A phase I study of bizelesin, a highly potent and selective DNA-interactive agent, in patients with advanced solid malignancies


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Background: The aim of this study was to assess the feasibility of administering bizelesin, a cyclopropylpyrroloindole with extraordinarily high potency as a bifunctional DNA-damaging agent and selectivity for specific AT-rich DNA sequences, as a single i.v. bolus injection every 4 weeks in patients with advanced solid malignancies. The study also sought to determine the maximum tolerated dose (MTD) of bizelesin, characterize its pharmacokinetic behavior, and seek preliminary evidence of anticancer activity.

Patients and methods: Patients with advanced solid malignancies were treated with escalating doses of bizelesin as an i.v. bolus injection every 4 weeks. The selection of the specific starting dose, 0.1 µg/m², which was equivalent to one-tenth the toxic dose low in dogs, factored in large interspecies differences in myelotoxicity as gauged using an ex vivo hematopoietic colony-forming assay. Due to concerns about the high potency of bizelesin and the large interspecies differences in toxicity, a conservative dose-escalation scheme was used for dose-level assignment to determine the MTD levels for both minimally pretreated (MP) and heavily pretreated (HP) patients. A variety of analytical assays were assessed to reliably measure bizelesin concentrations in plasma.

Results: Sixty-two patients were treated with 185 courses of bizelesin at eight dose levels ranging from 0.1 to 1.5 µg/m². Myelosuppression, principally neutropenia that was always brief, was the most common toxicity observed. Thrombocytopenia and anemia were uncommon and severe non-hematological effects were not observed. Severe neutropenia alone and/or associated with fever was consistently experienced by HP and MP patients at doses exceeding 0.71 and 1.26 µg/m², respectively. These doses also resulted in functionally non-cumulative myelosuppression as repetitive treatment was well-tolerated. A 40% reduction in measurable disease lasting 24 months was noted in a patient with advanced ovarian carcinoma. Various analytical methods were evaluated but none demonstrated the requisite sensitivity to reliably quantify the minute plasma concentrations of bizelesin and metabolites resulting from administering microgram quantities of drug.

Conclusions: The highly potent and unique cytotoxic agent, bizelesin can be feasibly administered to patients with advanced solid malignancies. The recommended doses for phase II studies of bizelesin as a bolus i.v. injection every 4 weeks are 0.71 and 1.26 µg/m² in HP and MP patients, respectively. The characteristics of the myelosuppression, the paucity of severe toxicities with repetitive treatment, the preliminary antitumor activity noted, and, above all, its unique mechanism of action as a selective DNA-damaging agent and high potency, warrant disease-directed evaluations of bizelesin in solid and hematopoietic malignancies and consideration of its use as a cytotoxic in targeted conjugated therapeutics.

Key words: bizelesin, cyclopropylpyrroloindole, DNA-active agent, phase I study

Introduction

The prototypical cyclopropylpyrroloindole (CPI) antitumor antibiotic, CC-1065 (Figure 1), was isolated from the fermentation products of the soil organism Streptomyces zelensis in the mid-1970s [1]. Although CC-1065 had a unique structure, unprecedented in vitro and in vivo potency (IC₉₀ for L1210 leukemia = 0.05 ng/ml), an apparently novel mechanism of action by non-intercalative covalent binding to specific AT-rich regions in the minor groove of DNA, and notable activity in several murine tumor screening systems, delayed lethality (>30 days after a single intravenous (i.v.) injection) accompanied by irreversible hepatic and renal toxicities within the therapeutic range, precluded its further development [2–9]. Other CPI analogs, including adozelesin, carzelesin and bizelesin, were

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subsequently synthesized as part of an effort to develop CPI analogs with mechanistic and cytotoxic features similar to CC-1065, but devoid of its delayed toxicity [9]. Although both adozelesin and carzelesin underwent clinical evaluation and major antitumor responses were observed, the overall magnitude of anticancer activity was unimpressive at doses associated with dose-limiting myelosuppression [9–19].

