Phase I Study of Topotecan, A New Topoisomerase I Inhibitor, in Patients With Refractory or Relapsed Acute Leukemia

By Hagop M. Kantarjian, Miloslav Beran, Amy Ellis, Leonard Zwedding, Susan O'Brien, Lorraine Cazenave, Charles Koller, Mary Beth Rios, William Plunkett, Michael J. Keating, and Elihu H. Estey

The purpose of this study was to define, in a phase I study in leukemia, the maximally tolerated dose (MTD), major toxicities, and possible antitumor activity of Topotecan, a new topoisomerase I (topo I) inhibitor. Topotecan was delivered by a 5-day continuous infusion every 3 to 4 weeks to patients with refractory or relapsed acute leukemia, at doses ranging from 3.5 mg/m² to 18 mg/m² per course. Twenty-seven patients were treated, including 17 patients with acute myelogenous or undifferentiated leukemia, 7 with acute lymphocytic leukemia, and 3 with chronic myelogenous leukemia in blastic phase. Severe mucositis was the dose-limiting toxicity occurring in two of five patients treated with Topotecan 11.8 mg/m² per course; a third patient had prolonged myelosuppression. At the MTD of 10 mg/m² per course, 1 of 12 patients had severe mucositis and 8 had mild-to-moderate mucositis. Nausea, vomiting, diarrhea, and prolonged myelosuppression were uncommon. Three patients (11%) achieved a complete response, two (7%) had a partial response, and one (4%) had a hematologic improvement. The overall complete plus partial response rate was 19%, and 24% in acute myelogenous or undifferentiated leukemia. A novel in vitro assay that quantifies Topotecan-stabilized topo I-DNA complexes in patient samples was used, which demonstrated heterogeneity in the ability of Topotecan to interact with topo I, the intracellular target of Topotecan. This phase I study defined the MTD of Topotecan to be 10 mg/m² by continuous infusion over 5 days every 3 to 4 weeks in patients with refractory or relapsed acute leukemia. Severe mucositis was the dose-limiting toxicity. Future studies will define the precise activity of Topotecan in different leukemia subsets, its efficacy in combination with other antileukemic drugs, and correlations between Topotecan-induced topo I-DNA complex formation and individual patient response to Topotecan.

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MATERIALS AND METHODS

Study population. Adults with a diagnosis of acute leukemia who were refractory or had relapsed following frontline induction or salvage chemotherapy were entered into this study. All patients signed an informed consent to participate in the study according to institutional guidelines. Eligibility required: (1) a performance status of 2 or better; (2) a bilirubin level of 1.5 mg% or less and a creatinine level of 1.5 mg% or less.

Pretreatment evaluation included a history and physical examination, complete blood counts (CBC), differential and platelet counts, SMA12 with liver and renal function tests; coagulation profile; bone marrow aspirate, biopsy, chemical and enzymatic stains, and cytogenetic studies. Diagnosis was confirmed by morphology and histo-
chemical stains and categorized according to the French-American-British criteria. Follow-up studies included CBC, differential and platelet counts three times weekly until remission, then at least once weekly; SMA12 at least weekly; and bone marrow aspiration on days 14 and 21 from start of therapy, every 3 to 7 days as indicated until remission, and before each maintenance course.

Treatment schedule. Topotecan was administered as a continuous infusion over 5 days every 3 to 4 weeks. A second induction course was delayed if there was no evidence of leukemia and persistence of marrow hypoplasia. The dose in the initial patients was 3.5 mg/m² per course. Dose escalations in subsequent patients were in 50% increments until evidence was seen of grade 2 toxicity based on the National Cancer Institute (NCI) toxicity grading system, then in 30% to 35% increments until the maximally tolerated dose (MTD) was reached.

The MTD was defined as one dose level below the one that produced, in at least two of a maximum of six patients receiving their first course of treatment, either (1) NCI grade 3 or greater nonhematologic toxicity, or (2) severe hematologic toxicity defined as persistent pancytopenia with less than 5% cellular bone marrow without evidence of residual leukemia, lasting for 6 weeks or more from the start of the course. A total of 12 patients were treated at the MTD to define the toxicity and antileukemic profile more precisely.

Patients who had neither antileukemic effect nor grade 2 or more toxicity on their first course received their second course at one dose level escalation. Patients manifesting grade 2 or more toxicity and antileukemic effect received a second course of the same level if toxicity was grade 2, and at 20% dose reduction if toxicity was grade 3. Patients achieving remission received maintenance therapy with Topotecan at the same dose level that induced the remission if it was below the MTD, or at 20% dose reduction from the MTD.

