A Phase I Study of Bizelesin (NSC 615291) in Patients with Advanced Solid Tumors[1]

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ABSTRACT

Purpose: **To evaluate the toxicities, characterize the pharmacokinetics, and determine the maximum-tolerated dose of bizelesin administered once every 4 weeks.**

Patients and Methods: **Patients with advanced solid tumors received escalating doses of bizelesin as an i.v. push every 4 weeks. Pharmacokinetic studies were performed with the first treatment cycle.**

Results: **Nineteen eligible patients received a total of 54 courses of bizelesin at doses ranging from 0.1 to 1 μg/m^2. Dose-limiting toxicity of neutropenia was seen in 2 of 4 patients treated at the 1 μg/m^2 dose level. Nonhematological toxicity was generally mild with maximum toxicity being ≤ grade 2 per National Cancer Institute Common Toxicity Criteria. No objective responses were seen in the 19 eligible patients. An L1210 bioassay was used to determine bizelesin plasma levels. The terminal elimination half-life was 140 min at the recommended Phase II dose. The area under the concentration time curve increased in proportion to administered dose, and the clearance remained constant over the dose range studied. Correlation analysis demonstrated relationships between dose and area under the concentration with cycle 1 hematological parameters, including absolute neutrophil and leukocyte nadirs.**

Conclusion: **Bizelesin administered every 4 weeks as an i.v. push is well tolerated with dose-limiting toxicity of neutropenia. The maximum-tolerated dose (and recommended Phase II dose) is 0.8 μg/m^2 administered once every 4 weeks.**

INTRODUCTION

Agents that interact with DNA have been a common source for drug development in clinical cancer research over many years. New drugs that exhibit novel mechanisms of action may prove important in advancing clinical cancer research. The CPI[4] antitumor antibiotic, CC-1065, was first isolated in the late 1970s from fermentation products of the bacteria *Streptomyces zelenis* (1). The structure of CC-1065 was determined by Martin *et al.* (2) and was found to bind selectively to A-T-rich regions of DNA interacting covalently at the N^3 position of adenine (3). Although CC-1065 showed promising efficacy in preclinical testing, development was terminated when irreversible hepatic and renal toxicity with delayed deaths were observed in mice (4).

Subsequent research was directed at the synthesis of CC-1065 analogues to find compounds, which would maintain drug potency but with less toxicity (5). Analogues that have been produced include carzelesin, adozelesin, and bizelesin (Fig. 1). Carzelesin and adozelesin are monofunctional analogues of CC-1065. Phase I testing with adozelesin identified myelosuppression (primarily thrombocytopenia and leukopenia) as the DLT (6). Bizelesin is a symmetrical dimer of CC-1065, in which the linker consists of two indole subunits separated by a ureido group (7). Bizelesin is unique among the CPI-related compounds in its bifunctional alkylating capability. The two chloromethyl moieties in each molecule can be converted to cyclopropyl alkylating species (via intermolecular rearrangement), both of which may interact with DNA. This product, U-77809, has been synthesized and found to be equipotent with bizelesin (8). pM concentrations of bizelesin effectively inhibited the growth of several human tumor cell lines (3, 9). The MTD of bizelesin in dogs, the most sensitive species, was 2 μg/m^2. Preliminary testing of *in vitro* myelotoxicity with bizelesin demonstrated significant (>100,000-fold) differences between species, which prompted a more detailed comparison of human, canine, and murine myeloid progenitor cells (10). The results of canine and murine *in vitro* clonal assays were consistent with those of the whole animal studies, indicating that bizelesin would be a potent myelosuppressive agent in the clinic. A starting dose of 0.1 μg/m^2 was chosen for this Phase I trial on the basis of greater *in vitro* myelotoxicity and lower MTD in dogs as compared with mice. This starting dose was 100–240-fold lower than the starting dose of other cyclopropylisoindole analogues (6, 11).

We performed a Phase I study of bizelesin administered as a brief i.v. injection every 4 weeks to determine the MTD,

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[4] The abbreviations used are: CPI, cyclopropyl-pyrroloindole; MTD, maximum-tolerated dose; DLT, dose-limiting toxicity; NCI, National Cancer Institute; CTC, Common Toxicity Criteria; AUC, area under the curve.