In contrast to CC-1065, adozelesin and carzelesin, bizelesin (Figure 1), which is made up of two chloromethyl functional groups linked by an indole-ureido-indole tether, is a bifunctional alkylating agent capable of forming DNA interstrand cross-links [9, 18–26]. Bizelesin, which is 10000 times more potent than its corresponding mono-CPI unit and is rapidly converted to the CPI form in vitro, alkylates the N2 position of adenines on opposite DNA strands in the minor groove in vitro and in vivo [9, 18, 20]. The interstrand cross-links resulting from bizelesin’s bifunctional alkylating capacity are highly lethal and differentiate the agent from other CPI analogs, which produce only monoadducts and are about 30-fold less potent than bizelesin [9, 18, 20]. In fact, bizelesin at picomolar concentrations produces appreciable cytotoxicity (>6.7 \log_{10} cell kill) against intraperitoneally implanted P388 and L1210 leukemia and 80% tumor-free survivors against subcutaneously implanted L1210. Impressive antitumor activity was also observed against a wide range of human tumor xenografts of colon, lung and breast origin at low microgram doses, and the agent was as active in tumors with acquired resistance to cisplatin and other alkylating agents as against the parental tumors [9, 28]. Bizelesin also demonstrated schedule-independent activity; however, greater total doses were tolerated on more protracted schedules.

The toxicology of bizelesin has been evaluated in rodents and dogs; however, difficulties associated with the development of suitable analytical assays capable of reliably measuring the concentrations achieved following administration of microgram doses precluded robust preclinical pharmacological evaluations. Hematopoietic, gastrointestinal and lymphoid tissues were most

Figure 1. Molecular structures of CC-1065 and bizelesin, and bizelesin’s active metabolite U-77809.
prone to the toxic effects of bizelesin, and non-cumulative myelosuppression was the principal dose-limiting toxicity (DLT) in both rodents and dogs [9, 28]. In rodents, recovery of weight loss was not attained until 16–30 days after treatment and therapeutic doses of bizelesin did not produce delayed deaths [9, 28]. Considerable interspecies differences in drug tolerance, with dogs being more susceptible to toxicity than rodents, prompted a more detailed comparison of the relative potency of bizelesin in human, canine and murine progenitor cell assays for starting-dose derivation in clinical trials [27].

The results of the aforementioned studies indicated that bizelesin may be a more attractive CPI analog based on its potency, unique DNA cross-linking features, and antitumor activity than other CPI analogs. The principal objectives of this study were to (i) determine the maximum-tolerated dose (MTD) of bizelesin administered as an i.v. bolus injection every 4 weeks in both minimally pretreated (MP) and heavily pretreated (HP) patients with advanced solid malignancies, and recommend doses for phase II trials; (ii) characterize the toxicities associated with this schedule of administration; (iii) describe the pharmacology of bizelesin administered in this schedule following development of a suitable analytical assay; and (iv) seek preliminary evidence for antitumor activity.

**Patients and methods**

**Patient selection**

Patients with histologically confirmed advanced solid malignancies that failed to respond to standard therapy or for whom adequate therapy was not available were eligible for this study. Eligibility criteria also included: age ≥18 years; an Eastern Cooperative Oncology Group performance status ≤2; life-expectancy ≥3 months; no prior chemotherapy, investigational medications or wide-field radiation therapy (>25% of the bone marrow) within 4 weeks of treatment (6 weeks for nitrosoureas and mitomycin C); adequate hematopoietic [absolute neutrophil count (ANC) ≥1500/µl, hemoglobin level ≥9.0 g/dl, platelet count ≥100000/µl]; hepatic (total bilirubin ≤1.5 mg/dl, hepatic transaminases ≤2.5 × institutional normal upper limit), and renal (serum creatinine within normal institutional limits or creatinine clearance ≥60 ml/min) functions; measurable or evaluable disease; prothrombin time ≤1.5 × institutional upper normal limit; no history of brain metastases and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients gave written informed consent according to federal and institutional guidelines before treatment.

