into this study was at least 2 weeks for major surgery and at least 3 weeks for radiotherapy and chemotherapy, with the exception of chloroethylnitrosoureas and mitomycin C, for which the minimum time interval was 6 weeks. In addition, complete recovery from the effects of any earlier intervention was required. Minimum eligibility requirements of the protocol included the following: an Eastern Cooperative Oncology Group performance status of ≤2; WBC count ≥ 3,000/µl; absolute neutrophil count ≥ 1,500/µl; platelet count ≥ 100,000/µl; prothrombin time within normal limits; serum creatinine ≤ 1.5 mg/dl or creatinine clearance ≥ 60 ml/min; total bilirubin < 1.5 mg/dl; and aspartate aminotransferase and alanine aminotransferase ≤ 2× the upper limit of normal. Patients with evidence of a primary or metastatic lesion in the central nervous system, acute ischemia or serious conduction abnormality in an electrocardiogram, occurrence of a myocardial infarction within the past 6 months, uncontrolled or bloody diarrhea, HIV infection, and pregnancy or breast feeding were excluded.
Drug Administration and Toxicity Assessments

The study protocol was approved by the institutional Scientific Review Committee and Human Protection Committee of Dana-Farber/Partners Cancer Care (Boston, MA). A signed written informed consent document satisfying all federal and institutional requirements was obtained as a condition of patient registration. Patients underwent a history, physical examination and performance status determination, an electrocardiogram and chest X-ray, a complete blood count with platelet and differential counts, coagulation tests (prothrombin time, partial thromboplastin time), a serum chemistry profile, and urinalysis within 14 days of initiating therapy.

KRN5500 (NSC 650426; M 589.70) was supplied by the NCI (Bethesda, MD) as a two-part kit, consisting of a solution of the drug in a vial and an ampule containing the initial diluent, which was stored at 5°C and protected from exposure to light. The vial contained 5.0 mg of KRN5500, 0.05 g of N,N-dimethylacetamide, 0.4 g of propylene glycol, 0.3 g of polysorbate 80, and ethanol (0.6 g) to provide a final volume of 1.5 ml. The ampule contained 1.0 ml of a solution of monoethanolamine (0.1 g) in water for injection (0.9 g). Adding the entire contents of the ampule to the vial provided a clear, almost colorless to very pale yellow viscous solution containing 2 mg/ml KRN5500 at a pH of 11. This solution is stable for at least 8 h at room temperature and further diluted with 0.9% Sodium Chloride for Injection, USP before infusion to a final drug concentration ranging from 4 to 50 μg/ml. The diluted infusion solutions are compatible with PVC i.v. infusion bags and are chemically stable for at least 4 h at room temperature.

KRN5500 was administered as a 1-h continuous i.v. infusion, through a central venous catheter, once every 24 h for 5 and are chemically stable for at least 4 h at room temperature. The study protocol was approved by the institutional Scientific Review Committee and Human Protection Committee of Dana-Farber/Partners Cancer Care (Boston, MA). A signed written informed consent document satisfying all federal and institutional requirements was obtained as a condition of patient registration. Patients underwent a history, physical examination and performance status determination, an electrocardiogram and chest X-ray, a complete blood count with platelet and differential counts, coagulation tests (prothrombin time, partial thromboplastin time), a serum chemistry profile, and urinalysis within 14 days of initiating therapy.

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Cohorts of three patients were scheduled for entry into each dose level. Escalation of the dose to the next higher level proceeded after all three patients had received the first cycle of therapy with the preceding dose and each was observed for at least 21 days without evidence of a DLT. An additional three patients were entered into a given dose level in cases where a single patient experienced a DLT during the first cycle of therapy. Dose escalation proceeded in the absence of a DLT in these three additional patients. The occurrence of a DLT in two patients from any cohort of three to six patients during the first cycle of therapy established the preceding dose level as the MTD. An additional six patients were enrolled at the MTD to better define the toxicity profile and confirm tolerance.

