The discovery of purine nucleoside phosphorylase (PNP) deficiency and T lymphocytopenia suggested that inhibition of this enzyme could serve as a therapeutic target. Inhibitors of PNP failed until structure-based synthesis of immucillin-H (BCX-1777, forodesine), a transition-state analog of PNP. The picomolar potency for PNP, T cell–selective cytotoxicity, and animal studies provided the rationale for use of forodesine in T-cell malignancies. Five patients were treated with an intravenous infusion of forodesine (40 mg/m²) on day 1; treatment continued on day 2; forodesine was administered every 12 hours for an additional 8 doses. Plasma and cellular pharmacokinetics and pharmacodynamics were investigated. Median peak level of forodesine (5.4 μM) was achieved at the end of infusion. This level was sufficient to increase plasma 2'-deoxyguanosine (dGuo) concentrations in all patients. Intracellular deoxyguanosine triphosphate (dGTP) increased by 2- to 40-fold in 4 of 5 patients (8 of 9 courses) and correlated with antileukemia activity in 4 patients. However, objective responses were not observed. This was the first clinical study in humans to demonstrate the plasma pharmacokinetics and the pharmacodynamic effectiveness of the PNP inhibitor, forodesine; however, regrowth of leukemia cells in the blood and marrow after course 1 suggested that a different therapeutic schedule should be considered for future studies. (Blood. 2005;106:4253-4260) © 2005 by The American Society of Hematology

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Several agents have been shown to inhibit PNP, and pharmacokinetic studies have demonstrated that more than 95% continuous inhibition of PNP is required to achieve significant reduction in T-cell levels. Acyclovir, a potent inhibitor of herpes simplex virus replication, also inhibits PNP, albeit to a lesser extent. The low inhibitory potency of this agent (eg, \( K_i \) of 90 \( \mu M \)) makes it unsuitable for clinical use. Similarly, allopurinol, 6-mercaptopurine, and 6-methoxypurines inhibit PNP, but only at very high drug concentrations. C-8–substituted analogs such as 8-iodoguanosine and 8-aminoguanosine have been used as inhibitors of PNP. Studies in cell lines clearly demonstrated that 8-aminoguanosine inhibited PNP activity and resulted in T-cell–selective cytotoxicity. Additional PNP inhibitors include analogs of dGuo such as 8-amino-9-(2-thienylmethyl)guanine (PD119229) and analogs of deazaguanine. However, the inhibitory activity was not as potent as that observed with N7 substituted congeners. A series of analogs with N7 substitution were found to be highly potent in inhibiting enzymatic activity of PNP. BCX-34 (peldesine) had a 50% inhibitory concentration (IC\(_{50}\)) of 30 nM; however, when used in clinical trials to treat patients with psoriasis and cutaneous T-cell lymphomas, there was no significant clinical activity. Enzymatic studies indicated that BCX-34 had a rapid off rate and could not inhibit PNP sufficiently to elevate plasma dGuo to levels necessary for T-cell suppression.

Schramm’s group used another strategy to design more potent PNP inhibitors by identification of the transition-state structure stabilized by the target enzyme. Geometric and electrostatic properties of the transition state of substrate were used as an atomic blueprint to design chemically stable isolomers to act as analogs. Using inosine as a substrate for transition-state analysis, a series of 9-deazaanucleoside analogs, termed immucillins, was designed to mimic the transition state. The immucillins have a carbon–carbon linkage between a cyclic amine moiety that replaces ribose, and either 9-deaza-hypoxanthine or 9-deaza-guanine (immucillin H and immucillin G, respectively). These analogs inhibited PNP with high potency; the \( K_i \) values were in the 20 to 80 pM range for human and bovine enzyme. In vitro studies using cell lines established that immucillin H (BCX-1777, forodesine; Figure 1) resulted in T-cell–selective cytotoxicity that was mediated by the intracellular accumulation of dGTP from exogenous dGuo. In vivo investigations in murine model systems further established utility and effectiveness of this agent in T-cell lysis.

Based on these observations, a phase 1 clinical trial was designed to test the hypothesis that forodesine would inhibit PNP in vivo, resulting in biochemical sequelae that would be an effective treatment strategy for T-cell malignancies. To serve this purpose, patients with previously treated T-cell malignancies such as T-cell lymphoblastic lymphoma (T-LL), T-cell acute lymphoblastic leukemia (T-ALL), and T-cell prolymphocytic leukemia (T-PLL) were entered into this protocol, the first study of forodesine in humans. The present investigation reports the clinical outcomes as were entered into this protocol, the first study of forodesine in leukemia (T-ALL), and T-cell prolymphocytic leukemia (T-PLL) patients with previously treated T-cell malignancies such as T-cell human and bovine enzyme. 34 In vitro studies using cell lines H and immucillin G, respectively). These analogs inhibited PNP and either 9-deaza-hypoxanthine or 9-deaza-guanine (immucillin utility and effectiveness of this agent in T-cell lysis.37 In vivo investigations in murine model systems further established that immucillin H (BCX-1777, forodesine; Figure 1) resulted in T-cell–selective cytotoxicity that was mediated by the intracellular accumulation of dGTP from exogenous dGuo.35,36 In vivo investigations in murine model systems further established utility and effectiveness of this agent in T-cell lysis.