Topotecan was supplied by the NCI for injection in 5 mg vials (as the base) with 100 mg of mannitol. The pH was adjusted to 3.0 with HCl/NaOH. Topotecan was constituted with 2 mL of sterile water for injection and further diluted to 0.1 mg/mL in 5% dextrose.

Response and toxicity criteria. A complete remission (CR) was defined as normalization of blood and bone marrow, with 5% or less blasts in a normocellular or hypercellular bone marrow, a granulocyte count above 10⁹/µL, and a platelet count above 100 × 10⁹/µL. Patients who met these criteria but still had 6% to 25% marrow blasts were considered to have a partial remission (PR). Hematologic improvement (HI) was defined as a CR but without recovery of the platelets for injection and further diluted to 0.1 mg/mL in 5% dextrose. Other responses were considered as failures and categorized as: (1) early death if death occurred within 2 weeks from start of therapy; (2) aplastic death if death occurred during therapy without evidence of hematologic recovery and with less than 20% marrow leukemic infiltrate (MLI = percentage of blasts × marrow cellularity); (3) secondary resistance if the MLI was reduced below 20% but increased later; and (4) primary resistance if the MLI did not decrease below 20%.

Toxicity was graded on a scale of 0 to 5 by the NCI criteria. Measurements of topo I-DNA complex formation in leukemia cells. The assay for detection of topo I-DNA complexes was based on previous studies indicating that protein-bound DNA is retained on glass fiber or nitrocellulose filters when applied to these filters. The amount of DNA (provided by Dr Grady Saunders) that had been radioactively labeled by random priming with [32P]dCTP (Amersham, Arlington Heights, IL) using the Amersham Multiprime Labeling System. Hybridization of Alu probe to DNA on the filter was quantified using

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
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<tr>
<td>AML/AUL</td>
<td>ALL</td>
</tr>
<tr>
<td>Treated</td>
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</tr>
<tr>
<td>Salvage status</td>
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<td>4</td>
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<td>Second</td>
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<td>6</td>
</tr>
<tr>
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</tr>
<tr>
<td>Diploid</td>
<td>6</td>
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<td>Translocation 15;17</td>
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<tr>
<td>Other abnormalities</td>
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<tr>
<td>Insufficient metaphases</td>
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</tr>
<tr>
<td>Not available</td>
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</table>

The assay for detection of topo I-DNA complexes was based on previous studies indicating that protein-bound DNA is retained on glass fiber or nitrocellulose filters when applied to these filters. The Alu sequence is ubiquitously distributed throughout the human genome. Details on the development and the validation of this assay in HL-60 leukemia cells have been published and will be published. This is the first application of the technique to leukemic cells freshly obtained from patients during a clinical study.

Ten milliliters of blood was drawn from patients with circulating leukemic cells before chemotherapy with Topotecan. Cells were separated from plasma by centrifugation 250g and resuspended in phosphate-buffered saline. Leukemic cells were isolated on Ficoll-Hypaque (5.7% Ficoll, 9% dextran sodium (Pharmacia, Piscataway, NJ) and resuspended to 5 × 10⁵/mL in Iscove's modified Dulbecco's medium (JHR Biochemicals, Lenexa, KS) before treatment with 100 µmol/L Topotecan, 2 µmol/L Topotecan, or vehicle (deionized water) for 1 hour at 37°C, 5% CO₂. Isolated, untreated cells (2.5 to 5 × 10⁶) were deposited on a slide by cyt centrifugation and stained with Wright's stain to confirm that the population of cells used in the assay were malignant. After drug treatment, 1 × 10⁶ cells were centrifuged at 13,000g for 1 minute (in triplicate) and the medium aspirated. The cells were immediately lysed with 100 µL 1.25% sodium dodecyl sulfate/5 mmol/L EGTA, pH 8, at 65°C. Lysates were vigorously vortexed and incubated at 65°C for 10 minutes before DNA was sheared with a 27-gauge needle. One milliliter of 65°C protein-binding buffer (0.4 guanidine HC1; 10 mmol/L Tris, pH 8; 10 mmol/L EGTA, pH 8; 0.01% Sarkosyl; 0.3 mol/L NaCl; 10 mmol/L MgCl₂) was added to the lysed cells. These lysates were applied to nitrocellulose filters using a dot blotter (both from Schleicher and Schuell, Keene, NH). Under the buffer conditions only protein-bound DNA is retained on the filter. DNA was fixed to the nitrocellulose filter by baking at 80°C for 2 hours in a vacuum oven. The amount of DNA on a filter was determined by hybridization to Alu DNA (provided by Dr Grady Saunders) that had been radio labeled by random priming with [32P]dCTP (Amersham, Arlington Heights, IL) using the Amersham Multiprime Labeling System. Hybridization of Alu probe to DNA on the filter was quantified using