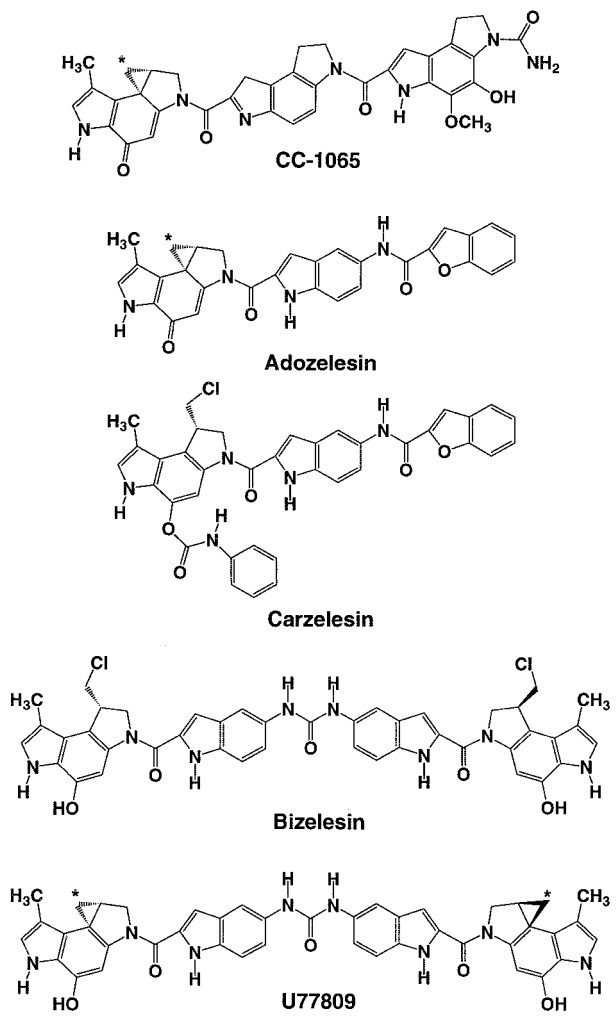

Fig. 1 Structure of bizelesin and other CPI analogues (*, active cyclopropyl functional group).

pharmacokinetic characteristics, and biological effects of this compound in patients with advanced solid tumors.

MATERIALS AND METHODS

Patients. All patients participating in this study had histological confirmation of a nonlymphoid, nonleukemic malignancy, for which there was no known standard therapy that was curative or capable of extending life expectancy. Additional eligibility criteria at study entry consisted of Eastern Cooperative Oncology Group performance status $\leq$2, age $\geq$18 years, WBC count $\geq$3,500/mm^3, absolute granulocyte count $>$1,500/mm^3, platelet count $\geq$130,000/mm^3, hemoglobin level $\geq$10 grams/dl, serum creatinine $\leq$0.3 mg/dl above the upper limit of normal, creatinine clearance $>$60 ml/min, normal direct bilirubin, aspartate aminotransferase $\leq$1.5 times the upper limit of normal, oral intake $\geq$1,200 calories/day, and life expectancy $>$12 weeks. Written informed consent was obtained from all patients before study entry. Patient exclusion criteria included prior chemotherapy or biological therapy within 4 weeks of study entry, any prior mitomycin C or nitrosourea chemotherapy, radiation therapy within the 3 weeks preceding entry or any previous radiation therapy to $>$10% of the bone marrow, uncontrolled infection, New York Heart Association class III or IV heart disease, pregnancy or lactation, unwillingness to practice adequate contraception by men or women of child-bearing age, central nervous system metastases, seizure disorder, or primary central nervous system tumor. The study was approved and monitored by the Institutional Review Board of the Mayo Clinic and Mayo Foundation.

Study Design. Bizelesin was administered as a single peripheral i.v. push into a free-flowing line of normal saline once every 4 weeks. The study drug was supplied by the NCI (Bethesda, Maryland) in a 2-ml sterile vial containing 5 μg/ml bizelesin in 1 ml of special diluent. The special diluent consisted of 1 ml of PET solvent (six parts PEG 400, three parts ethanol, and one part Tween 80), 1 mg of citric acid, and 1 ml of 0.9% sodium chloride. Patients were treated until disease progression or unacceptable toxicity. The starting dose of bizelesin was 0.1 μg/m^2. Doses were doubled until biological activity was noted (*e.g.,* grade-2 reproducible and reversible hematological or nonhematological toxicity). Additional doses were escalated by 25% increments. A minimum of 3 patients was treated at each dose level, and no intrapatient dose escalation was allowed. All patients at a given dose level were observed for at least 4 weeks before new patients were treated at a higher dose level.