![Figure 1. Chemical structures. (A) dGuo and (B) forodesine.](image)

### Patients and methods

#### Study group

Patients were eligible if they had previously treated relapsed or refractory T-LL, T- ALL, or T-PLL with a performance status 3 or better and adequate organ function with total bilirubin 1.5 mg/dL or less and creatinine 1.5 mg/dL or less (or if creatinine clearance was at least 45 mL/min). All patients were informed of the investigational nature of this protocol in accordance with institutional policies. An Institutional Review Board–approved informed consent form was signed by all patients for both clinical and pharmacology studies; approval was obtained according to the Declaration of Helsinki.

#### Therapy and statistical design

Forodesine was infused over 30 minutes on the first day. For days 2 to 5, the drug was administered twice daily at 12-hour intervals. The twice daily dosing schema of forodesine was chosen based on results of preclinical studies in primates suggesting a t\(_1/2\) for forodesine of approximately 3 hours and a t\(_1/2\) for plasma dGuo of 12 seconds. The starting dose of forodesine (40 mg/m\(^2\)) was based on data from preclinical studies in primates regarding activity and toxicity, suggesting maximum daily exposure in primates of 20 mg/kg/d (equivalent to 800 mg/m\(^2\)/d). Forodesine infusions were separated from citrated blood products by at least 30 minutes owing to unexplained deaths in preclinical animal studies when forodesine was given with citrate as the buffer vehicle. Additional courses of forodesine were allowed if stable disease or hematologic improvement was observed after course 1. Courses were repeated every 21 to 28 days. Intrapatient dose escalation was allowed for subsequent courses with 50% increments by protocol design if no clinically relevant drug toxicity was observed with the prior course.

The phase 1 statistical design was a continual reassessment method (CRM), with cohorts of 3 patients chosen for evaluation of toxicity frequency. The maximum tolerated dose (MTD) was projected to be the dose level associated with the probability of grade 3 or greater nonhematologic toxicity closest to 30%. The CRM was implemented by computerized entry of the toxicity profile observed with each patient evaluated at the 21-day time point from the start of forodesine. Toxicity was graded according to the National Cancer Institute Expanded Common Toxicity Criteria, version 2.0.

#### Response criteria

For T-ALL and T-PLL, complete remission (CR) was defined as 5% or fewer leukemia cells in a normocellular or hypercellular marrow with absolute neutrophil count (ANC) equal to or more than 10\(^9\)/L and platelet (PLT) count equal to or more than 100\(^9\)/L. Complete resolution of extramedullary disease was required. Partial response was defined as at least 25% increase in disease burden. For personal use only.
triphosphates (dNTPs) were obtained from Amersham Biosciences (Piscataway, NJ) and were used as standards. \[^3\text{H}\]-deoxyadenosine triphosphate (dATP) and \[^3\text{H}\]-deoxythymidine triphosphate (dTTP) were purchased from Perkin Elmer Life Sciences (Boston, MA) and MP Biomedicals (Irvine, CA), respectively.

### Samples for clinical pharmacology

Plasma, cellular, and urine pharmacokinetic studies were conducted in all patients. Blood sampling was performed before therapy; at the end of infusion (coi) of dose 1 (30 minute); and 1, 2, 3, and 4 hours, between 6 and 10 hours, and then 24 hours after the start of first dose. Sampling on days 2 to 5 was performed prior to and at the coi of doses 2, 4, 6, and 8 (first dose of the day). Collections were obtained via a separate intravenous line from that being used for forodesine infusions. Blood samples (3-10 mL) were obtained in green stopper Vacutainer tubes containing heparin and 50 μM BCX-3428 (BioCryst Pharmaceuticals) to inhibit PNP if not completely inhibited by forodesine. The tubes were placed immediately in an ice-water bath and transported to the laboratory.\(^4\) Urine samples were collected prior to drug infusion and during the first 48 hours of therapy while the patient was hospitalized. These samples were collected from 0 to 2 hours, 2 to 4 hours, 4 to 8 hours, 8 to 24 hours, and 24 to 48 hours after day 1 dosing of forodesine.

