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 ceroside-like antibiotic with broad spectrum activity against solid tumors, good performance status, and normal to near 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 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 biphasic 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 chemotherapy resistant 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 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 biphasic 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.

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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 NCI\textsuperscript{2} 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 $\leq 2$; WBC count $\geq 3,000/\mu l$; absolute neutrophil count $\geq 1,500/\mu l$; platelet count $\geq 100,000/\mu l$; prothrombin time within normal limits; serum creatinine $\leq 1.5$ mg/dl or creatinine clearance $\geq 60$ ml/min; total bilirubin $< 1.5$ mg/dl; and aspartate aminotransferase and alanine aminotransferase $\leq 2 \times$ 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.

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\textsuperscript{2} The abbreviations used are: NCI, National Cancer Institute; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; $C_{\text{max}}$, maximum plasma concentration; AUC, area under the plasma concentration-time profile from time 0 to infinity; CL, total plasma clearance.
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 differentia
counts, coagulation tests (prothrombin time, partial thrombo
toplastin 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 polysor
bate 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 mono-
ethanolamine (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
consecutive days. The dose was escalated from an initial level
of 0.8 mg/m²/day to predetermined levels of 1.2, 2.9, 3.7, and 4.9
mg/m²/day, with subsequent escalations at a constant rate of
30% from the preceding dose level as necessary. Treatment was
delivered on an outpatient basis whenever possible. Concurrent
supportive care, including narcotics and antiemetics, was per-
mitted as needed, although antiemetics were not given routinely
until patients began to experience nausea and vomiting. Addi-
tional cycles of therapy were administered at intervals of 21
weeks. 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. Pa-
tency 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 anticoagu-
lant (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

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. Reevalua-
tions 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 ev-
every two cycles of treatment. Disease assessments by any tech-
nique 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 dos-
ing and at 5, 15, 30, 60, 65, and 75 min and 1.5, 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 begin-
ing 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. Pa-
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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 thor-
oughly 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 Can-
cer 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-naph-
thoflavone, 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 tem-
perature, 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 stand-
ard 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 vor-
texing. 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 sample solution 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 meth-
anol-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, band-
width), 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 chro-
matographic system and data collection were completely con-
trolled 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 in-
tegrated 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 regres-
sion was performed using a weighting factor of 1/y, 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%;
or otherwise, the sample was reassayed. Specimens with an esti-
mated 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 dif-
terence 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 dis-
position 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 (Sci-
entific Consulting, Apex, NC), as described previously (13, 14).
Weighting according to 1/y^2 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, t1/2 (half-life of the initial disposition phase),
Cl (half-life of the apparent terminal disposition phase), mean
residence time, Vf (apparent volume of distribution of the sam-
pled 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, pre-
treatment 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 pa-
tients 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 Coop-
erative Oncology Group performance status of 0 or 1. Seventeen
patients had a diagnosis of colorectal carcinoma, three had

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Phase I Trial of KRN5500
cancer died 18 days after beginning the first course of KRN5500 dose level 4. A 63-year-old man with refractory colorectal ondansetron and lorazepam required expansion of the cohort at grade 3 cerebellar ataxia in a patient concurrently receiving therapy are summarized in Table 2. The only significant toxicities observed during the first cycle of third dose level of 2.9 mg/m²/day without any notable drug-only toxicity greater than grade 2 was grade 3 diarrhea in a patient with metastatic colorectal cancer, died suddenly at home after receiving the fourth daily dose of 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 because the event occurred only 1 week after completion of the first cycle, while the second course of KRN5500 was being delivered. There were no DLTs in any of these patients, and the only toxicity greater than grade 2 was grade 3 diarrhea in a single patient. Similarly, three patients were treated with the third dose level of 2.9 mg/m²/day without any notable drug-related toxicities aside from grade 4 lymphopenia in one patient.

Life-threatening and fatal toxicity occurred at dose levels 4 (3.7 mg/m²/day) and 5 (4.9 mg/m²/day). The occurrence of grade 3 cerebellar ataxia in a patient concurrently receiving ondansetron and lorazepam required expansion of the cohort at dose level 4. A 63-year-old man with refractory colorectal cancer died 18 days after beginning the first course 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.