Prestudy evaluations included a complete history and physical exam, including height, weight, performance score, and tumor measurement; complete blood count; serum chemistries; chest X-ray; urinalysis; electrocardiogram; short renal clearance; indicator lesion imaging (magnetic resonance imaging, computed tomography scan, or ultrasound); and a serum pregnancy test in women of child-bearing potential. All patients underwent an interim history and physical examination before each subsequent cycle of therapy. Complete blood counts were performed twice weekly during the interval between treatments and weekly after the patient was at a stable dose. Serum chemistry tests were performed weekly during the interval between treatments. The first cycle of therapy for each patient was administered on an inpatient basis in the Mayo General Clinical Research Center to expedite pharmacokinetic sampling, and subsequent cycles were administered on an outpatient basis.

The DLT was defined as grade-4 hematological toxicity for $\geq$5 days with or without febrile neutropenia or $\geq$grade-3 nonhematological toxicity as per NCI CTC, except for nausea and vomiting, for which grade-4 toxicity was dose limiting. DLTs were evaluated for the first cycle only. The MTD was defined as the dose level which, at most, 1 of 6 patients experienced DLT, and the next higher dose level produced DLT in at least 2 of 6 patients. Once the DLT was observed, 3 more patients were treated at the previous dose level to better characterize the MTD.

Growth Inhibition Assay. Allometric scaling of preclinical data (8) predicted patient bizelesin plasma concentrations far below values detectable by standard analytical methods; an L1210 bioassay was used to determine plasma concentrations. Exponentially growing (2–3 $\times$ 10^6 cells/ml) mouse leukemia L1210 cells (American Type Culture Collection, Rockville, MD) were maintained in RPMI medium supplemented with 10% FCS at 37°C under a humidified atmosphere (100% rela-

Table 1 Patient characteristics ($n = 19$)

Median age, years (range)	62 (35–77)
Gender (M/F)	12/7
ECOG PS 0/1[a]	12/7
Tumor type	
Colorectal	9
Upper gastrointestinal	3
Breast	2
Other (bile duct, lung, ovary, prostate, renal)	5
Prior therapy	
Chemotherapy	18
Radiation therapy	8
Immunotherapy	4
Hormonal therapy	3

[a] Eastern Cooperative Oncology Group Performance Score.

Table 2 Bizelesin dose levels

Dose level ($\mu g/m^2$)	No. of patients	No. of cycles
0.1	3	9
0.2	3	3
0.4	3	6
0.8	6	19
1.0	4	17

Table 3 Cycle 1 hematologic toxicity

		Leukocyte[a]				Neutrophil[a]			
Dose level ($\mu g/m^2$)	No. of patients	1	2	3	4	1	2	3	4
0.1	3	0	0	0	0	0	0	0	0
0.2	3	3	0	0	0	3	0	0	0
0.4	3	1	1	0	0	2	0	0	0
0.8	6	2	2	2	0	1	1	2	1
1.0	4	0	1	3	0	0	0	2	2

[a] NCI CTC grades.

tive humidity) of 5% CO_2:95% air. The day before analysis, culture medium was aspirated from the flask, and fresh medium was added to reduce the cell numbers to $5–8 \times 10^5$ cells/ml. Standard curve samples were prepared by diluting bizelesin (Pharmaceutical Resources Branch, Division of Cancer Treatment, NCI) stock solutions (100 μM in DMSO) to concentrations of 100-3200 pM in DMSO, followed by a dilution to 5–160 pM in a 1:1 mixture of cell culture medium:normal human plasma. Aliquots of these standards (0.158 ml/3-ml incubation) were added to flasks containing medium and 1.2×10^5 L1210 cells. The final concentration of drug in the flasks was 0.25–8 pM. All standard curve flasks contained 0.25% DMSO and 2.4% plasma, which have been shown to have no effect on L1210 cell growth (8).