**Dosage and drug administration**

The starting dose of bizelesin was 0.1 µg/m² every 4 weeks, which was equivalent to one-tenth the toxic dose low in dogs. The selection of this dose was based on the large interspecies differences in susceptibility to toxicity, principally hematological, with dogs being much more sensitive than rodents, and human hematopoietic cells being three times more sensitive than canine cells in ex vivo colony-forming assays [27, 28]. A similar rationale was successfully used to determine the starting doses for adozlesin and carzelesin for phase I evaluations [10–13, 15, 16]. A minimum of three patients were entered at dose levels that were not associated with DLT, with the first subject observed for at least 3 weeks before additional patients were treated. At least two patients must have completed 4 weeks of observation and a third patient must have completed 2 weeks of observation before dose escalation could proceed. Inpatient dose escalation was not permitted. In the event of drug-related toxicity that did not exceed grade 1 in severity, the dose could be increased in the next new patient by a maximum increment of 100%. In the event of grade ≥2 drug-related toxicity that was not dose-limiting, the maximum increment of dose escalation was 33%. If DLT was observed in any of the first three patients at a given dose level, up to six total new patients were to be treated at that specific dose level. The MTD was defined as the highest dose at which less than two of six new patients experienced DLT in the first course. Intermediate doses could be evaluated in the event of widely disparate rates of DLT between two successive dose levels. Following determination of the MTD, additional patients were to be treated to ascertain additional information about the tolerance of repetitive treatment at the MTD, which was utilized to recommend a dose for phase II studies. Intra-individual dose reduction by one level was permitted for individuals who experienced DLT. DLT was defined as: (i) grade ≥3 non-hematological toxicity (excluding nausea, vomiting or diarrhea without optimal premedication and/or supportive measures or grade 3/4 toxicity that is transient and not considered to be of sufficient severity to influence subsequent drug administration; idiosyncratic reactions); (ii) platelet count ≤25000/µl; (iii) ANC <1000/µl associated with fever (≥38.5°C); (iv) any ANC < 500/µl; and (iv) unresolved toxicity resulting in delay of retreatment ≥2 weeks. Toxicity was graded according to the National Cancer Institute (NCI) (Bethesda, MD, USA) Common Toxicity Criteria (Version 1.0).

The MTD was to be defined separately for MP and HP patients if it appeared that HP patients were more susceptible to DLT. HP patients were defined a priori as those who had been previously treated with greater than six courses of alkylating agent-containing chemotherapy (or greater than four courses of carboplatin); two or more courses of mitomycin C or a nitrosourea; or radiation therapy to >25% of hematopoietic reserves (with whole pelvic radiation equivalent to radiation to 30% of hematopoietic reserves). In ambiguous cases, classification was based on the discretion of the investigator.

Bizelesin was supplied by the NCI in a 2 ml vial containing bizelesin 5 µg in 1 ml diluent, which had been frozen at −70°C or lower. The diluent consisted of 100 µl solvent (6:3:1 v/v/v, polyethylene glycol 400:ethanol: polysorbate 80), 1 mg citric acid and 0.9% saline qs to 1 ml. The contents of the vial were allowed to thaw at room temperature. After thawing, 0.5 ml drug solution was added to 2 ml 0.9% sodium chloride injection USP in a glass vial and mixed. The resultant solution, which contained 1 µg bizelesin/ml, was stable for at least 24 h at room temperature. An appropriate volume of the stock solution to yield the required dose was administered rapidly through a free-flowing i.v. infusion of 0.9% saline.