Evaluation of Response

A baseline assessment of all measurable disease using any appropriate radiological technique was performed within 21 days of starting the first cycle of therapy. Biochemical markers of disease, such as carcinoembryonic antigen, prostate-specific antigen, or CA-125, were also determined whenever applicable and repeated after every second cycle of therapy. Reevaluations of malignant disease that was initially measured by physical examination or plain radiographs were performed after each cycle of therapy. Tumors measured by computed tomography or magnetic resonance imaging were similarly evaluated after every two cycles of treatment. Disease assessments by any technique were also performed 1 month after administration of the last dose of KRN5500. Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions. Standard WHO response criteria were used. Progressive disease was scored by a >25% increase in tumor burden or the appearance of any new lesion.

Pharmacokinetic Studies

Sample Collection. The plasma pharmacokinetics of KRN5500 was characterized in patients during the first cycle of therapy. Blood samples were collected immediately before dosing and at 5, 15, 30, 60, 65, and 75 min and 1, 2, 2.5, 3, 4, 5, 6, 7, and 8 h after starting the infusion for the first daily dose of the drug. Samples were obtained immediately before the beginning and end of the daily drug infusions for the remainder of the week. The volume of blood obtained at each time point was 7 ml. Blood specimens were acquired from an arm vein, either by venipuncture or with the use of a peripheral venous catheter, depending on the frequency of collections during the day. Patency of the catheter was maintained with the use of a heparin lock or slow normal saline drip. Specimens were collected in Vacutainer tubes with freeze-dried sodium heparin anticoagulant (Becton Dickinson, Franklin Lakes, NJ). The sample tubes were mixed by inversion and placed on wet ice until centrifuged (1800 × g, 10 min, 4°C) within 15 min after collection. Plasma was promptly separated from the blood cells and stored in polypropylene cryotubes at −70°C for subsequent analysis. The reading of a battery-powered digital timer was recorded when the infusion pump was started or stopped and when blood samples were collected. Urine was also collected and pooled during the 24-h period from the time that administration of the first daily dose was started until just before the second dose was
given. The urine was kept in an opaque container over ice. The total volume of urine was accurately measured, and, after thoroughly mixing, a 10-ml aliquot was removed and stored in a polypropylene centrifuge tube and stored at −70°C for analysis.

**Analytical Method.** An analytical reference sample of KRN5500 was provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI. A stock solution of the compound was prepared in methanol at a concentration of 0.10 mg/ml and stored at 0°C–5°C. Standard solutions were made daily by serially diluting the KRN5500 working solution with human donor plasma (MGH Blood Transfusion Service, Boston, MA) to concentrations of 50, 37.5, 25, 12.5, 5.0, 2.5, 1.0, and 0.0 ng/ml. A stock solution of the internal standard, α-naphthoflavone, was made in methanol at a concentration of 0.1 mg/ml. The working solution of the internal standard used in the assay was made on a daily basis by diluting the stock solution to a concentration of 3.5 μg/ml with methanol. Stock solutions of the drug and internal standard were stored in a refrigerator at 5°C and used for several months without any evidence of degradation.

Frozen samples were allowed to thaw at ambient temperature, mixed on a vortex stirrer, and centrifuged for 10 min at 8,160 × g (10,000 rpm) to separate particulates and lipids. An aliquot of plasma (1,000 μl) was pipetted into a polypropylene centrifuge tube and mixed with 2,000 μl of methanol on a vortex stirrer to precipitate proteins. After centrifuging the mixture at 3,500 × g for 10 min, the clear supernatant was transferred into a silanized, borosilicate glass, screw-top centrifuge tube, to which 1,000 μl of water and 5 ml of hexane were added. The tube was vigorously mixed on a reciprocating shaker for 10 min and then centrifuged for 5 min at 2,500 × g, upon which the upper organic phase was aspirated and discarded. Internal standard working solution (5 μl) was added to the sample, which was extracted twice with 7 ml of methyl tert-butyl ether for 10 min on a reciprocating shaker. The mixture was centrifuged (2,500 × g, 5 min) before removing each organic extract. The combined extracts were evaporated to dryness in a silanized glass culture tube under a stream of nitrogen at 40°C and reconstituted by adding 150 μl of methanol followed by 150 μl of 50 mM ammonium acetate buffer (pH 4.7), assisted by vortexing. After centrifuging in a polypropylene microcentrifuge tube (8,000 × g, 10 min), the clear solution was transferred into a borosilicate glass insert residing within an autosampler vial and sealed with a silicone/Teflon-lined septum closure.