Patient plasma samples were diluted with cell culture medium or 1:1 cell culture medium:normal human plasma to maintain 2.5–3% plasma/flask. Plasma samples were diluted 1:2–1:20 with a 1:1 mixture of cell culture medium and normal plasma as needed to achieve cell growth inhibition in the linear range of the standard curve. Aliquots (0.158 ml) of these dilutions were added to flasks containing 3 ml of medium and 1.2×10^5 L1210 cells. Patient urine samples were analyzed in a similar manner, except that standard curve samples and patient sample dilutions were made in 1:1 cell culture medium:normal human urine. After 48-h incubation in 5% CO_2:95% air at 37°C, culture aliquots (1 ml) were diluted with Isoton II (19 ml), and cell numbers were determined with a Coulter Counter (Coulter Corp., Miami, FL).

L1210 bioassay standard curves were linear in the range of 25–160 pM bizelesin in plasma, which corresponds to 0.75–4 pM in the culture flasks, attributable to the 40-fold dilution during preparation of assay samples. IC_{50}s for bizelesin standard curves (2.4–3% plasma in culture medium) ranged from 40 to 68.5 pM with a mean ±SD value of 53.2 ± 7.73 pM ($n = 31$) based on the concentration of drug in undiluted plasma. The corresponding IC_{50} of 1.6 pM bizelesin in the culture flask was similar to the IC_{50}s of 1.5 pM found when bizelesin was added directly to culture medium (8). The value of the coefficient of variation of the slopes of standard curves was 10.2% ($n = 31$). Plasma quality assurance samples (32, 90, 120, and 3000 pM) had a coefficient of variation values of 13.7–18.5%, and mean calculated values were within ±10% of expected values during the course of the trial. The limit of quantitation was 25 pM. The linear ranges (25–160 pM) and limit of quantitation (25 pM) for the urine assays were similar to those for the plasma assays.

Pharmacokinetics. On day 1 of the first cycle of treatment, blood (5 ml) was collected from patients in heparinized tubes before administration and at the following times after i.v. administration of bizelesin: 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 360, 480, 720, 960, and 1440 min. Plasma specimens were stored at −70°C until analysis after centrifugation to separate plasma from RBCs. Urine was collected in separate plastic containers during the intervals of 0–4, 4–8, and 8–24 h after drug administration. After the urine volume was recorded, a 20-ml aliquot was frozen for drug analysis.

Data Analysis. Growth inhibition was expressed as a percentage of cells in drug-treated flasks relative to the number of cells in control flasks. The linear portion of the graph of percentage growth inhibition *versus* log (drug concentration) was used to determine IC_{50}s and calculate nM concentrations of drug equivalents in patient plasma and urine samples. Plasma drug concentrations were fitted by noncompartmental analysis using WINNONLIN (version 1.5; Scientific Consulting, Inc., Covy, NC).

Statistical Analysis. Toxicity and pharmacology data were analyzed primarily in a descriptive fashion. The number and severity of toxic incidents indicated the level of tolerance for bizelesin in the treatment of advanced cancer. Hematological toxicity measures of neutropenia, leukopenia, and thrombocytopenia were assessed using the continuous variables as outcome measures (primarily nadir and percentage change from baseline values), as well as categorization via NCI CTC standard toxicity grading. Nonhematological toxicities were evaluated via the ordinal NCI CTC standard toxicity grading only. Frequency distributions and other descriptive measures formed the basis of the analysis of these variables.

Normality testing was carried out via standard Shapiro-Wilk testing (12). Correlation among the pharmacodynamic outcome measures and other hematological values (neutrophil nadir and percentage change in neutrophil counts from baseline)

