A Phase I and Pharmacological Study of Topotecan Infused over 30 Minutes for Five Days in Patients with Refractory Acute Leukemia 1

Eric K. Rowinsky, 2, 3 Scott H. Kaufmann, 4 Sharyn D. Baker, 3 Carrole B. Miller, Susan E. Sartorius, M. Kathy Bowling, Tian-Ling Chen, Ross C. Donehower, and Steven D. Gore


ABSTRACT

The principal objectives of this study were to determine the feasibility of escalating doses of the hydrophilic topoisomerase I (topo I) inhibitor topotecan (TPT) as a 30-min infusion daily for 5 days in adults with refractory or relapsed acute leukemia and to study the pharmacokinetic behavior of high doses of TPT and pharmacodynamic determinants of TPT activity. Fourteen patients received 27 courses of TPT at doses ranging from 3.5 to 5.75 mg/m2/day every 3 weeks. A constellation of unusual adverse effects, consisting of high fever, rigors, precipitous anemia, and hyperbilirubinemia, was the principal dose-limiting toxicity of high doses of TPT on this schedule. These toxicities were consistently intolerable at the 5.75 mg/m2/day dose level; however, they were neither severe nor common at lower doses. Although the precise etiology of these effects is not known, high doses of TPT may induce acute hemolytic reactions in this patient population. Severe, albeit transient, mucositis was experienced by two of eight patients in 2 of 17 courses at the next lower dose level, 4.5 mg/m2/day, which was determined to be the maximum tolerated dose and the dose recommended for further trials. The pharmacokinetic behavior of TPT at high doses was not dose dependent and resembled that at lower doses. In view of preclinical data suggesting that TPT sensitivity might correlate with topo I levels, topo I content in leukemia blasts was assessed by Western blotting. Variations in topo I content were observed. Moreover, strong correlations were evident between topo I content and two markers of proliferation, proliferating cell nuclear antigen and nuclear protein B23, raising the possibility that differences in topo I content observed among various leukemia specimens might reflect differences in the proliferating fractions of cells in various leukemia samples. Although complete clearance of circulating leukemia blasts occurred in most courses, neither sustained responses nor hematopoietic recovery were observed in the heavily pretreated, poor-risk patients enrolled in this study, and it was not possible to correlate these differences in topo I content with clinical response.

These results indicate that substantial dose escalation of TPT as a 30-minute infusion for a 5-day schedule above myelosuppressive doses is feasible in adults with refractory or relapsed leukemias; however, further development of alternate high-dose schedules in leukemia may be warranted in view of the nature of the dose-limiting toxicity and the lack of sustained clinical responses in this preliminary investigation.

INTRODUCTION

TPT 5 is a semi-synthetic, water-soluble analogue of CPT that stabilizes covalent complexes formed between DNA and the nuclear enzyme topo I (1). This interaction results in enzyme-linked, reversible, single-strand DNA breaks that are subsequently converted to double-strand breaks by the replication apparatus of the cell (1–6). In preclinical studies, TPT demonstrated broad activity in both leukemia and solid tumors, and TPT was superior to the prototypic topo I inhibitor, CPT, in mice bearing L1210 leukemia and in other tumor models (1, 2, 4–8). An optimal administration schedule was not adequately determined for TPT in preclinical studies. However, continuous and frequent dosing schedules generally resulted in superior antitumor activity compared with single bolus schedules, and the clinical antitumor activity with TPT to date has generally been observed on continuous drug exposure or frequent dosing schedules (2, 5, 7–12). In Phase I and II studies of TPT given over 30 min daily for 5 days, which has been the most common

1 The abbreviations used are: TPT, topotecan; 9-dimethylaminomethyl-10-hydroxycamptothecin; CPT, camptothecin; topo, topoisomerase; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; G-CSF, granulocyte colony-stimulating factor; AML, acute myeloid leukemia; CML-B, blast crisis of chronic myeloid leukemia; CR, complete response; AUC, area under the time-concentration curve; PCNA, proliferating cell nuclear antigen; PARP, poly(ADP-ribose) polymerase.
schedule evaluated to date, antitumor activity has been noted in glioma, sarcoma, carcinomas of the ovary, lung (both small and non-small cell), breast, head and neck, and cervix, as well as other malignancies (2, 4, 7–11, 13–22).

In addition to its preclinical antitumor spectrum encompassing a variety of leukemia models, there are several reasons for evaluating TPT in leukemia: (a) neutropenia is the principal DLT of TPT on most schedules in patients with normal hematopoietic function (2, 5, 7–22); (b) this myelosuppression is brief, noncumulative, and rarely associated with treatment delays, suggesting that TPT is not irreversibly toxic to early hematopoietic cells; and (c) nonhematological effects are not usually significant at TPT doses associated with severe myelosuppression. This toxicity profile indicates that escalation above doses that are not tolerated by solid tumor patients may be feasible in patients with leukemia.