A 250-μl aliquot of the solution sample was injected onto a Nova-Pak Phenyl stainless steel column (15 cm × 3.9 mm, inner diameter; 4 μm; particle size; Waters, Milford, MA) preceded by a 15 × 3.2-mm inner diameter Brownlee Phenyl NewGuard (7 μm, particle size) cartridge (Alltech Associates, Deerfield, IL) and a 0.5 μm inline filter (Upchurch Scientific, Oak Harbor, WA). Chromatography was performed at ambient temperature using an isocratic mobile phase composed of methanol-50 mM ammonium acetate buffer (pH 4.7) (33:67, v/v) delivered at 1.0 ml/min. UV absorbance of the effluent from the analytical column was monitored at 266 nm (6.5 nm, bandwidth), with a 4-s response time, using a Hewlett-Packard (Palo Alto, CA) series 1100 Variable Wavelength Detector fitted with a 14-μl flow cell (10 mm, pathlength). Operation of the chromatographic system and data collection were completely controlled with HP ChemStation for LC software, rev. A.04.01, operating under Microsoft Windows95 on a Vectra XM 5/90 Series 3 computer (Hewlett-Packard). Chromatograms were integrated to provide peak areas using the data analysis functions of the software.

Each study sample was independently assayed in duplicate, on different days, together with a series of calibration standards. Standard curves were constructed by plotting the KRN5500 to internal standard chromatographic peak area ratio against the known concentration of KRN5500. Linear least squares regression was performed using a weighting factor of 1/5, without inclusion of the origin. Values of the slope and y intercept for the best fit line were used to calculate the analyte concentration in the study samples. Results were considered to be acceptable if the two determinations differed from their average by ≤10%; otherwise, the sample was reassayed. Specimens with an estimated concentration above the upper limit of the standard curve were reassayed in duplicate on appropriate dilution with drug-free plasma.

**Data Analysis.** Sample times were calculated as the difference between the blood collection interval midpoint and starting time of the infusion. The model-independent equation for zero-order i.v. drug input and first-order biexponential disposition was fit to the KRN5500 plasma profiles determined in individual patients for the first daily dose by weighted nonlinear regression using WinNonlin version 1.1 software package (Scientific Consulting, Apex, NC), as described previously (13, 14). Weighting according to y^2 weighting invariably yielded the best fit of the data. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic variables [i.e., Cmax, AUC, CL, t1/2,z (half-life of the apparent terminal disposition phase), t1/2,1 (half-life of the initial disposition phase), mean residence time, V1 (apparent volume of distribution of the sampled compartment), and Vss (apparent volume of distribution at steady state)] according to standard equations (15). Mean values of pharmacokinetic variables were calculated as the geometric mean of the individual patient values (16). SDs for the geometric mean values were estimated by the jackknife method (17). Parametric statistical tests of pharmacokinetic variables were performed after logarithmic transformation of the data (16, 18). Pearson sample correlation coefficients (r) were calculated to identify relationships between pharmacokinetic parameters, pretreatment laboratory values, and the maximum percentage change in laboratory values observed during the first cycle of therapy. The suggestion of a significant correlation, as indicated by |r| ≥ 0.4, was substantiated by examining a scatter plot of the data and regression line, ascertaining whether the P of the slope of the regression line was >0.05, and determining whether the Spearman correlation coefficient (r_s) was ≥r.