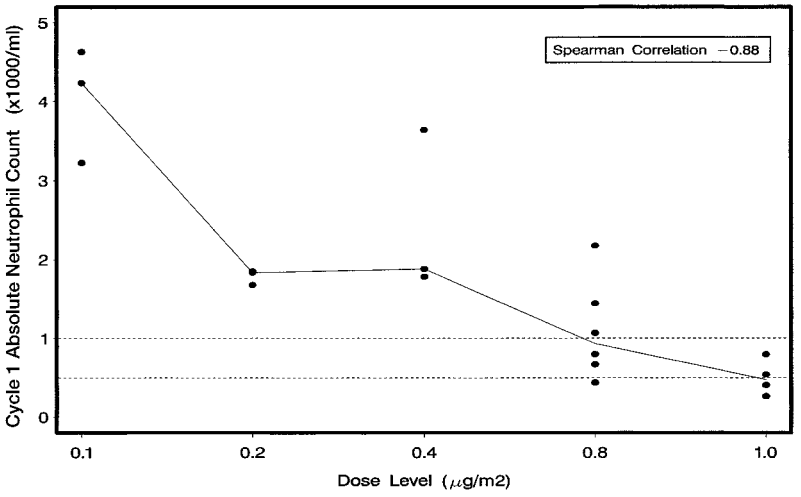

Fig. 2 Cycle 1 absolute neutrophil count nadir *versus* dose level. Median ANC represented by ——— and grades 3/4 NCI hematological CTC illustrated by ----.

was carried out via simple graphics and Spearman's ρ coefficient. Paired *t* tests and Wilcoxon procedures were used to assess average intrapatient changes in these and other variables. Exploratory analysis was also carried out on time-related variables, including time until any treatment-related toxicity, time until treatment-related grade ≥3 toxicity, and time until hematological nadirs (leukocyte, neutrophil, and platelet). Summary statistics were supplemented by Kaplan-Meier survival estimates and related confidence intervals (13, 14).

RESULTS

Toxicity. A total of 19 patients with advanced cancer was entered and treated in this study. Patient characteristics are summarized in Table 1. The dose escalation scheme and number of courses for bizelesin are shown in Table 2. A total of 54 assessable courses of bizelesin was administered (median: 2 courses; range: 1–12 courses). No objective responses were observed among the 19 evaluable patients. One patient with renal cell carcinoma receiving 0.8 μg/m^2 had stable disease for nine cycles of protocol treatment, whereas another patient with rectal carcinoma receiving 1 μg/m^2 remained on treatment for 12 cycles. The DLT of neutropenia was observed at the 1 μg/m^2 dose level (Table 3). Two of 4 patients at this dose level experienced grade-4 neutropenia lasting 7 and 5 days. Only 1 of 6 patients at 0.8 μg/m^2 experienced grade-4 neutropenia lasting 4 days. The cycle 1 neutrophil nadirs for all patients are presented in Fig. 2. The median time to neutrophil nadir for patients experiencing grade-3 or -4 neutropenia during the first course ($n = 7$) was 14 days (range: 12–16 days) with the median time to neutrophil recovery being 5 days. Other hematological toxicities were mild with one episode of grade-2 anemia and one episode of grade-1 thrombocytopenia at the 0.4 and 0.8 μg/m^2 dose levels, respectively. Nonhematological toxicity was generally mild and limited to ≤NCI CTC grade 2 (data not shown). These mild toxicities tended to be gastrointestinal symptoms, including anorexia, diarrhea, nausea, and weight loss. Patients also reported malaise, facial flushing, and headache, which were all mild in nature and felt to be related to treatment.

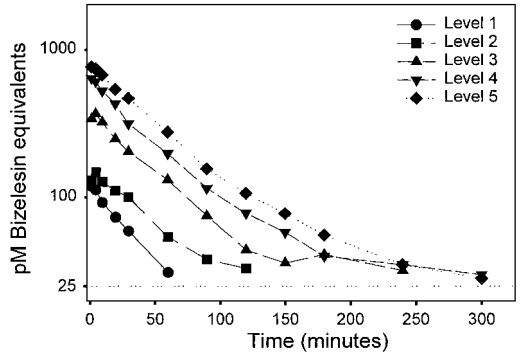

Fig. 3 Representative plasma drug concentrations for patients treated with bizelesin. *Level 1,* 0.1 μg/m^2; *Level 2,* 0.2 μg/m^2; *Level 3,* 0.4 μg/m^2; *Level 4,* 0.8 μg/m^2; and *Level 5,* 1 μg/m^2. The lower limit of assay sensitivity is illustrated by the ···· at 25 pM bizelesin equivalents.