Previous Phase I studies of TPT in adults with refractory or recurrent leukemias evaluated the feasibility of administering TPT on a continuous 5-day infusion schedule. Severe, prolonged oropharyngeal and perianal mucositis was the principal DLT of TPT, precluding dose escalation above 2.1 mg/m²/day, which is approximately 3-fold higher than the MTD achieved in solid tumor patients (23, 24). In contrast, mucositis has not been a prominent toxicity of TPT administered as a 30-minute infusion daily for 5 days. The administration of G-CSF has also permitted a 3-fold dose escalation of TPT on this schedule in patients with solid tumors, suggesting that even further dose escalation of TPT on this schedule might be feasible in leukemia patients, in whom severe myelosuppression is much less prohibitive (25).

Preclinical studies (reviewed in Refs. 2–6) have raised the possibility that cells with higher topo I levels might be more sensitive to CPT and TPT. In addition, it has been proposed that elevation of topo I levels might be associated with decreased expression of topo II, the enzyme that is targeted by epipodophyllotoxins and anthracyclines. Accordingly, there has been considerable interest in assessing expression of topo I and topo II in samples from patients receiving this class of agents.

The principal goals of this study were to: (a) describe the nonhematological DLTs of higher myelosuppressive doses of TPT administered as a 30-minute infusion daily for 5 days; (b) determine the MTD and recommended Phase II dose of TPT on this schedule in leukemia patients; (c) seek preliminary evidence of activity in patients with refractory or recurrent acute leukemia; (d) describe the pharmacokinetic and pharmacodynamic behavior of high doses of TPT; and (e) examine topo I and topo II expressions in leukemic blasts relative to clinical response.

PATIENTS AND METHODS

Patient Selection. Patients with histologically documented AML, acute lymphoblastic leukemia, and CML-B who failed to respond to intensive conventional chemotherapy or relapsed after achieving a CR were eligible for the study. For patients with AML without a known antecedent myelodysplastic syndrome or acute lymphocytic leukemia in first relapse, the first remission duration was required to be have been less than 1 year, and patients had to be ineligible for potentially curative high-dose chemotherapy regimens using allogeneic or autologous bone marrow transplantation. Patients must have had at least 30% leukemic blasts on bone marrow examination and evidence of progressive leukemia accompanied by signs of bone marrow failure such as anemia, neutropenia, and/or thrombocytopenia. Eligibility criteria also included age ≥18 years; Eastern Cooperative Oncology Group performance status ≤2; life expectancy enabling the completion of at least one course of therapy; adequate hepatic (total bilirubin ≤2.0 mg/dl) and renal (creatinine ≤2.0 mg/dl) functions unless organ dysfunction was directly due to leukemia; and no prior history of hemorrhagic cystitis or other coexisting medical problems of sufficient severity to prevent full compliance with the study. Informed written consent was obtained according to federal and institutional guidelines.

Dosage and Administration. The starting dose of TPT was 3.5 mg/m²/day given as a 30-minute infusion daily for 5 days. This dose was selected because it was the recommended Phase II dose of TPT on an identical dosing schedule with G-CSF in a previous Phase I study in solid tumor patients (25), with further dose escalation resulting in an unacceptable incidence of severe prolonged grade 4 neutropenia, albeit with minimal nonhematological effects. Successive dose levels in the present study were to be approximately 20 to 30% higher than preceding levels: 4.5, 5.75, 7.0, and 8.5 mg/m²/day. At least three new patients were entered at each dose level. Re-treatment was permitted at a minimum interval of 3 weeks. Dose escalations were permitted in an individual patient if two new patients completed treatment at the next higher dose without DLT. Dose reductions by one level were permitted for DLT. As the MTD was approached and potential DLT was observed, at least six new patients were entered. The MTD was defined as one dose level below the dose that induced DLT in greater than one-third of new patients (at least two of a maximum of six new patients). Intolerable (grades 3 and 4) nonhematological toxicities were considered dose-limiting; however, isolated and brief grade 3 nonhematological toxicity of some types (e.g., mucositis) were considered acceptable in this group of heavily pretreated leukemia patients. Prolonged (>21 days) severe aplasia (absolute neutrophil count [ANC] ≤500/µl or platelets ≤25,000/µl) was considered dose-limiting if pretreatment counts were sufficiently higher and disease progression was not evident.

TPT (SmithKline Beecham, King of Prussia, PA) was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in vials containing a lyophilized mixture of 9 mg of TPT and 100 mg of mannitol. The pH was adjusted previously to 3 to 4 with hydrochloric acid and sodium hydroxide. TPT was reconstituted with 2 ml of sterile water. The total daily dose was diluted with 50 ml of a 5% dextrose solution and administered over 30 min.