**RESULTS**

**Patient Characteristics.** Characteristics of the 26 patients evaluable for toxicity assessments are listed in Table 1. There were 13 males and 13 females, with a median age of 54.5 years (range, 40–70 years), 24 of whom had an Eastern Cooperative Oncology Group performance status of 0 or 1. Seventeen patients had a diagnosis of colorectal carcinoma, three had...
Phase I Trial of KRN5500
due to presumed abdominal sepsis. An autopsy revealed no source other than widely metastatic cancer for the clinical presentation of fever, hypotension, and severe abdominal pain. Moderate (grade 2) nonhematological toxicities were noted in five of the six patients in this expanded cohort, including nausea/vomiting, diarrhea, fatigue, dyspnea, neurological symptoms, and hyperbilirubinemia. One of these patients also experienced grade 4 nausea and vomiting. DLTs developed in both of the patients entered into dose level 5 (grade 4 hyperbilirubinemia in one patient and grade 3 dyspnea in the other). Consequently, further evaluation of this dose level was discontinued. When four additional patients were subsequently enrolled for treatment at dose level 4 as the putative MTD, a second patient developed rapidly progressive, fatal respiratory failure due to interstitial pneumonitis within 2 days after receiving the final dose of the first cycle of KRN5500. In addition, grade 3–4 lymphopenia occurred in 6 of the 11 patients evaluated at this dose level, and 1 patient had grade 4 thrombocytopenia. Accordingly, 2.9 mg/m²/day was established as the MTD for KRN5500 delivered as a short i.v. infusion on a daily × 5 schedule.

**Antitumor Activity.** Nine patients received fewer than two cycles of therapy and were not evaluable for response. Disease progression was evident in 13 patients when evaluated after receiving the second cycle of therapy. Another patient was removed from the study due to progressive disease during the third cycle. Disease stabilization persisting for five to six cycles of therapy was evident in 3 of the 17 patients who were evaluable for response. These patients were among a group of 13 patients with colorectal cancer that was refractory to prior treatment with fluoropyrimidines and irinotecan.

**Toxicities and Determination of the MTD.** A total of 26 patients were treated at five dose levels ranging from 0.8 to 4.9 mg/m²/day for 5 days. The designation and grade of all significant clinical toxicities observed during the first cycle of therapy are summarized in Table 2. The only significant toxicities experienced by the three patients who received the starting dose of 0.8 mg/m²/day were grade 2 fatigue in two patients together with grade 3 or 4 lymphopenia. The first patient entered into the second dose level, 1.2 mg/m²/day, a 52-year-old woman with metastatic colorectal cancer, died suddenly at home after receiving the fourth daily dose of the second cycle of therapy. The cause of death was uncertain, and the family declined an autopsy. The patient appeared to tolerate the first cycle of therapy well, with the most severe toxicities being limited to grade 2 nausea and vomiting, fatigue, and an increase in serum lactate dehydrogenase activity. Although there was no clear evidence to suggest that the death was drug-related, the dose level was expanded to evaluate an additional six patients being treated at dose level 4 as the putative MTD, a second patient developed rapidly progressive, fatal respiratory failure due to interstitial pneumonitis within 2 days after receiving the final dose of the first cycle of KRN5500. In addition, grade 3–4 lymphopenia occurred in 6 of the 11 patients evaluated at this dose level, and 1 patient had grade 4 thrombocytopenia. Accordingly, 2.9 mg/m²/day was established as the MTD for KRN5500 delivered as a short i.v. infusion on a daily × 5 schedule.

**Assay Validation.** The assay used to measure the concentration of KRN5500 in plasma specimens acquired during the pharmacokinetic studies was adapted from a previously reported method developed for preclinical pharmacology studies of the drug (19). Major modifications were made in the sample preparation procedure to improve selectivity and eliminate interferences associated with strongly retained endogenous compounds present in human plasma. The new assay was thoroughly validated according to currently recommended guidelines (20). The drug and internal standard eluted with retention times of 7.0 and 10.5 min, respectively. Liquid chromatograms of plasma from 6 anonymous donors and study specimens acquired from 26 patients before treatment with KRN5500 showed no peaks, either of endogenous origin or attributable to a concurrently administered medication, that interfered with the detection of the drug or internal standard. Similarly, there was no evidence of interfering peaks in study specimens obtained during or after the administration of KRN5500.

The lower limit of quantitation was 1 ng/ml using a sample volume of 1000 µl. Standard curves of KRN5500 in plasma at concentrations ranging from 1 to 50 ng/ml exhibited excellent linearity. In this concentration range, the grand mean ± SD absolute recovery of the drug was 68.7 ± 2.3%. Within-day accuracy of the assay ranged from 94.9% to 101.4%, and the precision was 2.9–4.9% in quality control plasma solutions with KRN5500 concentrations of 4.1, 20.7, and 41.3 ng/ml. Between-day accuracy and precision of the analytical method were assessed by analyzing the interpolated drug concentrations from a

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
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<tr>
<td>Age (yrs)</td>
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<tr>
<td>Median</td>
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<tr>
<td>Range</td>
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<td>2</td>
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<tr>
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</tr>
<tr>
<td>≥3</td>
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</tr>
<tr>
<td>Prior radiotherapy</td>
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* Eastern Cooperative Oncology Group performance status.