### Plasma and urine pharmacology

The plasma was removed after centrifugation and stored at \(-70^\circ\text{C}\) until high-pressure liquid chromatography (HPLC) analyses. Forodesine and inosine were determined by a validated liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) method. They were extracted using a Waters Oasis “HLB” affinity solid phase extraction cartridge on a Zymark RapidTrace Workstation (Caliper Life Sciences, Hopkinton, MA). Chromatography was performed isocratically using a Zorbax SB C-3 column (Agilent Technologies, Lake Forest, CA) with isocratic elution using 5 mM ammonium formate and 5% methanol, pH 3.0, in water on an Agilent 1100 HPLC system. Column effluent was analyzed by positive ion multiple reaction monitoring using a PE Sciex API 3000 MS/MS equipped with Turbo Ion Spray in positive ion mode. The concentrations of forodesine and inosine were then determined by weighted (1/\(y\)) quadratic regression analysis of peak areas produced from the standard curve spanning 5 to 1000 ng/mL for forodesine and 15 to 5000 ng/mL for inosine.

#### Cellular pharmacology

Cell pellets from blood samples were diluted with phosphate-buffered saline and leukemia cells were isolated by Ficoll-Hypaque density gradient step-gradient centrifugation procedures.\(^5\) A Coulter channelizer (Coulter Electronics, Hialeah, FL) was used to determine cell number and the mean cell volume. The nucleotides in the leukemia cells were extracted by 60% methanol as described,\(^6\) and the DNA polymerase assay as modified by Sherman and Fyfe\(^7\) was used to quantitate dNTPs in the cell extracts.

### Table 2. Clinical outcomes after therapy with forodesine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>No. of courses</th>
<th>BM disease, %</th>
<th>WBC count, (\times 10^9/L)</th>
<th>Absolute PB leukemia, (\times 10^9/L)</th>
<th>Other observations</th>
<th>Overall response, course 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Detectable only by flow cytometry</td>
<td>Detectable only by flow cytometry</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>94</td>
<td>12</td>
<td>4.4</td>
<td>6.1</td>
<td>Detectable only by flow cytometry</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>55</td>
<td>69</td>
<td>21.3</td>
<td>45.8</td>
<td>92.2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>90</td>
<td>ND</td>
<td>87.1</td>
<td>200.5(^6)</td>
<td>44.4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>78</td>
<td>97†</td>
<td>14.8</td>
<td>0.7</td>
<td>10.9</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>91</td>
<td>19</td>
<td>150.6</td>
<td>17.2</td>
<td>102.4</td>
</tr>
</tbody>
</table>

| No improvement in transfusion requirements were observed (all patients were transfusion-dependent prior to forodesine treatment). Details are provided for course 1 only, with posttherapy assessments at day 21 unless otherwise indicated. Refer to text and Figure 2A-C for additional details. |

SD indicates stable disease; PD, progressive disease; ND, not done.

\(^{†}\)Day 10.

\(^{†}\)Day 14 bone marrow aspiration showed 3% blasts without morphologically detectable LL cells.
multifactorial in nature. and inevaluable for neutropenia owing to subsequent chemotherapy. Toxicities in patient no. 1 (renal failure, depressed level of consciousness) observed at day 21 and

Calculated in the first 24 hours (ie, milligrams) was determined
due to the small number of data points. The cumulative
amount excreted in the first 24 hours (ie, milligrams) was determined for each patient where possible. Linear or rectangular hyperbola analyses for r values and for each patient where possible. Linear or rectangular hyperbola analyses

determined due to the small number of data points. The cumulative
amount excreted in the first 24 hours (ie, milligrams) was determined for each patient where possible. Linear or rectangular hyperbola analyses for r values and for each patient where possible. Linear or rectangular hyperbola analyses

Calculations and statistical analysis
Noncompartmental analysis was used to determine the pharmacokinetic parameters from the plasma concentration-time data. Urine excretion rate curves were generated for forodesine and inosine using noncompartmental analysis. Rate of drug or biomarker was plotted against the midpoint time of urine collection. The half-life (t1/2, terminal elimination t1/2) could not be determined due to the small number of data points. The cumulative

Results
Study group
Five patients with relapsed or refractory T-cell malignancies were treated with forodesine; prior therapy and patient characteristics are
detailed in Table 1. Three patients had T-PLL and 2 had T-ALL. Forodesine (40 mg/m²) was administered according to protocol in all 5 patients. Patient nos. 2 and 5 received additional courses of forodesine, the latter with dose escalation (Tables 2-3). After the first 5 patients were enrolled, review of the clinical and pharmacology data suggested that an alternative dosing schedule of forodesine should be considered, and enrollment in the phase 1 portion of the study ceased, although the MTD had not been reached.