Follow-up Studies. Histories, physical examinations, complete blood counts, electrolytes, chemistries, clotting studies, and indices of fibrinolysis were performed prior to treatment and at least weekly. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (26). Bone marrow aspirations and biopsies were obtained before each course to assess tumor response; to gauge cellularity; to predict hematopoietic recovery; and to determine if the next course should be...
administered on day 22 or delayed for an additional 7 days, at which time a repeat bone marrow examination was performed. Patients were retreated if thecellularity of leukemic blasts was unchanged or reduced during the prior course; hematopoietic recovery was felt to be maximal; and nonhematological toxicities resolved. A CR was defined as a cellular bone marrow with ≤5% blasts without Auer rods, the absence of circulating blasts, and the reconstitution of normal hematopoiesis, as manifested by adequate peripheral blood counts (ANC ≥1500/µL and platelets ≥100,000/µL; Ref. 27). A partial response was defined as a reduction in bone marrow blasts to less than 25% of cellular constituents for at least 1 month (27).

Pharmacokinetic Studies. To study the pharmacokinetic behavior of high doses of TPT, whole-blood samples in heparinized tubes were obtained from indwelling venous catheters placed in the arm contralateral to the drug infusion. Samples were collected before the infusion, 15 min during the infusion, immediately before the end of infusion, then at 2, 10, 30, and 60 min and 2, 3, 4, 8, and 24 h after the end of the infusion on day 1 (9-11, 25). TPT pharmacokinetic studies were not performed on day 5 of treatment because no differences in the pharmacokinetic behavior of TPT on days 1 and 5 have been demonstrated in prior studies of TPT on this schedule (9, 10). Samples were immediately placed in heparinized tubes; transported on ice to the laboratory; and centrifuged at 4°C to separate plasma. To each 1 ml of plasma sample, 3 ml of cold methanol were added; the mixture was centrifuged; and the supernatant was frozen at −70°C.

Specimen processing, extraction, and high-performance liquid chromatographic quantitation of both the lactone TPT and total drug were performed using a method described previously (9, 25). Retention times and peak areas were calculated using the Nelson 2600 chromatographic data system (P.E. Nelson, Cupertino, CA). Drug concentrations were calculated from calibration curves made with pretreatment plasma of each subject at TPT concentrations ranging from 5 to 200 nm. Calibration curves were linear with correlation coefficients ≥0.997. The coefficients of variation for intra-day reproducibility was 11 and 6% at plasma concentrations of 5 and 50 nm, respectively. The coefficient for inter-day reproducibility, as assessed by evaluating slopes from 10 calibration curves assayed in a 2-month period, was 10%.

Pharmacokinetic Analysis. A two-compartment model was fitted to individual TPT lactone and total TPT concentration data using maximum-likelihood estimation (Adapt II; Ref. 28). A power variance model was used with the maximum-likelihood analysis (29). Estimated structural model parameters included volume of distribution for the central compartment (Vd₁), elimination rate constant (kₑ₁), and the intercompartment rate constants (Kₑ₂ and Kₑ₃). Calculated pharmacokinetic parameters included the AUC from time zero to infinity, systemic clearance (Cl), α and β half-lives (t₁/₂α and t₁/₂β), and the volume of distribution at steady-state (Vdₛ). Maximal plasma concentration (Cₘₐₓ) was determined using the model estimated structural parameters.

In Vitro Studies. Materials and antibodies used for Western blotting were obtained from the following sources: C-21 monoclonal anti-topo I from Dr. Y-C. Cheng (Yale University School of Medicine, New Haven, CT); Ki-S1 anti-topo IIα from Dr. Udo Kellner (University of Kiel, Kiel, Germany); C-2-10 monoclonal anti-PARP from Guy Poirier (Laval University, Quebec City, Quebec, Canada); monoclonal anti-PCNA from Boehringer Mannheim (Indianapolis, IN); and monoclonal anti-histone H1 from Dr. James Sorace (Veterans Administration Hospital, Baltimore, MD; Refs. 30–33). The chicken polyclonal serum against the nucleolar protein B23 was prepared as described previously (34). Peroxidase-coupled affinity-purified goat antimouse IgG, antihouse IgM, and antichicken IgG were purchased from Kirkegaard and Perry Laboratories (Gaithersburg, MD).

Marrow Samples and Cell Lines. Marrow samples were harvested by percutaneous aspiration from the posterior iliac crest prior to chemotherapy. Mononuclear cells were isolated by Ficoll-Hypaque sedimentation, sedimented at 300 × g for 10 min, and resuspended in RPMI 1640 containing 10 mM HEPES (pH 7.4 at 20°C; Ref. 33). Aliquots for Western blotting were sedimented at 300 × g for 10 min and solubilized by sonication in 250 mM Tris-HCl (pH 8.5) containing 6 mM guanidine hydrochloride, 10 mM EDTA, 150 mM 2-mercaptoethanol, and 1 mM freshly added α-phenylmethylsulfonyl fluoride (35). Other aliquots were removed for histochemical examination.

HL-60 human AML cells were grown at densities of <1 × 10⁶ cells/ml in medium consisting of RPMI 1640, 5% fetal bovine serum, 50 units/ml penicillin G, 50 µg/ml streptomycin, and 2 mM l-glutamine (35). Nonviable cells were removed by sedimentation on Ficoll-Hypaque step gradients (density, 1.119 g/ml) prior to solubilization as described above.