**Pretreatment and follow-up studies**

Histories that included recording of performance status and concurrent medications, physical examinations and routine laboratory evaluations were performed before treatment and weekly. Routine laboratory evaluations included complete blood counts, differential white blood cell count (WBC), electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, hepatic transaminases, prothrombin time and urinalysis. Pretreatment studies also included an electrocardiogram, pregnancy testing in all relevant females and pertinent radiological studies for evaluation of all measurable or evaluable sites of malignancy, as well as an assessment of relevant tumor markers. Complete blood counts and differential WBC counts were carried out every other day if ANC <1000/µl or platelets <50000/µl, and chemistries and/or electrolytes were assessed twice weekly for patients who developed abnormalities of at least one grade above their pretreatment values. Radiological studies for disease status assessments were repeated after every other course or as needed to confirm response. Patients were able to continue treatment if they did not develop progressive disease. A complete response was scored if there was disappearance of all active disease on two measurements separated by a minimum period of 4 weeks and a partial response required at
least a 50% reduction in the sum of the product of the bidimensional measurements of all documented lesions separated by at least 4 weeks. Any concurrent increase in the size of any lesion by 25% or more or the appearance of any new lesion was considered disease progression.

Plasma and urine sampling and assay

Blood samples in 5-ml heparinized tubes were collected before the first infusion of bizelesin and at 15, 30 and 45 min and 1, 1.5, 2, 6, 12 and 24 h after the end of infusion. Urine was collected continuously for 24 h following drug administration in 0–6, 6–12, 12–18 and 18–24 h aliquots. Following collection, the blood samples were placed on ice and centrifuged within 10 min at 2500 rpm at 4°C. The plasma was transferred to a polypropylene cryo-tube and frozen at −80°C until quantitative analysis. After the urine collections were shaken, 2-ml aliquots were drawn off and frozen at −80°C in a labeled sample tube.

Various analytical assay methods, including high performance liquid chromatography, mass spectroscopy coupled with liquid chromatography and gas chromatography using several types of chemical derivations, were evaluated but no method demonstrated the requisite sensitivity and reliability to quantify the minute concentrations of bizelesin and metabolite concentrations in plasma and urine resulting from administering microgram quantities of drug.

Results

General

Sixty-two patients, whose pertinent characteristics are displayed in Table 1, were treated with 185 courses of bizelesin at eight dose levels ranging from 0.1 to 1.5 µg/m². Two patients who were treated with bizelesin 0.95 µg/m² and died due to rapid disease progression in the first course were not fully evaluable for the determination of the MTD. The total numbers of new patients and courses at each dose level, the rates of DLT experienced by patients in first and all courses at each dose level, and the overall dose-escalation scheme are shown in Table 2. The median number of courses administered was two (range one to 16). Nine patients required dose reduction on one occasion for dose-limiting myelosuppression at the following bizelesin dose levels: 0.95 µg/m² (three patients after one, two and three courses); 1.26 µg/m² (three patients after one, one and two courses); and 1.5 µg/m² (three patients after one, one and five courses).

No adverse events of severity grade >1 occurred in patients treated with bizelesin at the first two dose levels; however, the lead patient in the third dosing cohort (0.4 µg/m²) experienced grade 2 neutropenia and anemia and a second subject developed brief (<5 days), uncomplicated grade 4 neutropenia. Therefore, six total patients were treated with bizelesin 0.4 µg/m², but no additional dose-limiting events were noted and dose escalation proceeded thereafter in maximum increments of 33%.

No further dose-limiting events were observed until the sixth dose level (0.95 µg/m²), at which time the third patient experienced brief (<5 days) uncomplicated grade 4 neutropenia on day 16 of course 1. Since the first courses of the first two patients treated at the 0.95 µg/m² dose level had been uneventful, and the first 2 weeks following treatment of the third subject had also been uneventful, a single individual was enrolled at the next higher bizelesin dose level, 1.26 µg/m², before the DLT at the lower dose was noted. This patient also experienced brief (<5 days), uncomplicated grade 4 neutropenia. Since both subjects who had experienced DLT had received extensive prior myelosuppressive therapy and were classified as HP according to the criteria established de novo, the dose-escalation process diverged into distinct schemes for MP and HP patients. For MP patients, the incidence of DLT was acceptable in the first six patients treated with bizelesin at doses of 0.95 and 1.26 µg/m², whereas an unacceptably high rate of hematological DLT, specifically uncomplicated grade 4 neutropenia, was observed in the first course of two of five MP patients at the 1.50 µg/m² dose level. In contrast, two of six evaluable HP patients experienced DLT, consisting of one episode each of uncomplicated grade 4 neutropenia alone and grade 4 neutropenia plus fever, in course 1 following treatment with bizelesin at the 0.95 µg/m² dose level. At the next lower dose, 0.71 µg/m², there were no dose-limiting events among seven evaluable HP subjects.