The plasma pharmacokinetics of KRN5500 was studied in all 26 patients during the first cycle of therapy. Pharmacokinetic parameters could not be reliably estimated from one patient treated with daily doses of 3.7 mg/m² due to problems encountered during administration of the drug that significantly altered the plasma profile. Plasma levels of the drug declined biexponentially after the end of the 1-h infusion in all patients evaluated. The mean plasma concentration-time profile for the first daily dose of KRN5500 for the group of patients treated with the MTD of 2.9 mg/m²/day is shown in Fig. 2. Mean values of the pharmacokinetic parameters of KRN5500 for the groups of patients evaluated at each dose level are presented in Table 3. The pharmacokinetics of KRN5500 was linear on day 1 at doses ranging from 0.8 to 4.9 mg/m² as indicated by dose-proportionate increases in both the $C_{\text{max}}$ ($r = 0.99$) and AUC ($r = 0.97$). Grand mean values for the biological half-life (1.29 ± 0.37 h) and mean residence time (1.07 ± 0.31 h) of the drug, calculated for the entire group of 25 patients, were similar. The grand mean ± SD CL, 6.15 ± 2.37 liters/h/m², was approximately 15% of the normal hepatic blood flow for adults. Although KRN5500 is a highly lipophilic molecule, its apparent volume of distribution at steady state was relatively low (6.56 ± 1.98 liters/m³), being only 20–30% total body weight. There was no detectable parent drug in urine collected over a 24-h period after administering the first daily dose.

The plasma concentration of KRN5500 was invariably below the 1.0 ng/ml lower limit of quantitation of the analytical method in samples acquired shortly before each successive daily dose was given in all patients. Relationships between the daily $C_{\text{max}}$ of KRN5500 measured on days 1 and 5 in individual patients at each dose level are illustrated in Fig. 3. Regression analysis of the $C_{\text{max}}$ values for each daily dose revealed a significant trend toward increasing values in only 1 of the 13 patients treated with doses of 0.8–2.9 mg/m²/day. There were no significant differences between the mean $C_{\text{max}}$ values on days 1 and 5 in the cohorts evaluated at these doses (Table 3). Interpatient variability in the $C_{\text{max}}$ values ranged from 7.8% to 23.7% at the three initial dose levels. In contrast, as shown in Fig. 3, for the 10 patients treated with daily doses of 3.7 mg/m², the mean $C_{\text{max}}$ on day 5 (563 ± 142 ng/ml) was significantly ($P = 0.017$) higher than on day 1 (448 ± 94 ng/ml), suggestive of an alteration in drug disposition on repeated daily dosing. Correlation coefficients afforded from regression analysis of the daily $C_{\text{max}}$ values were ≥0.5 for 9 of the 11 patients with complete data sets at the two highest dose levels.

![Fig. 2](image-url) Mean plasma concentration-time profile for the first dose of KRN5500 in the cohort of three patients treated with the MTD of 2.9 mg/m²/day. Points (●) are the geometric mean values of the observed KRN5500 plasma concentrations at each sample time, and the solid line is the best-fit curve determined by nonlinear regression analysis of the data.

### Table 2 Summary of clinical toxicities during the first cycle of therapy

<table>
<thead>
<tr>
<th>No. of patients (grade 2/grade 3/grade 4) at dose levels of</th>
<th>0.8 mg/m²</th>
<th>1.2 mg/m²</th>
<th>2.9 mg/m²</th>
<th>3.7 mg/m²</th>
<th>4.9 mg/m²</th>
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</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>3</td>
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<td>3</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
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<td>0/0/0</td>
</tr>
<tr>
<td>Fatigue</td>
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<td>2/0/0</td>
<td>4/1/0</td>
<td>0/0/0</td>
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* DLTs occurring in the same patient who died due to respiratory failure.
* DLTs occurring in the same patient.
* Only one of these adverse events was classified as a drug-related DLT.
levels. The incidence of severe toxicity was not associated with the magnitude of the increase in $C_{\text{max}}$ on day 5 relative to day 1.