Electrophoresis, Western Blotting, and Quantitation. After alkylation of free sulphydryl groups with iodoacetamide, samples were dialyzed sequentially into 4 M urea and 0.1% (w/v) SDS prior to lyophilization (33, 36). Immediately

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of courses (fully evaluable)</td>
<td>27 (27)</td>
</tr>
<tr>
<td>Median no. of courses/patient (range)</td>
<td>2 (1–6)</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>50 (37–74)</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>7:7</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Leukemia type</td>
<td></td>
</tr>
<tr>
<td>CML-B</td>
<td>4</td>
</tr>
<tr>
<td>Acute lymphoblastic</td>
<td>1</td>
</tr>
<tr>
<td>Acute myeloid</td>
<td>9</td>
</tr>
<tr>
<td>Primary</td>
<td>3</td>
</tr>
<tr>
<td>Secondary</td>
<td>6</td>
</tr>
<tr>
<td>No. of prior intensive chemotherapy regimens</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

* ECOG, Eastern Cooperative Oncology Group.
* Intensive chemotherapy or preparative chemotherapy regimens for bone marrow transplantation producing prolonged (>25 days) aplasia. Does not include chronic oral therapies, vincristine/prednisone, and IFN.
before electrophoresis, samples were reconstituted at a concentration of $5 \times 10^7$ cellular equivalents/ml in sample buffer [2% (w/v) SDS, 4 mM urea, 62.5 mM Tris-HCl (pH 6.8 at 21°C) and 1 mM EDTA] and dissolved by heating to 65°C for 20 min. Samples containing $5 \times 10^7$ cellular equivalents were applied to 5–15% (w/v) acrylamide gradients surmounted by 5% (w/v) acrylamide stacking gels. After electrophoresis (10 mAmp/gel for 5 h), polypeptides were electrophoretically transferred to nitrocellulose (90 V for 6 h) using a Hoefer TE52 transfer apparatus (San Francisco, CA) containing 25 mM Tris, 192 mM glycine, 20% (v/v) methanol, and 0.2% (w/v) SDS. The nitrocellulose was stained with fast green FCF to confirm efficient transfer of proteins and treated for 7.5 min.

The polypeptides were subsequently reacted with an 1gM monoclonal antibody that consisted of sequential infusions of high doses of l-13-D-tubuline in each sample was compared to a standard curve constructed by running serial dilutions of HL-60 cells on the same blot (36). To correct for differences in loading, blots were subsequently reacted with an IgM monoclonal antibody to histone H1, a polypeptide that is proportional to DNA content.

RESULTS

Fourteen patients whose characteristics are displayed in Table 1 were entered in this study. All patients received extensive prior chemotherapy, and two patients previously had bone marrow transplantations. Six patients had primary refractory leukemia, six patients had achieved CRs at some juncture and relapsed, and two patients received no prior treatment. Prior extensive chemotherapy was usually "timed-sequential" therapy that consisted of sequential infusions of high doses of 1-B-d-arabinofuranosylcytosine, daunomycin, and amsacrine, which usually produced greater than 25 days of deep aplasia (37). The two patients who were not treated previously with extensive chemotherapy included a patient with a concurrent diagnosis of metastatic breast and a patient with CML-B who had received prior therapy with hydroxyurea and IFN. These patients were treated with 27 courses of TPT through three dose levels, as shown in Table 2. The median number of courses administered per patient was two (range, 1–6).

Toxicity. A constellation of severe toxic effects, consisting of fever, rigors, precipitous anemia, and hyperbilirubinemia, was the principal nonhematological DLT of TPT in these heavily pretreated patients. The frequencies of these toxicities at each TPT dose level are displayed in Table 2.

Dose-Limiting Toxicity. As detailed in Table 3, a constellation of severe DLTs, which occurred during all four courses administered to three patients at the 5.75 mg/m²/day dose level and one of 17 courses at the 4.5 mg/m²/day dose level, precluded dose escalation of TPT on this administration schedule. The constellation of manifestations included: (a) fever beginning within minutes to 3 h after the first dose of TPT and resolving within 24 h after the last dose. Maximal individual temperature elevations during the treatment period ranged from 39.9°C to 41.8°C. At the 5.75 mg/m² dose level, grade 3 to 4 fever (>40°C) that was not associated with hypotension was experienced by two patients during three courses, whereas a third subject experienced a maximal temperature elevation to 39.9°C (grade 2). Infectious sources were never documented by chest X-rays, computed tomographic scans of lung and abdomen, or multiple cultures of blood and other body fluids obtained during all febrile episodes; (b) rigors that were temporally related to fever with respect to onset, intensity, and resolution. Severe rigors occurred in all three patients treated at the 5.75 mg/m²/day dose level, and one patient experienced minimal rigors with a second course of TPT at the 4.5 mg/m²/day dose level after developing severe fever and rigors following a first course at the 5.75 mg/m²/day dose level. Parenteral administration of propoxyphene was often required for symptomatic relief of severe rigors; and one patient who developed intolerable rigors refused further treatment after the third dose of the second course; (c) severe precipitous fall in the hematocrit values and increasing RBC transfusion requirements in the peritreatment period despite stable transfusion requirements prior to treatment. Although the pattern resembled drug-induced hemolysis, laboratory findings were not always supportive. Direct and indirect Coombs testing was negative during all five episodes; elevations of serum levels of lactic acid dehydrogenase were documented during two courses in