Toxicity

Myelosuppression, particularly neutropenia, was the principal adverse effect and DLT of bizelesin on this administration schedule. The distributions of relevant toxicity grades of neutropenia, as well as hematological dose-limiting events, as functions of the bizelesin dose level, are displayed in Table 3. The ANC nadir was experienced relatively late, typically on day 15; however, delay in retreating patients due to unresolved neutropenia on day 29 was never required in MP or HP patients. Overall, grade 4 neutropenia was observed in 29 courses (16%), and 10 (5%) of these episodes occurred in the first course of treatment. The duration of severe neutropenia (<500/µl) was always <5 days. Furthermore,
fever associated with grade 3/4 neutropenia was experienced by only four patients (6%) involving four courses (2%). Although HP patients appeared to be more prone to severe neutropenia than MP patients, there were no clinically significant cumulative effects on the depth of ANC nadirs in the dose range evaluated in this study.

Severe effects on platelets and red blood cells occurred less frequently than severe neutropenia and were always noted concurrently in HP subjects. Grade 3 anemia, possibly related to drug and requiring red blood cell transfusions, was noted in one course (0.5%). Similarly, thrombocytopenia was uncommon, with grade 2 toxicity occurring in three courses [one patient at 0.53 µg/m² (course 16), two patients at 1.26 µg/m² (course 1)] and grade 3 toxicity occurring in three courses (one patient at 0.95 µg/m² during course 8, one patient at 1.26 µg/m² during courses 2 and 3).

Table 2. Dose-escalation scheme

<table>
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<th>Cohort number</th>
<th>Bizelesin dose (µg/m²)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>Median ANC nadir, cells per µl (range)</th>
<th>Total courses with neutropenia (course 1)</th>
<th>New patients with DLT</th>
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<td></td>
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<td>Grade 4</td>
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<tr>
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<td>3</td>
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<td>1974 (1938–2257)</td>
<td>0 (0)</td>
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<tr>
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<td>1462 (480–3675)</td>
<td>0 (0)</td>
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<tr>
<td>5</td>
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<tr>
<td>5</td>
<td>0.71 (HP)</td>
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<td>2700 (1064–4340)</td>
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Table 3. Hematological toxicity

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*Two patients who died due to progressive disease were not fully evaluable for toxicity.

DLT, dose-limiting toxicity; HP, heavily pretreated; MP, minimally pretreated.

*Includes only patients in whom therapy was initiated at the dose level.

*Median ANC nadir values for course 1.

*Mutually non-exclusive (grade 4 events with fever are tabulated in both columns).

ANC, absolute neutrophil count; HP, heavily pretreated; MP, minimally pretreated.
Nausea, fatigue, anorexia and diarrhea were the most common non-hematological effects of bizelesin; however, these toxicities were always mild (grade 1) or moderate (grade 2). Furthermore, non-hematological events were noted across the entire bizelesin dosing range and definite temporal relationships could not be discerned for any of these potential toxicities, indicating that the underlying malignant process may have been contributory.