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

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<th>Table 1 Patient characteristics</th>
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<td>Characteristic</td>
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<td>Pancreatic</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Prior chemotherapy regimens</td>
</tr>
<tr>
<td>0–2</td>
</tr>
<tr>
<td>≥3</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
</tr>
</tbody>
</table>

<sup>a</sup> Eastern Cooperative Oncology Group performance status.
total of 15 standard curves run during a 12-week period. Mean values ± SD of the regression parameters for these standard curves were as follows: slope, 0.0322 ± 0.0057; y intercept, −0.013 ± 0.013; and correlation coefficient, 0.9985 ± 0.0015. The grand mean between-day accuracy was 99.7–102.7%, and the precision ranged from 3.8% to 14.2%. At the lowest concentration of KRN5500 included in the standard curves, values of the accuracy and precision were 102.7% and 14.2%, respectively.

Pharmacokinetics. 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/m²), 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 27.9% on day 1 and 19.6% to 32.7% on day 5. The coefficient of variation for the average $C_{\text{max}}$ in individual patients ranged from 4.4% 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

---

**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>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>0/0/0</td>
<td>4/0/0</td>
<td>0/0/0</td>
<td>2/0/1</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0/0/0</td>
<td>1/0/0</td>
<td>1/0/0</td>
<td>1/0/0</td>
<td>1/0/0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2/0/0</td>
<td>3/0/0</td>
<td>2/0/0</td>
<td>4/1/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>0/1/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Transaminis</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>0/1/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0/0/0</td>
<td>1/0/0</td>
<td>1/0/0</td>
<td>2/1/0</td>
<td>1/0/0</td>
</tr>
<tr>
<td>Pulmonary/dyspnea</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>1/0/0</td>
<td>2/0/1b</td>
<td>0/1/0</td>
</tr>
<tr>
<td>Neurologic/ataxia</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>1/1/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>0/1/1</td>
<td>1/0/0</td>
<td>0/0/0</td>
<td>1/4/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0/0/0</td>
<td>0/0/0</td>
<td>1/0/0</td>
<td>0/0/1</td>
<td>0/0/0</td>
</tr>
</tbody>
</table>

---

a DLTs occurring in the same patient who died due to respiratory failure.
b 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 (\( P < 0.01 \), two-tailed t test) in the eight patients who were at least 65 years old (4.6 ± 1.6 liters/m\(^2\)) in comparison with the 18 younger patients (7.1 ± 2.3 liters/h/m\(^2\)). 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., \( r > 0.4 \)) 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/m\(^2\)/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 death. The elevated bilirubin and serum transaminase levels
that were noted occurred predominantly in the context of pro-
gressive growth of preexisting liver metastases. Clinically sig-
nificant 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, al-
though these effects could be effectively controlled by pretreat-
ment with antiemetic drugs, especially 5-hydroxytryptamine
antagonists. Moderate diarrhea, lymphopenia, and thrombocy-
topenia were also frequently noted. There were no objective
responses to therapy with KRN5500 in the 17 evaluable pa-

tients. However, some patients appeared to experience clinical
benefit, indicated by disease stabilization persisting for 5–6
months in 3 of the 13 evaluable patients with colorectal cancer.

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 previ-
ously reported value of 5.41 liters/h/m² for the parameter in a
population-based pharmacokinetic analysis of data from a co-
hort 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 per-
formed in Japan (21). There was no significant difference be-
tween 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 enhanced
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 phar-
macokinetic 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 investiga-
tor 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. Sub-
group analysis revealed that the mean CL of KRN5500 in these
elderly patients was 35.2% lower than that observed in the youn-
ger 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 rea-
sonable 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
\( (i.e., M_r > 300) \) and the absence of measurable concentrations of
the parent drug in urines obtained from treated patients. Unfor-
tunately, 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 com-
prising 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, respec-

\( ^4 \) 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 \( (i.e., 60 \text{ ng/ml} ) \) for only 6 h. The dura-
tion of exposure to the drug appears to be a critical parameter for
inducing cytotoxicity in human tumor cell lines \( \text{in vitro} \) (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.\(^5\) Moreover, the concentration of drug necessary to effect
cell death decreases significantly on prolonging the exposure
period.

KRN5500 was formulated as a suspension and adminis-
tered by i.p. injection on a daily × 5 schedule during \( \text{in vivo} \)
efficacy studies against human tumor xenograft models per-
formed 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 maxi-

\( ^6 \) Results for the evaluation of KRN5500 in the NCI human tumor cell
line screen were obtained by accessing public data provided by the
Developmental Therapeutics Program, Division of Cancer Treatment
and Diagnosis, NCI at the following internet address: http://tp.nci.
.nih.gov.

\( ^3 \) Dr. David T. Vistica, personal communication.

\( ^4 \) Dr. Sherman S. Stinson, personal communication.
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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