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Dose escalation scheme and principal toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose level (mg/m²/day)</strong></td>
<td><strong>No. of patients</strong></td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
</tr>
<tr>
<td>4.5</td>
<td>8</td>
</tr>
<tr>
<td>5.75</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
</tr>
</tbody>
</table>

*a Documented to be due to reactivation of herpes simplex type I virus. Did not recur in a subsequent course in which acyclovir was given concurrently.

*b Severe mucositis concurrent with the development of sepsis, renal dysfunction related to sepsis, and extremely high TPT total AUC in one of the two courses.
which both pre- and posttreatment measurements were performed: peripheral blood smears reflected multiple prior transfusions during all courses, confounding the evaluation of hemolysis; and hemosiderin was present in the urine of one patient in whom this parameter was measured; (d) isolated elevations in serum bilirubin, with no or minimal elevations in hepatocellular enzymes. Abrupt elevations in serum bilirubin were noted on days 2-5 during the peritreatment period, with levels peaking on days 2-12, and complete resolution before retreatment on day 22. In two of the four courses that resulted in acute elevations in serum bilirubin, the composition was primarily of conjugated (direct) bilirubin, whereas both unconjugated (indirect) and conjugated bilirubin were increased in the two other courses. Grade 3 to 4 elevations in total bilirubin concentrations were observed in three patients during three of four courses of TPT administered at the 5.75 mg/m²/day dose level. Despite the appearance of drug-associated hemolysis, hapten-associated reactions could not be documented in an *ex vivo* assessment using TPT from the same drug supply lot and RBCs and plasma from an affected subject. Elevations in serum bilirubin were not observed in subjects who did not experience this constellation of dose-limiting toxic effects.

Although all three affected patients were treated with TPT from the same lot, two of these patients also experienced identical toxic manifestations following treatment with TPT supplied from two other lots. In addition, most patients who did not develop the aforementioned toxic manifestations were treated with TPT from the principal supply lot used in the affected patients. All of the affected patients received RBC and platelet transfusions on multiple occasions, and two of the three individuals had massive splenomegaly associated with CML-B.

**Mucositis.** Severe (grade 3) mucositis lasting longer than 3 days occurred during three courses involving three patients treated with TPT doses of 3.5 mg/m²/day (one patient) and 4.5 mg/m²/day (two patients). The etiology of the mucositis in the patient treated with TPT at the 3.5 mg/m²/day dose level appeared to be due to Herpes Simplex Type I since the virus was cultured from the patient’s oral lesions and serological studies were positive. Mucositis did not recur during a subsequent course of TPT at the same dose level in which i.v. acyclovir was administered prophylactically. Another patient who was treated at the 4.5-mg/m²/day dose level developed severe mucositis concurrent with sepsis and renal failure. This patient, who expired on day 15 due to sepsis, had the lowest clearance rates of both total TPT and lactone of any patient in the study, presumably due to reduced renal TPT clearance. Therefore, although two of eight patients treated at the 4.5-mg/m²/day level

### Table 3 Characteristics of fever, rigor, anemia, and hyperbilirubinemia

<table>
<thead>
<tr>
<th>PT no./Dx†</th>
<th>Dose (mg/m²/day)</th>
<th>Course no.</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Days</th>
<th>Rigors</th>
<th>Δ Hct</th>
<th>Day</th>
<th>Other</th>
<th>Bilirubin (mg/dl) (Total/Direct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/CML-B</td>
<td>5.75 (days 1–5)</td>
<td>1</td>
<td>40.8°C</td>
<td>1–6</td>
<td>Severe</td>
<td>24 to 17%</td>
<td>1</td>
<td>(−)Coombs</td>
<td>1.5/0.5 (pre)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>40.1°C</td>
<td>1–4</td>
<td>Severe</td>
<td>24 to 15%</td>
<td>2</td>
<td>(−)Coombs</td>
<td>2.2/0.9 (pre)</td>
</tr>
<tr>
<td>8/CML-B</td>
<td>5.75 (days 1–5)</td>
<td>1</td>
<td>39.9°C</td>
<td>1–5</td>
<td>Severe</td>
<td>28 to 23%</td>
<td>2</td>
<td>(−)Coombs</td>
<td>1.1/0.5 (pre)</td>
</tr>
<tr>
<td>9/AML</td>
<td>5.75 (days 1–5)</td>
<td>1</td>
<td>41.8°C</td>
<td>1–6</td>
<td>Severe</td>
<td>33 to 19%</td>
<td>4</td>
<td>(−)Coombs</td>
<td>0.0/0.3 (pre)</td>
</tr>
<tr>
<td></td>
<td>4.5 (days 1–5)</td>
<td>2</td>
<td>39.9°C</td>
<td>1–6</td>
<td>Mild</td>
<td>28 to 22%</td>
<td>5</td>
<td>(−)Coombs</td>
<td>No change</td>
</tr>
</tbody>
</table>