**Antineoplastic activity**

Although subjective benefit in disease-related symptoms was noted in several patients, neither partial nor complete antitumor responses were documented. A 66-year-old female patient with advanced ovarian carcinoma whose disease had recurred 1 year after primary treatment with the combination of carboplatin and paclitaxel, and during treatment with topotecan and carboplatin as single agents and the combination of vinorelbine and gemcitabine, experienced a 40% reduction in measurable disease involving abdominal lymph nodes and intra-abdominal carcinomatosis. Her maximum antitumor effect was noted following eight courses of bizelesin at the 0.53 µg/m² dose level, and 16 total courses were administered over 16 months. Treatment was discontinued because of protracted, albeit moderate (grade 2) thrombocytopenia at nadir, following her last course of treatment. The platelet count remained <100000/µl for 10 weeks following treatment. However, the antineoplastic response persisted for 8 months (total response duration 24 months). Following objective evidence of progressive disease, she was treated with paclitaxel and carboplatin as single agents and the combination of vinorelbine and gemcitabine, experienced a 40% reduction in measurable disease involving abdominal lymph nodes and intra-abdominal carcinomatosis. Her maximum antitumor effect was noted following eight courses of bizelesin at the 0.53 µg/m² dose level, and 16 total courses were administered over 16 months. Treatment was discontinued because of protracted, albeit moderate (grade 2) thrombocytopenia at nadir, following her last course of treatment. The platelet count remained <100000/µl for 10 weeks following treatment. However, the antineoplastic response persisted for 8 months (total response duration 24 months). Following objective evidence of progressive disease, she was treated with several types of chemotherapeutic agents, including paclitaxel, liposomal doxorubicin, and docetaxel as single agents, without remarkable hematological toxicity. Seven patients (renal cell carcinoma in six patients, carcinoma of unknown primary in one patient) experienced stable disease for at least 6 months during bizelesin treatment, and two subjects (the aforementioned patient with ovarian carcinoma and another with renal cell carcinoma) did not experience disease progression for at least 1 year during treatment.

**Discussion**

The CPI bizelesin was selected for clinical development because of its unique mechanism of action as a DNA-damaging agent, lack of cross-resistance to other alkylating agents, extraordinarily high potency, and broad preclinical antitumor activity [9, 21–26, 28]. Bizelesin is the only known DNA-active agent that preferentially induces interstrand DNA cross-linking in a qualitatively and quantitatively specific manner in proliferative regions of the genome [21–26]. This study was performed to assess the feasibility of administering bizelesin as a single i.v. bolus injection every 4 weeks in patients with advanced solid malignancies.

As predicted from preclinical studies, myelosuppression, particularly neutropenia, was the principal DLT of bizelesin on this schedule, whereas non-hematological toxicities were much less common and never functionally preclusive. Severe, albeit non-dose-limiting, thrombocytopenia and anemia were occasionally noted, especially in patients who developed severe neutropenia, but these events were not clinically relevant. Although grade 3/4 neutropenia was common at bizelesin doses >0.71 µg/m², the duration of severe (grade 4) neutropenia was always brief and not commonly associated with fever. The extent of prior treatment with myelosuppressive therapies influenced drug tolerance to a modest extent, with HP individuals developing an equivalent level of toxic effects at 75% of the bizelesin dose compared with MP patients. Bizelesin dose levels exceeding 0.95 and 1.50 µg/m² were associated with unacceptably high incidences of DLT in HP and MP patients, respectively. In contrast, repetitive treatment of HP and MP patients at bizelesin doses of 0.71 and 1.26 µg/m², respectively, was well-tolerated and these doses are therefore recommended for HP and MP patients participating in phase II studies. Since grade 4 neutropenia, which was rarely consequential, was the predominant DLT in this trial, it is possible that, by modifying the definition of DLT to include only protracted (e.g. >5 day) grade 4 neutropenia, further bizelesin dose escalation could have been accomplished. However, the rationale to use a more conservative definition of DLT (i.e. isolated grade 4 neutropenia) was largely based on concerns that the high potency of bizelesin and profound interspecies differences in tolerance would potentially lead to more erratic toxicity in the clinic. On a similar note, the probability of observing erratic and unpredictable toxicity is amplified by the additional variability incurred in the pharmaceutical preparation and use of such minute (microgram) quantities of drug. Similar findings were reported by Pitot et al. [28] who observed myelosuppression as the principal DLT in a similar study. Using a nearly identical definition of DLT and MTD, these investigators determined that 0.8 µg/m² was the MTD and the dose recommended for phase II studies, but patients were not classified de novo according to the extent of prior myelosuppressive therapy, which probably accounted for the higher phase II dose recommendation for MP patients in the present study.