Clinical outcomes
Overall, no objective responses were observed (Table 2). Three patients (nos. 1, 2, and 5) had stable disease after one course of forodesine; additional courses of forodesine were administered in the latter 2 patients. Patient no. 4 had initial reduction in tumor burden with regrowth of the leukemia, whereas patient no. 3 had progressive disease.

Patient no. 1 had a minor (25%) reduction in peripheral adenopathy during forodesine therapy and decrease in bone marrow involvement (from 94% to 12%) identified on day 21 (Tables 1-2). For patient no. 2, response to course 1 of forodesine was observed with reduction in white blood cell (WBC) count from 121.1 × 10⁹/L to 33.8 × 10⁹/L by day 6, with stability until approximately day 28 when proliferation recurred (Figure 2A). Cytoreduction was again observed with course 2 of forodesine 40 mg/m² with reduction in WBC count from 81.7 × 10⁹/L to 46.7 × 10⁹/L with stability thereafter (Figure 2A). The patient discontinued therapy owing to recurrence of neurologic toxicities at approximately day 21 of course 2. Patient no. 3 developed a progressive increase in WBC count (from 87.1 × 10⁹/L to 200.5 × 10⁹/L) by day 10, with a proportional increase in lymphocytes and decrease in absolute prolymphocytes (Table 2). Patient no. 4 had clearance of circulating blasts by day 10 and absence of detectable bone marrow disease on day 14. However, day 21 bone marrow aspiration revealed regrowth of T-ALL with persistent cytopenias (Table 2 and Figure 2B). For patient no. 5, the WBC count decreased from 150.6 × 10⁹/L to 17.2 × 10⁹/L by day 21 of

All patients inevaluable for hematologic toxicity other than neutropenia owing to baseline cytopenias; patient no. 3 off-study day 10 of therapy for progression of disease and inevaluable for neutropenia owing to subsequent chemotherapy. Toxicities in patient no. 1 (renal failure, depressed level of consciousness) observed at day 21 and multifactorial in nature.

During days of forodesine infusion.
†Present at baseline, progressed to grade 2 with course 2, then improved with subsequent courses, suspected related to prior vincristine.

Table 3. Toxicities observed during and after therapy with forodesine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose, mg/m²</th>
<th>Grades 1-2</th>
<th>Grades 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>course 1</td>
<td>40</td>
<td>Hypertension, hypotension</td>
</tr>
<tr>
<td>2</td>
<td>course 1</td>
<td>40</td>
<td>Hypocalcemia,* headache,* nausea/vomiting,* constipation, orthostasis (autonomic), tremors</td>
</tr>
<tr>
<td>3</td>
<td>course 2</td>
<td>40</td>
<td>Orthostasis (autonomic), tremors</td>
</tr>
<tr>
<td>4</td>
<td>course 1</td>
<td>40</td>
<td>Headache,* nausea/vomiting*</td>
</tr>
<tr>
<td>5</td>
<td>course 1</td>
<td>40</td>
<td>Stomatitis, peripheral neuropathy†</td>
</tr>
<tr>
<td>6</td>
<td>course 2</td>
<td>40</td>
<td>Peripheral neuropathy,† orthostasis, (autonomic)†</td>
</tr>
<tr>
<td>7</td>
<td>course 3</td>
<td>60</td>
<td>Peripheral neuropathy,† orthostasis, (autonomic)†</td>
</tr>
<tr>
<td>8</td>
<td>course 4</td>
<td>90</td>
<td>Peripheral neuropathy,† orthostasis (autonomic)†</td>
</tr>
</tbody>
</table>
course 1 of forodesine. No change in lymphadenopathy or splenomegaly was observed. Figure 2C details the pattern of change in the absolute prolymphocyte count with each subsequent cycle of forodesine (40 mg/m², 60 mg/m², 90 mg/m²), characterized by initial increase in the WBC count during days of forodesine infusion, with gradual nadir by day 10 to 14 of each cycle, and initial increase in the WBC count during days of forodesine infusion.

Table 4. Pharmacokinetic parameters for forodesine after a single intravenous infusion of forodesine