† Dx, diagnosis; LDH, lactate dehydrogenase.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Plasma disposition curves of TPT total (●, ■) and lactone (○, □) for two representative patients treated with TPT 3.5 mg/m² (●, □) and 5.75 mg/m² (○, □). The lines represent the best-fit curves using model estimated parameters from the individual subject data.
developed severe mucositis, dose-limiting mucositis could be considered an inherent effect of this specific dose level in one individual only, since mucositis was, in part, related to the concurrent development of renal dysfunction in the other subject. Grades 1 and 2 mucositis were also experienced by three other patients each during five courses at the 4.5-mg/m²/day dose level. Other toxicities included pruritis (two courses), myalgia (one course), and partial and complete alopecia in several patients who did not have complete alopecia before retreatment.

Miscellaneous. In addition to the three patients who developed fever as part of their DLT, fever without a clear infectious source was experienced by eight patients during 12 courses. A diffuse, erythematous, macular rash involving the face, trunk, and upper extremities developed in five patients during seven courses of TPT, all administered at a dose of 4.5-mg/m²/day. The rash usually occurred by day 7 and resolved by days 10 to 15. Four patients also experienced grade 1 (three courses) and grade 2 (1 course) diarrhea following treatment with TPT at 4.5 mg/m²/day. Mild to moderate nausea and/or vomiting was experienced during the treatment period during 11 courses involving five patients at the 4.5-mg/m²/day dose level and two courses involving two patients at the 5.75-mg/m²/day dose level. Other toxicities included pruritis (two courses), myalgia (one course), and partial and complete alopecia in several patients who did not have complete alopecia before treatment with TPT.

Antileukemic Activity. Antileukemic activity was noted during 11 of 23 courses involving 8 of 11 patients with leukemic blasts in the peripheral blood. Reductions in the numbers of circulating blasts was usually observed by days 4 to 5 and were maximal between days 5 and 15. In those courses administered to patients with circulating blasts, complete clearance of circulating blasts occurred in two of four courses at 3.5 mg/m²/day; five of 15 courses at 4.5 mg/m²/day; and in all four courses at 5.75 mg/m²/day. However, these effects were not sustained in these heavily pretreated patients with heterogeneous diseases, and progressive disease was typically noted before or soon after day 22, the scheduled time of retreatment. No patient experienced a sustained CR with complete hematopoietic recovery.

Pharmacokinetic Studies. Plasma sampling for pharmacokinetic studies was performed in 12 subjects. Characteristic plasma concentration–time curves are displayed in Fig. 1. Pertinent pharmacokinetic parameters for both total TPT and the lactone are displayed in Table 4. In this high-dose range, dose-dependent pharmacokinetic behavior was not evident. The clearance of the TPT lactone was approximately 3-fold greater than that of the total TPT forms; however, the primary determinant of the observed differences in plasma exposure was the dose level, which varied over a 3-fold range.

[Table 4: Pharmacokinetic parameters]

<table>
<thead>
<tr>
<th>Dose (mg/m²/day)</th>
<th>Cmax (nm)</th>
<th>AUC (μM/min)</th>
<th>CL (liters/min/m²)</th>
<th>( t_{1/2b} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAC</td>
<td>Total</td>
<td>LAC</td>
<td>Total</td>
</tr>
<tr>
<td>3.5 (n = 3)</td>
<td>62</td>
<td>163</td>
<td>11.3</td>
<td>43.8</td>
</tr>
<tr>
<td>4.5 (n = 6)</td>
<td>117</td>
<td>217</td>
<td>13.9</td>
<td>53.1</td>
</tr>
<tr>
<td>5.75 (n = 3)</td>
<td>185</td>
<td>329</td>
<td>22.0</td>
<td>54.1</td>
</tr>
<tr>
<td><strong>MEAN (SE)</strong></td>
<td><strong>31</strong></td>
<td><strong>77</strong></td>
<td><strong>0.31</strong></td>
<td><strong>0.40</strong></td>
</tr>
</tbody>
</table>

\[ \text{Values represent means (ranges).} \]

In addition, symptoms began soon after the end of the dosing period (days 5–8); both symptoms and physical findings were maximal on days 8–15; and complete resolution usually occurred by day 22 posttreatment.
that of total TPT; respective mean (± SE) clearance values were 0.77 ± 0.05 liters/min/m² and 0.23 ± 0.02 liters/min/m², and the ratio of the AUC of the lactone to that of total TPT averaged 0.31 ± 0.03 μm/min. \( t_{1/2} \) was 190 ± 20 and 219 ± 18 min for the TPT lactone and total TPT, respectively, and \( V_{\text{dss}} \) were 176 ± 17 and 65 ± 5 liters/m². Overall, plasma drug concentrations exceeded TPT concentrations that have been associated with significant cytotoxic effects in vitro. For examples, TPT \( LD_{50} \) for human leukemic blasts in a colony-forming assay ranged from 6 to 22 nM in a previous Phase I study (17), whereas \( C_{\text{max}} \) for the lactone and total TPT at the MTD were 117 nM (range, 93–136) and 217 nM (range, 94–325), respectively.