Patient Pharmacokinetics. The patient plasma and urine bizelesin concentration data are reported in molar drug equivalents that represent combined antiproliferative activity of bizelesin and its cytotoxic metabolites. The mean plasma profiles of bizelesin for each dose level are illustrated in Fig. 3. Bizelesin drug equivalents were detectable for 60 min after administration of the lowest dose (0.1 μg/m^2) and for 5 h after administration of the highest doses (0.8–1 μg/m^2). Whereas mono-exponential decline of plasma bizelesin equivalents was observed at the lowest dose (0.1 μg/m^2), a biexponential decline was observed at all subsequent doses (0.2–1 μg/m^2). Noncompartmental analysis was performed to compare the pharmacokinetic parameters among all patients (Table 4) over the five dose levels. The apparent terminal elimination half-life of bizelesin increased during dose escalation and was associated with detection of bizelesin for longer periods of time. The mean half-life observed at the recommended Phase II dose was 140 min. Bizelesin pharmacokinetics were linear based on the dose proportional increase in AUC values and constant mean plasma clearance values for the five dose levels (Fig. 4). Urinary

Table 4 Summary of bizelesin pharmacokinetic parameters

Level	Dose ($\mu g/m^2$)	Patient	AUC (pM × min)	$t_{1/2z}$ (min)	Cl (ml/min/m^2)	V_{ss} (ml/m^2)
1	0.1	1	4570	32.7	26.7	1160
		2	6770	38.4	18.1	990
		3	13300	104	9.17	1430
Mean			8220	58.4	18.0	1200
SD			4570	39.7	8.8	220
2	0.2	4	12000	74.3	20.3	1910
		5	11900	56.7	20.1	1710
		6	12200	69.5	20.2	1800
Mean			12000	66.8	20.2	1810
SD			135	9.12	0.1	100
3	0.4	7	19100	85.7	25.7	2200
		8	19000	60.0	25.8	1720
		9	35900	117	13.8	1600
Mean			24600	87.8	21.7	1840
SD			9800	28.9	6.9	320
4	0.8	10	47400	197	20.8	3250
		11	44300	56.2	22.1	1640
		12	54200	188	18.1	2610
		17	50800	120	19.6	2090
Mean			49200	140	20.1	2400
SD			4280	66	1.7	690
5	1.0	13	60600	134	20.2	2140
		14	53400	92.3	22.9	2150
		15	41400	135	29.6	3040
		16	45900	57.4	27.1	2220
Mean			50300	105	24.9	2390
SD			8450	37	4.2	440

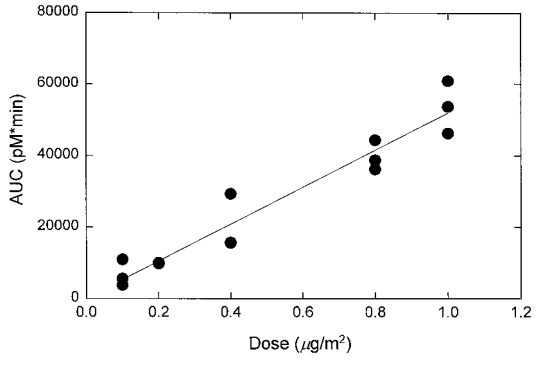

Fig. 4 AUC *versus* dose level.

recovery was determined in 3 patients who received 1 $\mu g/m^2$ bizelesin. Excretion of bizelesin drug equivalents during 24 h as a percentage of administered dose was <1%. No further analysis of urine from patients was performed because there was no evidence of significant urinary excretion at the highest dose level.

An exploratory analysis was performed to evaluate the relationship between pharmacokinetic parameters and toxicity. All relationships between dose and AUC with leukocyte and neutrophil cycle 1 nadirs and percentage changes from baseline were statistically significant with Spearman correlation coefficients ranging from 0.66 to 0.88 (Table 5). These data are preliminary given the size of the sample and inherent variability in correlation data. No relationships were found between any pharmacokinetic parameters and nonhematological toxicities.