<table>
<thead>
<tr>
<th>Patient no. and dose</th>
<th>Dose, mg/m²</th>
<th>t1/2, h</th>
<th>Cmax, µM</th>
<th>AUCinf, h·µM</th>
<th>Clearance, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 70 mg 40 mg/m²</td>
<td>40</td>
<td>17</td>
<td>7.8</td>
<td>60.2</td>
<td>39.1</td>
</tr>
<tr>
<td>2 67 mg 40 mg/m²</td>
<td>40</td>
<td>8</td>
<td>4.9</td>
<td>45.2</td>
<td>52.1</td>
</tr>
<tr>
<td>3 64 mg 40 mg/m²</td>
<td>40</td>
<td>6</td>
<td>5.4</td>
<td>33.1</td>
<td>35.5</td>
</tr>
<tr>
<td>4 110 mg 40 mg/m²</td>
<td>40</td>
<td>10</td>
<td>5.5</td>
<td>31.7</td>
<td>38.3</td>
</tr>
<tr>
<td>5 96 mg 40 mg/m²</td>
<td>40</td>
<td>11</td>
<td>4.0</td>
<td>37.2</td>
<td>47.3</td>
</tr>
<tr>
<td>6 94 mg 60 mg/m²</td>
<td>60</td>
<td>11</td>
<td>7.7</td>
<td>64.9</td>
<td>83.3</td>
</tr>
<tr>
<td>7 142 mg 40 mg/m²</td>
<td>40</td>
<td>15</td>
<td>5.3</td>
<td>41.8</td>
<td>61.4</td>
</tr>
<tr>
<td>8 212 mg 90 mg/m²</td>
<td>90</td>
<td>15</td>
<td>7.5</td>
<td>72.3</td>
<td>112.9</td>
</tr>
</tbody>
</table>

Cmax indicates maximum observed concentration; AUCinf, area under the curve for a single dose over a 24-hour period; AUCt∞, area under the curve extrapolated to infinity; Vz, volume of distribution based on terminal elimination phase.

Toxicity

Details of the toxicities observed with forodesine are provided in Table 3. The most common toxicity observed was grade 3-4 neutropenia, associated with infection or fever of unknown origin (or both) in 2 cases. Grade 1-2 headaches, hypocalcemia, and nausea/vomiting were observed during days of infusion of forodesine. Autonomic dysfunction (orthostasis) was observed as a late event (approximately day 21 or later) in 2 patients; this was attributed to forodesine in patient no. 2 and to prior vincristine treatment in patient no. 5.

Plasma pharmacology

The typical pharmacokinetic profile of forodesine revealed that the peak level of forodesine was achieved at the end of the infusion (Figure 3A). The forodesine elimination was slow; median t1/2 was 11 hours. At 24 hours, a detectable level of the parent drug was observed. Assessments at the end of infusions 2, 4, 6, and 8 indicated that there was similar accumulation of the drug after subsequent infusions. The median concentration of the parent drug in the 5 patients after the first infusion was 1440 ng/mL or 5.4 mM (Table 4).

The concentration of dGuo increased throughout the treatment period, reaching a maximum of 20 µM at the end of the last infusion (Figure 3B). For T-PLL patients nos. 2, 3, and 5 the dGuo levels peaked at the end of the last infusion, whereas that was achieved prior to completion of treatment for T-ALL patients nos. 1 and 4 (Table 5). The dGuo Cmax values ranged from 701.5 ng/mL (2.6 µM) to 9155 ng/mL (34 µM) for the patients treated with 40 mg/m² and increased with dose escalation of forodesine from 4245 ng/mL (16 µM) for course 2 of 40 mg/m² to 9835 ng/mL (37 µM) for course 4 of 90 mg/m² treatment (Table 5; patient no. 5). There was a rapid increase in plasma inosine concentrations after the initial dose of forodesine that was maintained throughout the treatment periods (Figure 3C) for all patients except patient no. 4 whose pattern of inosine response mirrored the dGuo response.

Urine pharmacology

The summary of the forodesine urine excretion data after the initial infusion of forodesine and up to the next 24 hours is provided in Table 6. The maximum rate of excretion ranged from 3.7 mg/h to 10.7 mg/h for the 6-hour mid-time point. Three hours was peak time for the median maximum excretion rate. The majority (54%-73%) of the forodesine was cleared by the kidneys (Table 6). The cumulative amount excreted did not plateau and the total forodesine excreted over 24 hours underestimated the total forodesine excreted via the kidneys. The excretion rate for dGuo was similar to that of forodesine, whereas the inosine excretion rate was significantly greater (Table 7). Unlike forodesine, the excretion rates for both dGuo and inosine remained the same throughout the 24-hour period.