Relationships between the pertinent pharmacokinetic parameters and principal clinical effects were also evaluated. The propensity to develop mucositis of any grade was related to the AUC of both lactone and total TPT; mean lactone and total TPT AUCs for patients who developed mucositis were 17.0 and 55.1 μm/min, respectively, which were greater than comparable AUC values (11.7 and 42.8 μm/min) in patients who did not develop mucositis (one-tailed \( t \) test, \( P = 0.05 \) for both). Scatterplots of these data are shown in Fig. 2. However, the grade of mucositis did not correlate with the AUCs of either TPT lactone or total TPT. With respect to the constellation of fever, rigors, and severe precipitous decreases in hematocrit, there was substantial overlap in TPT systemic exposure in patients who did and did not develop these adverse effects. Although significant, persistent antileukemic responses were not observed; there were no pharmacodynamic relationships evident between patients who did and did not experience complete clearance of circulating leukemic blasts.

**Topo I Content of Leukemic Marrows.** In view of the preclinical data suggesting a relationship between topo I levels and TPT sensitivity (see “Introduction”), bone marrows from 10 patients (6 AML and 4 CML-B) were examined for topo I content by Western blotting. In agreement with previous results, topo I content varied widely (Fig. 3A; Ref. 24). To search for an explanation for this variation, the blots were probed with antibodies to PCNA, a subunit of DNA polymerase δ that is observed only in cycling cells, and B23, a nucleolar polypeptide whose levels have been closely associated with replication (38–40). As controls, the same samples were probed with antibodies to PARP, a DNA repair-related enzyme with a subnuclear distribution similar to that of topo I, and topo IIα, an enzyme that has been reported to diminish in amount when topo I levels increase (41–43). The results of these blots are shown in Fig. 3, B–E, and are summarized in Fig. 4.

There was a strong correlation \( (r = 0.79, P < 0.01) \) between topo I content and levels of the proliferation marker PCNA (Fig. 4A). Likewise, there was a strong correlation \( (r = 0.77, P < 0.01) \) between topo I content and levels of the proliferation-associated polypeptide B23 (Fig. 4B). In contrast, there was no correlation between topo I content and levels PARP (Fig. 4C) or topo IIα (Fig. 4D). The relationship between topo I content and two established markers of proliferation raises the possibility that differences in topo I content might reflect differences in the proliferating fractions of cells in various leukemic samples.

**DISCUSSION**

This study demonstrates that substantial dose escalation of TPT above doses that are intolerable in patients with solid tumors is feasible in patients with leukemia when TPT is administered as a 30-minute infusion for 5 days every 3 weeks. A constellation of unusual adverse effects, consisting of high fever, rigors, precipitous decreases in hematocrit, and hyperbilirubinemia, was the principal DLT of TPT on this schedule and was consistently intolerable at the 5.75-mg/m²/day dose level. These effects, particularly fever, also occurred in patients treated with lower TPT doses, but they were neither severe nor common at the lower doses. Although severe (grade 3) mucositis occurred in two of 17 courses involving two of eight patients treated at the next lower dose level, 4.5 mg/m²/day, the course of one of the subjects was confounded by the concurrent devel-
Development of sepsis and renal toxicity, which may have contributed to the mucositis by reducing the clearance of TPT.

The nature of the DLT in the present study was surprising. However, both fever and anemia have been commonly observed in the pretreatment period in patients with solid tumors who received TPT doses that were much lower than those used in the present study. In a previous Phase I study of TPT (0.5–2.5 mg/m²/day) on an identical schedule in patients with solid tumors, severe anemia, which occurred early (often by day 8), abruptly, and often required the transfusion of at least two units of packed RBCs, was noted in 45% of patients (9). The acute and early onset of fever, rigors, precipitous decline in hematocrit, and hyperbilirubinemia suggested drug-induced hemolysis. On several occasions, other laboratory findings also supported the possibility that TPT itself induces hemolytic anemia. Interestingly, although experience with TPT doses of this magnitude is limited, similar toxicities were not noted in an early phase I study involving solid tumor patients who were treated with TPT doses as high as 22.5 mg/m² as a single 30-min infusion (44). Although this observation conflicts with the notion that high doses of TPT may cause hemolysis, patients with leukemia may be more susceptible to the potential hemolytic effects of TPT, possibly due to a greater likelihood of alloimmunization and hypersplenism. In fact, two of three patients who developed DLT associated with precipitous anemia had massive splenomegaly. No pharmacodynamic relationships that could account for these toxicities were evident.