DISCUSSION

Bizelesin is an extremely potent bifunctional alkylating agent with preclinical activity against a variety of tumor cell lines. The current study was performed to determine the toxicity and MTD of bizelesin administered as an i.v. push once every 4 weeks. We observed neutropenia as the DLT in this study. Myelosuppression has also been the major toxicity observed in clinical trials with other CPI compounds, including adozelesin (6, 15). Another Phase I study of bizelesin has demonstrated similar findings to our study with myelosuppression as the DLT (16). Dose-limiting side effects were observed at a similar dose level of 1.26 $\mu g/m^2$ as compared with 1 $\mu g/m^2$ in our study.

Bizelesin plasma concentrations were determined using an extremely sensitive, but nonspecific, bioassay of drug cytotoxicity against L1210 murine leukemia cells. The assay is useful because it measures the presence of potentially active and toxic species present after administration of the parent molecule. Bizelesin is converted in aqueous solution to the stable dicyclopropylpyrroloindole molecule U-77809 (Fig. 1) via intermolecular rearrangement. Both molecules have equivalent potency against L1210 tumor cells (8).

The L1210 bioassay has been used to characterize the pharmacokinetics of bizelesin (8) and other potent antitumor agents in preclinical studies (17, 18) and of dolastatin-10 in a Phase I clinical trial (19). It should be emphasized that the bioassay lacks specificity and does not distinguish between parent drug and cytotoxic degradation products and/or metabolites, which may contribute to growth inhibitory activity. This is important because bizelesin is rapidly hydrolyzed to the corresponding

Table 5 Spearman toxicity correlation coefficients

Hematology value	Dose level correlation coefficients	Dose level *P*	AUC correlation coefficients	AUC *P*
Neutrophil percentage change from baseline	0.85	0.0001	0.76	0.0007
Leukocyte percentage change from baseline	0.87	0.0001	0.72	0.0016
Neutrophil cycle 1 nadir	−0.88	0.0001	−0.73	0.0014
Leukocyte cycle 1 nadir	−0.85	0.0001	−0.66	0.0058

active cyclopropylisoindole molecules (8). Bizelesin pharmacokinetics in this group of patients with solid tumors were linear in the dose range studied in this trial. Although plasma half-life appeared to increase with increasing dose, AUC increased in proportion to dose, and clearance remained constant. This result is consistent with being able to adequately characterize the terminal elimination phase of the plasma profile at higher dose levels but not at the lower ones. The low volume of distribution values suggest that bizelesin distributes principally in total blood volume with modest tissue uptake. We found little evidence for urinary excretion of bizelesin or cytotoxic metabolites.

The lower MTD in humans (1 $\mu g/m^2$) compared with mice (30 $\mu g/m^2$) after a single dose of bizelesin is most likely attributable to greater sensitivity of human myeloid progenitor cells to bizelesin, as compared with murine cells, rather than differences in pharmacokinetics. The bizelesin clearance value in humans was ~50% of the value found in mice (8). The bizelesin concentration at which 100% colony inhibition occurred was three logs lower for human granulocyte-macrophage formation than for mouse granulocyte-macrophage formation (10).

We have demonstrated that bizelesin can be given readily and safely administered as an i.v. push every 4 weeks. The DLT on this schedule was neutropenia. The MTD and recommended Phase II dose for additional studies are 0.8 $\mu g/m^2$ given every 4 weeks. As neutropenia was dose limiting without significant thrombocytopenia or other toxicities, studies of this agent in acute leukemia may be warranted.

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REFERENCES

1. Hanka, L. J., Dietz, A., Gerpheide, S. A., Kuentzel, S. L., and Martin, D. G. CC-1065 (NSC-298223), a new antitumor antibiotic production, *in vitro* biological activity, microbiological assays and taxonomy of the producing microorganism. J. Antibiot., *31:* 1211–1217, 1978.

2. Martin, D. J., Chidester, C. G., Duchamp, D. J., and Mizsak, S. A. Structure of CC-1065 (NSC- 298223), a new antitumor antibiotic. J. Antibiot., *33:* 902–903, 1980.

3. Lee, C. S., and Gibson, N. W. DNA damage and differential cytotoxicity produced in human carcinoma cells by CC-1065 analogues, U-73,975 and U-77,779. Cancer Res., *51:* 6586–6591, 1991.

4. McGovren, J. P., Clarke, G. L., Pratt, E. A., and DeKoning, T. F. Preliminary toxicity studies with the DNA-binding antibiotic, CC-1065. J. Antibiot., *37:* 63–70, 1984.