Table 5. Pharmacokinetic parameters for dGuo after 8 intravenous infusions of forodesine

<table>
<thead>
<tr>
<th>Patient no. and dose</th>
<th>T max, h</th>
<th>Cmax, µM</th>
<th>AUCall, h·µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 40 mg/m²</td>
<td>48</td>
<td>34.3</td>
<td>1663</td>
</tr>
<tr>
<td>2 40 mg/m²</td>
<td>97</td>
<td>4.3</td>
<td>222</td>
</tr>
<tr>
<td>3 40 mg/m²</td>
<td>97</td>
<td>2.6</td>
<td>134</td>
</tr>
<tr>
<td>4 40 mg/m²</td>
<td>73</td>
<td>13.9</td>
<td>853</td>
</tr>
<tr>
<td>5 40 mg/m²</td>
<td>97</td>
<td>16.9</td>
<td>444</td>
</tr>
<tr>
<td>6 40 mg/m²</td>
<td>96</td>
<td>15.9</td>
<td>741</td>
</tr>
<tr>
<td>7 60 mg/m²</td>
<td>96</td>
<td>20.1</td>
<td>952</td>
</tr>
<tr>
<td>8 90 mg/m²</td>
<td>96</td>
<td>36.8</td>
<td>1164</td>
</tr>
</tbody>
</table>

T max indicates time to achieve maximum level; Cmax, maximum observed concentration; AUCall, area under the curve for a single dose over 24-hour period.

Table 6. Forodesine urine excretion

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose, mg/m²</th>
<th>Dose, mg</th>
<th>T max, h</th>
<th>Maximum rate, mg/h</th>
<th>Volume, mL</th>
<th>Amount recovered, mg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>79</td>
<td>3</td>
<td>4.4</td>
<td>1275</td>
<td>42 (54)</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>67</td>
<td>1</td>
<td>5.7</td>
<td>2475</td>
<td>49 (73)</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>64</td>
<td>6</td>
<td>3.7</td>
<td>2655</td>
<td>47 (72)</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>110</td>
<td>3</td>
<td>10.7</td>
<td>3500</td>
<td>77 (70)</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>212</td>
<td>1</td>
<td>9.6</td>
<td>1600</td>
<td>88 (41)</td>
</tr>
</tbody>
</table>
Cellular pharmacology

An increase in dGuo concentration in plasma suggested that there was inhibition of PNP in all patients. When analyzed for a concomitant increase in intracellular dGTP levels, except for lymphoblasts from patient no. 3, all had a gradual increase in dGTP (Figure 4). The endogenous pretreatment dGTP concentrations varied substantially, with a median of 15 μM (range, 1-28 μM; n = 5). Blasts from patients nos. 1 and 3 had only 1 μM and 1.5 μM endogenous dGTP levels, respectively.

After infusion of forodesine on day 1, the circulating leukemia cells had similar increases in dGTP concentration in patients nos. 2, 4, and 5. The pretreatment levels of 15, 27, and 28 μM increased by 5- to 10-fold within the first 8 hours and by 10- to 20-fold within 24 hours after start of therapy, and with the additional infusions, there was a gradual increase in dGTP levels; the highest concentrations were about 1 mM. Patient no. 1 started with 1 μM dGTP, which increased by 3- and 8-fold at 8 and 24 hours and continued during the 5 days of therapy. In contrast to the other 4 patients, leukemia cells from patient no. 3 showed no significant change in cellular dGTP levels.

Leukemia cells obtained from patients nos. 2 to 5 were analyzed for other deoxynucleotides. As with dGTP, there was a gradual increase in dATP levels for the first 24 hours after start of therapy and by 46 hours a plateau was achieved. For illustration purposes, data in one patient (no. 5) are presented (Figure 5). In contrast to dATP levels, deoxycytidine triphosphate (dCTP) and dTTP levels remained unchanged throughout the 5 days of therapy, suggesting the effect was only on purine deoxynucleotides (Figure 5). The endogenous concentrations of dATP were 31 and 66 μM in patients nos. 2 and 4, respectively. As in patient no. 5, dATP increased in these 2 patients without any effect on pyrimidine deoxynucleotides. For patient no. 3, there was no increase in intracellular dATP level (1 μM at the start of therapy), similar to the absence of increase in intracellular dGTP concentration.

To test whether the perturbation of purine deoxynucleotide pool was maintained from one course to the next, 16 peripheral blood samples from patients nos. 2 and 5 were collected during course 1 and course 2. In both cases, the pattern of dGTP and dATP increase was similar from one course to the next (Figures 5A-B), suggesting that circulating leukemia cells maintain the phosphorylation and elimination characteristics and result in similar levels of increase in dGTP and dATP with retreatment. Again, pyrimidine deoxynucleotide pools were un perturbed after forodesine administration in each course.

To investigate whether there was a dose-response relationship, pharmacology samples were collected from patient no. 5 after 40, 40, 60, and 90 mg/m² forodesine. Sixteen samples per course were collected to compare the accumulation of dGTP, dATP, dCTP, and dTTP. The starting level of dGTP was similar in each course: 28, 25, 37, and 32 μM, respectively. Increasing the dose from 40 to 90 mg/m² did not further augment intracellular dGTP accumulation in these prolymphocytes (Figure 6) even though there was a proportional increase in dGuo in plasma (Table 4).