Based on the results of this study, the MTD and recommended dose for further studies of TPT on this schedule is 4.5 mg/m²/day, which is 3-fold higher than the MTD (1.5 mg/m²/day) previously established in patients with solid tumors, in whom neutropenia was the principal DLT (9–11). This dose is also 1.3-fold higher than the MTD of TPT (3.5 mg/m²/day) given with G-CSF support to minimally pretreated or untreated solid tumor patients, in whom further dose escalation is precluded by severe myelosuppression (25).

In patients with refractory acute leukemia, the magnitude of TPT dose escalation above the MTD established for solid tumor patients does not appear to be influenced by schedule. In previous studies of TPT as a 5-day continuous infusion in acute leukemia, the MTD was also 3-fold higher than the MTD established for solid tumor patients (23, 24, 45). Therefore, the selection of an administration schedule for further development in leukemia cannot be based solely on the potential for dose escalation above myelosuppressive doses because both schedules are equivalent in this regard. On the other hand, a similar potential for dose escalation in leukemia patients above MTDs established in solid tumor patients is a characteristic of only a few types of antineoplastic agents, principally antimetabolites and alkylating agents. In addition, it has not been possible to escalate the doses of other topo I inhibitors to a similar extent (2, 5). For the prototypic topo I inhibitor, CPT, and the semisynthetic, water-soluble CPT analogue, irinotecan, severe nonhematological effects are at least as prominent as myelosuppression, precluding dose escalation on almost all dosing schedules (2, 5, 46, 47). With irinotecan, although the aggressive use of antidiarrheal and antiemetic agents has been reported to

![Fig. 4 Relationship between topo I content and other nuclear polypeptides. A, PCNA; B, B23; C, PARP; D, topo II c. Blots shown in Fig. 3 were scanned as described. The content of each polypeptide in each clinical sample was compared to the content in HL-60 cells. After correction for loading (division by relative H1 content), the relative content of each polypeptide in each sample was compared to relative topo I content in the same sample. A value of 1.0 indicates that a clinical sample contains as much of the measured polypeptide as an equal number of HL-60 cells; a value of 0.1 indicates that the clinical sample contains 10% as much polypeptide as an equal number of HL-60 cells. O, AML; ●, CML-B.](image)
reduce the rate and severity of nonhematological toxicity, these measures have not resulted in the ability to escalate doses more than 2-fold (2, 5, 46, 47). Thus, TPT is unique among topo I inhibitors in its ability to be escalated in leukemic patients, suggesting that further investigations of this agent in the treatment of leukemia or as part of intensive cytoreductive regimes prior to hematopoietic stem cell re-infusion might be warranted.

The factors that determine topo I content in human leukemia cells are currently unknown. Recent results suggest that the topo I content in normal myeloid cells might be related to the degree of proliferation or maturation (48). In particular, high levels of topo I have been observed in early myeloid precursors, and low topo I levels have been observed in the mature postmitotic cells. The present study also sought to evaluate whether variations in topo I might also reflect differences in proliferation. As reported previously, there were differences in topo I content between various leukemic samples (Fig. 3A; 24). In contrast to tissue culture cell lines, where it has been reported that topo II content increases when topo I content decreases (42, 43), a negative correlation between levels of these polypeptides was not observed (Fig. 4D).

Instead, there was a strong positive correlation between topo I content and two markers of proliferation, PCNA (Fig. 4A) and B23 (Fig. 3B), suggesting that variations in topo I content reflect variations in the size of the proliferative cell fraction in various specimens. Because of differences in the doses of TPT administered to various patients as well as the lack of clinical responses in the present trial, it was not possible to correlate these differences in topo I content with differences in clinical response. The administration of more homogeneous doses to a more homogeneous patient population in the Phase II setting might allow such a correlation to be observed.

The feasibility of substantial dose escalation of TPT on a 30-min infusion daily for a 5-day schedule in leukemia patients is encouraging, particularly since positive concentration-response and dose-response effects are clearly evident in both in vitro and animal studies (1, 2, 5, 7, 8). However, the development of alternate high-dose TPT schedules in leukemia, such as the 5-day continuous infusion schedule, may be warranted in view of the nature of the DLT and the lack of sustained clinical responses noted in this preliminary investigation.

ACKNOWLEDGMENTS

Kind gifts of antibodies from Y-C. Cheng, Udo Kellner, Guy Poirier, and James Sorace were essential for this study. The technical assistance of Wendy Berry, Christopher Buckwalter, Phyllis Svingen, and Timothy Kotke, as well as the editorial assistance of Deb Strauss, are gratefully acknowledged.

REFERENCES

17. Eisenhauer, E. A., Wainman, G., Boos, G., Macdonald, V., and Bramwell, V. Phase II trials of topotecan in patients with malignant...
A phase I and pharmacological study of topotecan infused over 30 minutes for five days in patients with refractory acute leukemia.