The CL of KRN5500 was significantly lower ($P < 0.011$, two-tailed t-test) in the eight patients who were at least 65 years old (4.6 ± 1.6 liters/m²) in comparison with the 18 younger patients (7.1 ± 2.3 liters/h/m²). There were no significant associations between any other patient characteristic, including gender and body surface area, or pretreatment serum chemistry tests and any pharmacokinetic parameter. There was no distinct relationship between the most severe drug-related toxicity that occurred during the first cycle of therapy and the AUC for the first daily dose of KRN5500 (Fig. 4). Mean values of the AUC as well as the range of AUC values were similar for patients experiencing grade 2, 3, and 4 toxicity ($P = 0.52$, single-factor ANOVA). However, it is notable that the one patient who succumbed to grade 5 toxicity had an AUC (1056 ng·h/ml) that was considerably greater than that observed in any other patient (range, 83–880 ng·h/ml). The only significant relationships (i.e., $r \geq 4$) between the AUC of KRN5500 and the maximum percentage change in any hematological or biochemical parameter indicative of organ toxicity were moderate correlations with the peak blood urea nitrogen ($r = 0.59$) and total bilirubin ($r = 0.60$) elevations.

**DISCUSSION**

KRN5500 was selected for Phase I clinical trials by the NCI on the basis of impressive preclinical activity, a novel mechanism of action, and tolerable toxicity at therapeutically effective doses against tumor models. In this Phase I clinical trial, the MTD of the drug given as a 1-h i.v. infusion once every 24 h for 5 consecutive days was found to be 2.9 mg/m²/day. All of the DLTs were nonhematological and included pulmonary toxicities, hyperglycemia, fatigue, hepatotoxicity, and ataxia. Pulmonary toxicities and hyperbilirubinemia were the most prevalent adverse events. However, the pulmonary effects appeared to be the most severe and problematic toxic manifestation of the drug, resulting in severe dyspnea, respiratory failure, and...
and death. The elevated bilirubin and serum transaminase levels that were noted occurred predominantly in the context of progressive growth of preexisting liver metastases. Clinically significant ataxia occurred in two patients, both of whom were receiving lorazepam as an antiemetic, although a neurological examination proved to be normal in the patient experiencing grade 3 ataxia. Moderate fatigue was noted frequently at doses above 1.2 mg/m²/day, even in patients with stable disease in whom these symptoms could not be attributed to progressive cancer. The drug induces significant nausea and vomiting, although these effects could be effectively controlled by pretreatment with antiemetic drugs, especially 5-hydroxytryptamine antagonists. Moderate diarrhea, lymphopenia, and thrombocytopenia were also frequently noted. There were no objective responses to therapy with KRN5500 in the 17 evaluable patients. However, some patients appeared to experience clinical benefit, indicated by disease stabilization persisting for 5–6 months in 3 of the 13 evaluable patients.

Pharmacokinetic studies revealed that the CL of KRN5500 is independent of the administered dose when determined on the first day of treatment with daily doses ranging from 0.8 to 4.9 mg/m². The grand mean CL of KRN5500 determined in this study, 6.15 ± 2.37 liters/h/m², was comparable with the previously reported value of 5.41 liters/h/m² for the parameter in a population-based pharmacokinetic analysis of data from a cohort of 18 patients that received doses ranging from 3 to 21 mg/m² given as a 2-h i.v. infusion during a Phase I trial performed in Japan (21). There was no significant difference between the plasma concentration of drug achieved at the end of the 1-h i.v. infusion with the first and fifth daily doses in patients treated with doses up to 2.9 mg/m²/day. In contrast, there was evidence of a significant change in drug disposition consistent with decreased CL on repeated daily dosing in the expanded cohort of patients receiving the 3.7 mg/m²/day dose level. However, this did not appear to be associated with an increased risk for the occurrence of severe toxicity. Nevertheless, the clinical observations coupled with the pharmacokinetic data supported establishing the 2.9 mg/m²/day dose as the MTD for this administration schedule of KRN5500.