Discussion

The purpose of this phase 1 trial was to determine the maximum tolerated dose of BCX-1777 (a transition-state analog inhibitor of PNP) and to relate pharmacodynamics of the drug to the administered dose. Because prior investigations of another PNP inhibitor (BCX-34 or peldesine) had failed to achieve this objective, this study had both clinical and biologic end points (sufficient drug to maintain increased levels of plasma dGuo and intracellular dGTP in malignant T cells without excessive toxicity). Pharmacokinetic and pharmacodynamic parameters were used to determine the maximum biologically effective dose.

Pharmacokinetic investigations of the parent drug in plasma showed that concentrations between 4 and 8 μM BCX-1777 were achieved with 40 mg/m² dosing (Table 4). This starting dose was thus likely sufficient to achieve an effective inhibitory level of forodesine in plasma, given that the concentration needed to inhibit the human PNP enzyme is in the picomolar range. Previous pharmacokinetic investigations in primates indicated that the terminal elimination rate was less than 3 hours, prompting

Table 7. Urine excretion of dGuo and inosine after a single intravenous infusion of forodesine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose, mg/m²</th>
<th>Amount excreted, mg</th>
<th>Maximum rate, mg/h</th>
<th>T_max, h</th>
<th>Amount excreted, mg</th>
<th>Maximum rate, mg/h</th>
<th>T_max, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>79.2</td>
<td>213.9</td>
<td>16</td>
<td>11.5</td>
<td>151.5</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>67.2</td>
<td>95.1</td>
<td>16</td>
<td>4.8</td>
<td>578.1</td>
<td>16</td>
</tr>
<tr>
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<td>64.4</td>
<td>172.6</td>
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<td>9.5</td>
<td>652.8</td>
<td>6</td>
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<td>110</td>
<td>321.2</td>
<td>16</td>
<td>15.3</td>
<td>1833.1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>212</td>
<td>91.1</td>
<td>16</td>
<td>3.9</td>
<td>1142.9</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 4. Accumulation of dGTP in all 5 patients after infusions of 40 mg/m² forodesine. Peripheral blood samples were collected and leukemia cells were isolated to extract dNTPs. Intracellular levels of dGTP were quantitated and plotted for patient nos. 1 (A), 2 (●), 3 (○), 4 (□), and 5 (*).

Figure 5. Comparison of deoxynucleotide level after forodesine therapy. Patient no. 5 received 2 courses of forodesine 40 mg/m² day 1, then twice daily for 4 days. Samples were assayed after infusions 2, 4, 6, and 8. Intracellular concentrations of dCTP (●), dTTP (○), dATP (□), and dGTP (●) were quantitated by DNA polymerase assay and are plotted after the first (A) and second (B) courses.
selection of a twice daily dosing schema for initial study. However, the observed peak level of forodesine (median 5.4 μM); and the long t1/2 (median 10 hours) in this study suggested that once daily dosing of 40 mg/m2 might be sufficient to provide adequate and maintained drug exposure for inhibition of PNP.

Forodesine is a potent inhibitor of PNP activity. In vitro studies showed Kᵢ values ranging from 0.5 to 1.2 nM with purified murine, canine, and primate enzymes37 and 20 to 80 pM for purified human response (Figure 3 and Table 5). Consistent with these observations, infusions of forodesine for 30 minutes resulted in a rapid increase of both plasma dGuo and inosine, and a single dose produced a sustainable 24-hour dGuo (36 ng/mL) with only a 10-fold increase in dGTP. No additional accumulation of dGTP was observed; the observed peak level of forodesine (median 5.4 μM); and the long t1/2 (median 10 hours) in this study suggested that once daily dosing of 40 mg/m2 might be sufficient to provide adequate and maintained drug exposure for inhibition of PNP.

Forodesine is a potent inhibitor of PNP activity. In vitro studies showed Kᵢ values ranging from 0.5 to 1.2 nM with purified murine, canine, and primate enzymes37 and 20 to 80 pM for purified human and bovine enzymes.34 Although the concentration of PNP in large body organs is not known, the high plasma concentrations of forodesine in the micromolar range and greater volume of distribution than total body water (Table 4) suggested that complete inhibition of PNP would occur. The kidneys appeared to be the major route of elimination of forodesine with clearance rates approximating calculated creatinine clearance. Hence, there was competition between binding to tissue PNP and renal elimination. Consistent with these observations, infusions of forodesine for 30 minutes resulted in a rapid increase of both plasma dGuo and inosine, and a single dose produced a sustainable 24-hour dGuo response (Figure 3 and Table 5).