The wide interspecies variability in the susceptibility to bizelesin toxicity in preclinical toxicological studies and the high potency of bizelesin argued for a highly conservative and cautious approach to the selection of a starting dose and overall dose-escalation scheme [9]. The lower MTD in humans (0.71–1.26 µg/m²) than in mice (30 µg/m²) after a single dose of bizelesin is probably due to the greater sensitivity of human myeloid precursors to the cytotoxic effects of bizelesin [27]. Interestingly, this observation was predicted by a preclinical study in which the relative myelotoxic effects of bizelesin on human, canine and murine hematopoietic progenitor cells were assessed using ex vivo hematopoietic colony-forming assays. There was a 3-log difference in drug concentration in which 100% colony inhibition occurred for granulocyte–macrophage colony formation versus that for humans and dogs [27].

Although a principal objective of this study was to characterize the pharmacokinetic behavior of bizelesin, allometric scaling of preclinical data predicted that bizelesin concentrations in plasma would be far below the range of values that are accurately measured with standard analytical assays [28]. This prediction was substantiated by the failure of conventional analytical methods, including high-performance liquid chromatography, mass spectroscopy coupled with liquid chromatography, and gas...
chromatography using several types of chemical derivation reactions to yield the requisite sensitivity to reliably quantify the minute concentrations of bizelesin and metabolites in plasma resulting from administering microgram quantities of drug. Similar to the situation with other highly potent cytotoxics, such as the natural products maytansine and calicheamicyn, which are also administered in minute quantities as the toxin component of immunoconjugates, it is likely that immunoassays and/or radioimmunoassays with much greater sensitivity could be developed to reliably measure bizelesin in plasma [29]. However, any single immunological assay would probably be too specific for the parent compound, and additional assays would be required to measure the active dicyclopropylpyrroloindole metabolite (U-77809) formed spontaneously by the intermolecular rearrangement of bizelesin [9, 20]. This consideration is particularly important in view of the fact that the cytotoxic potencies of bizelesin and U-77809 against L1210 leukemia cells in vitro are equivalent [20]. Interestingly, an L1210 bioassay was utilized by Pitot et al. [28] to measure bizelesin concentrations in plasma in a complementary phase I study. These investigators reported dose-proportional pharmacokinetic behavior, plasma half-life values averaging 105 ± 37 min, and a low volume of distribution at steady-state (mean 2390 ± 440 ml/m²). However, the L1210 bioassay cannot distinguish between parent drug and cytotoxic degradation products and metabolites, and instead measures the sum of all cytotoxic bizelesin species present [28]. The non-specificity of the bioassay also precludes its usefulness in clinical evaluations of bizelesin in combination with other systemic modalities that are cytotoxic to L1210 leukemia cells.

The CPI anticancer agents are undoubtedly some of the most interesting and unique DNA-damaging agents that have ever undergone clinical development, particularly in view of the non-random distribution of DNA lesions produced following treatment of cancers with these agents [21–26]. The sequence-specific DNA targeting capabilities of the CPIs is perhaps best exemplified by the bifunctional DNA targeting capabilities of bizelesin, which forms interstrand DNA cross-links that are probably responsible for its extraordinarily high potency in both preclinical and clinical evaluations [21–26]. Although the development of agents that alkylate DNA does not seem to be a high priority at this time, bizelesin’s unique mechanistic characteristics, predictable toxicological profile, and lack of non-hematological toxicities at clinically relevant doses, warrant further evaluations of its potential role in the treatment of both solid and hematopoietic malignancies. Furthermore, in view of the many recent advances in targeting tumors with antibodies and other highly specific modalities, as well as the paucity of highly potent cytotoxins for immunoconjugate development, bizelesin is a logical candidate to be considered in this regard [29].

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References