The opportunity to identify age-related effects on the pharmacokinetic behavior of new anticancer drugs is not often presented in Phase I clinical trials because elderly patients are typically underrepresented due to referral patterns and investigator bias, even though >50% of all cancers are presented by patients older than 65 years (22). Eight of the 26 (31%) patients entered into this clinical trial were at least 65 years old. Subgroup analysis revealed that the mean CL of KRN5500 in these elderly patients was 35.2% lower than that observed in the younger patients, which was a statistically significant difference (P = 0.01). There are several implications of this finding on the continued clinical development of KRN5500. It would be reasonable to independently establish a MTD of the drug for elderly patients rather than accruing them into a Phase II study using a dosage defined in a predominantly younger patient population. In addition, identifying the mechanism responsible for, or factors associated with, the diminished CL of KRN5500 in elderly patients would also be worthwhile. It appears likely that KRN5500 is largely eliminated by the liver, either by direct biliary excretion and/or metabolism, in consideration of its size (i.e., Mᵣ > 300) and the absence of measurable concentrations of the parent drug in urines obtained from treated patients. Unfortunately, relatively little is known about the extent of biliary excretion or hepatic metabolism of the drug, other than its intracellular conversion to the cytotoxic species SAN-Gly as noted previously. Age-related changes in renal and hepatic function appear to be among the more important physiological factors that are responsible for clinically significant differences in the elimination of anticancer drugs between older and younger patient populations (23, 24). In particular, normal aging is accompanied by a 25–35% decrease in liver volume and a 35–40% decrease in hepatic blood flow (25). Determining the degree to which quantitative measures of liver function, mass, and blood flow correlate with the CL of KRN5500 may prove to be very informative.

Against the full panel of 60 human tumor cell lines comprising the NCI anticancer drug screen, average concentrations of KRN5500 producing 50% growth inhibition, total growth inhibition, and 50% cell death after 48 h of continuous exposure were approximately 6 ng/ml, 60 ng/ml, and >60 ng/ml, respectively. In comparison, peak plasma concentrations of KRN5500 achieved in cancer patients treated with daily doses of 2.9 mg/m², the MTD of the 1-h i.v. infusion daily 5 schedule, were in the 350–400 ng/ml range. Plasma levels of the drug required for cytotoxicity were maintained for less than 2 h and remained above the concentration required for cytostatic antiproliferative effects (i.e., 60 ng/ml) for only 6 h. The duration of exposure to the drug appears to be a critical parameter for inducing cytotoxicity in human tumor cell lines in vitro (7). Exposing cells to KRN5500 for less than 48 h, even at relatively high concentrations, only serves to inhibit cell growth because at least 48 h of continuous exposure is required to induce cell death. Moreover, the concentration of drug necessary to effect cell death decreases significantly on prolonging the exposure period.

KRN5500 was formulated as a suspension and administered by i.p. injection on a daily 5 schedule during in vivo efficacy studies against human tumor xenograft models performed by investigators at the NCI (5). Pharmacokinetic studies in non-tumor-bearing mice revealed that administration of the most efficacious dose in this manner resulted in plasma levels of the drug that ranged from a minimum of 100 ng/ml to a maximum near 1000 ng/ml on days 2 through 5 of the repeated daily dosing regimen. Thus, the most effective dosing regimen identified in these preclinical efficacy studies actually mimicked a continuous i.v. infusion, most likely due to slow, dissolution rate-limiting absorption of the drug suspension from the peritoneal cavity.

In summary, the MTD of KRN5500 administered as a short i.v. infusion on 5 consecutive days was 2.9 mg/m²/day. The only
suggestion of therapeutic activity observed in this study was disease stabilization in three patients with chemorefractory colorectal cancer. Because cytotoxic concentrations of the drug were only briefly achieved in plasma, administering KRN5500 in a manner that would significantly prolong systemic exposure to potentially effective concentrations may be therapeutically advantageous. On the basis of these findings, a Phase I trial of KRN5500 given as a 72-h continuous i.v. infusion has been initiated, the results of which will be reported in a separate communication.

REFERENCES


Phase I Clinical Trial and Pharmacokinetic Study of the Spicamycin Analog KRN5500 Administered as a 1-Hour Intravenous Infusion for Five Consecutive Days to Patients with Refractory Solid Tumors


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