5. Warpehoski, M. A. CC-1065 analogs: therapeutic potential of a sequence specific drug-DNA reaction. Proc. Am. Assoc. Cancer Res., *30:* 668–669, 1989.

6. Shamdas, G. J., Alberts, D. S., Modiano, M., Wiggins, C., Power, J., Kasunic, D. A., Elfring, G. L., and Earhart, R. H. Phase I study of adozelesin (U-73,975) in patients with solid tumors. Anti-Cancer Drugs, *5:* 10–14, 1994.

7. Ding, Z. M., and Hurley, L. H. DNA interstrand cross-linking, DNA sequence specificity, and induced conformational changes produced by a dimeric analog of (+)-CC-1065. Anti-Cancer Drug Design, *6:* 427–452, 1991.

8. Walker, D. L., Reid, J. M., and Ames, M. M. Preclinical pharmacology of bizelesin, a potent bifunctional analog of the DNA-binding antibiotic CC-1065. Cancer Chemother. Pharmacol., *34:* 317–322, 1994.

9. Carter, C. A., Waud, W. R., Li, L. H., DeKoning, T. F., McGovren, J. P., and Plowman, J. Preclinical antitumor activity of bizelesin in mice. Clin. Cancer Res., *2:* 1143–1149, 1996.

10. Volpe, D. A., Tomaszewski, J. E., Parchment, R. E., Garg, A., Flora, K. P., Murphy, M. J., and Grieshaber, C. K. Myelotoxic effects of the bifunctional alkylating agent bizelesin on human, canine and murine myeloid progenitor cells. Cancer Chemother. Pharmacol., *39:* 143–149, 1996.

11. Awada, A., Punt, C. J., Piccart, M. J., Van Tellingen, O., Van Manen, L., Kerger, J., Groot, Y., Wanders, J., Verweij, J., and Wagener, D. J. Phase I study of carzelesin (U-80,244) given (4-weekly) by intravenous bolus schedule. Br. J. Cancer, *79:* 1454–1461, 1999.

12. Shapiro, S. S., and Wilk, M. B. An analysis for variance test for normality. Biometrika, *52:* 591–610, 1965.

13. Lee, E. T. (eds.). Statistical Methods for Survival Analysis, Ed. 2, pp 66–92. New York: John Wiley & Sons, 1992.

14. Pepe, M. S., and Fleming, T. R. Weighted Kaplan-Meier statistics. Large sample and optimality considerations. J. Stat. Soc. B, *53:* 341–352, 1991.

15. Burris, H., Earhart, R., Kuhn, J., Shaffer, D., Smith, L., Weiss, G., Kasunic, D., Radbury, G., Capmbell, L., and Von Hoff, D. A phase I trial of adozelesin, a novel DNA sequence-specific alkylating agent. Proc. Am. Assoc. Cancer Res., *33:* 520, 1992.

16. Schwartz, G. H., Aylesworth, C., Stephenson, J., Johnson, T., Campbell, E., Hammond, L., Von Hoff, D. D., and Rowinsky, E. K. Phase I trial of bizelesin using a single bolus infusion given every 28 days in patients with advanced cancer. Proc. Am. Soc. Clin. Oncol., *19:* 235A, 2000.

17. Reid, J. M., Walker, D. L., and Ames, M. M. Preclinical pharmacology of ecteinascidin 729, a marine natural product with potent antitumor activity. Cancer Chemother. Pharmacol., *38:* 320–334, 1996.

18. Reid, J. M., Walker, D. L., and Ames, M. M. Evaluation of an L1210 bioassay and HPLC for determination of dolastatin-10 in human and murine plasma. Proc. Am. Assoc. Cancer Res., *36:* 364, 1995.

19. Pitot, H. C., McElroy, E. A. Jr, Reid, J. M., Windebank, A. J., Sloan, J. A., Erlichman, C., Bagniewski, P. G., Walker, D. L., Rubin, J., Goldberg, R. M., Adjei, A. A., and Ames, M. M. Phase I trial of dolastatin-10 (NSC 376128) in patients with advanced solid tumors. Clin. Cancer Res., *5:* 525–531, 1999.