With these high and maintained plasma levels of dGuo, it was expected that circulating T cells would also accumulate high levels of intracellular dGTP (Figure 4). In 3 patients (nos. 2, 4, 5), the leukemia cells accumulated concentrations of dGTP, which were 40- to 60-fold greater than pretreatment levels. Patient no. 1, on the other hand, had a 10-fold increase, whereas patient no. 3 had no augmentation in intracellular dGTP level. These data, albeit in a limited number of patients, strongly suggested heterogeneity regarding accumulation of dGTP, which is not directly related to plasma levels of dGuo. Patient no. 2 had a dGuo level of 4 μM with a 60-fold increase in dGTP, whereas patient no. 1 had a high level of dGuo (36 μM) with only a 10-fold increase in dGTP. No increase in dGTP level was observed in patient no. 3 despite a plasma dGuo concentration of 3 μM (Table 5). Similarly, intracellular accumulation of dGTP was not dependent on the excretion rate of dGuo; patients nos. 2, 4, and 5 with highest increases in dGTP had 5, 15, and 4 mg/h excretion of dGuo, respectively (Table 7). Hence, it appeared that the accumulation of dGTP was dependent on the inherent capability of circulating T cells to phosphorylate dGuo and maintain dGTP. This is in keeping with the observation that leukemia cells with higher levels of endogenous dGTP accumulated even greater levels of dGTP after forodesine was infused. For example, lymphoblasts or prolymphocytes from patients nos. 2, 4, and 5 started with intracellular dGTP at 15, 27, and 28 μM, respectively, and further increased dGTP to 40- to 60-fold. Thus, the initial cellular dGTP concentration may serve as an indicator of potential for dGTP augmentation on forodesine administration and, perhaps, predict clinical response.

Dose escalation of forodesine did not appear to increase intracellular dGTP further. By increasing the dose of forodesine from 40 to 60 or to 90 mg/m2, a corresponding increase in plasma dGuo from 15 to 20 to 37 μM, respectively, was observed; however, the extent of dGTP increase was not further augmented by these higher levels of dGuo (Figure 6). These data, although limited owing to evaluation in a single patient, suggested that dGTP accumulation was saturated at or below 15 μM dGuo in plasma.

In addition to increased intracellular dGTP levels, there was a less pronounced, yet significant, effect on dATP concentration (Figure 5). The mechanism for this increase is not known, but it was also observed in T-lineage cell lines when incubated with forodesine and exogenous dGuo in vitro.36 The human PNP lacks significant substrate potential for deoxyadenosine (dAdo). Therefore, inhibition of PNP activity on adenosine or dAdo, resulting in an increase in dAdo followed by intracellular phosphorylation to dATP, does not seem to be the route for this increase in dATP. It is more likely that dATP is generated intracellularly.

It has been postulated that the mechanism by which PNP inhibition kills T cells is through an increase in dGTP followed by inhibition of reduction of cytidine diphosphate (CDP) and uridine diphosphate (UDP) to pyrimidine deoxynucleotides by RNR. In addition, as mentioned, dATP is a global inhibitor of RNR. Hence, the pyrimidine deoxynucleotide pool would be expected to decrease after forodesine treatment. However, data in the present trial clearly demonstrated that pyrimidine pools were not affected, suggesting (1) no effect on the activity of RNR, (2) activity of the enzyme may be too low in more indolent leukemias, or (3) pyrimidine deoxynucleotides were maintained by the salvage route.

In summary, although no objective clinical responses were observed, there was clear antileukemia activity with forodesine, which correlated with intracellular accumulation of dGTP (Figures 2 and 4). In the patient with progression of disease during therapy, no intracellular accumulation of dGTP was observed. In contrast, the other 4 patients had cytoreduction of disease, which correlated with marked increases in intracellular dGTP. In patients who received more than one course of forodesine, recurrent T-cell proliferation noted prior to subsequent courses was associated with predose levels of intracellular dGTP. Despite dose escalation of forodesine, no additional accumulation of dGTP was observed compared with the initial course, suggesting that duration of therapy may be of more clinical relevance than dose. Although cytoreduction data demonstrated a relationship between intracellular accumulation of dGTP/dATP and death of T-lineage cells; proliferation of the remaining population of T cells after therapy suggested that this effect was not maintained with the current dosing schema of forodesine. Thus, ongoing phase 2 clinical trials are exploring the efficacy of protracted single daily dosing of intravenous forodesine,36-40 while phase 1 studies with oral formulation have just been initiated.

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References

A proof-of-principle pharmacokinetic, pharmacodynamic, and clinical study with purine nucleoside phosphorylase inhibitor immucillin-H (BCX-1777, forodesine)

Varsha Gandhi, John M. Kilpatrick, William Plunkett, Mary Ayres, Leigh Harman, Min Du, Shanta Bantia, Jan Davisson, William G. Wierda, Stefan Faderl, Hagop Kantarjian and Deborah Thomas