<table>
<thead>
<tr>
<th>Topotecan Dose (mg/m² continuous infusion over 5 d)</th>
<th>No. of Patients</th>
<th>Subsequent Courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same Dose</td>
<td>Increased</td>
<td>Reduced</td>
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<td>3.5</td>
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<td>10.0</td>
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<td>5</td>
<td>—</td>
</tr>
<tr>
<td>18.0</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>5</td>
</tr>
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</table>
in 8 patients. Nineteen of the 27 patients (70%) received Topotecan as their second or subsequent salvage. Two of the eight patients treated in first salvage had chronic myelogenous leukemia in blastic phase (CML-BP), one had Philadelphia chromosome (Ph)-positive acute lymphocytic leukemia (ALL), one had erythroleukemia with abnormalities in chromosome 5 (5q-), and one had a first CR duration of less than 1 year.

### RESULTS

The characteristics of the 27 patients entered in the study are shown in Table 1. Their median age was 51 years (range 23 to 65 years), 8 (30%) were 60 years or older, and 13 were women. Performance status was 0 or 1 in 19 patients and 2 in 8 patients. Nineteen of the 27 patients (70%) received Topotecan as their second or subsequent salvage. Two of the eight patients treated in first salvage had chronic myelogenous leukemia in blastic phase (CML-BP), one had Philadelphia chromosome (Ph)-positive acute lymphocytic leukemia (ALL), one had erythroleukemia with abnormalities in chromosome 5 (5q-), and one had a first CR duration of less than 1 year.

#### Toxicity

The dose escalation scheme of Topotecan with the number of patients treated and of courses delivered are shown in Table 2. Because no grade 2 or greater toxicity was observed with Topotecan administered at 7.9 mg/m² by continuous infusion over 5 days, the dose level was increased by 50% to 11.8 mg/m² per course. The first three patients entered did not experience toxicities over the next 2-week observation period, therefore one patient was treated at 18 mg/m² per course. The third patient who entered at 11.8 mg/m², developed delayed grade 3 mucositis as did the patient treated at 18 mg/m². Of the two additional patients treated subsequently at 11.8 mg/m², one had grade 3 mucositis and the other had prolonged myelosuppression. Patients were then treated with 10 mg/m² of Topotecan by continuous infusion over 5 days, which was later determined as the MTD.

The major toxicity encountered was mucositis, which was severe at a dose level of 11.8 mg/m² per course or above. Moderate (grade 2) mucositis was described as painful erythema, edema, or ulcers in the oral mucosa, the patient still being able to eat. Severe (grade 3) mucositis was described as similar lesions that prevented the patient from eating. Severe mucositis was observed in two of five patients administered 11.8 mg/m² on their first course (Table 3). At the dose level of 10 mg/m², grade 3 mucositis was seen in 1 of 12 patients, and grade 1 or 2 mucositis in 5 additional patients. Mucositis involved the oral mucosa, esophagus, or both. Severe esophagitis associated with gastrointestinal bleeding was observed in one of the two patients with severe mucositis treated at 11.8 mg/m².

No other severe nonhematologic toxicities were observed. Mild-to-moderate nausea and vomiting were reported in two patients (at 3.5 and 10 mg/m²); whereas mild-to-moderate diarrhea was observed in four patients (2 at 10 mg/m² and 2 at 11.8 mg/m²).

Elevations of creatinine levels above 2 mg% were observed in two patients and bilirubin levels above 2 mg% in two other patients. In all four instances, the changes were noted during febrile episodes: sepsis in one patient; pneumonia, two patients; fever of unknown origin, one patient.

Prolonged myelosuppression, defined as marrow hypoplasia for longer than 6 weeks from the start of treatment, was noted in one of five patients treated with 11.8 mg/m² of Topotecan.

Febrile episodes were observed in 20 patients during periods of neutropenia. These included febrile episodes of unknown origin in 7 patients and documented infections in 13 patients: sepsis in 3 patients, pneumonia, 8 patients; fungemia, 1 patient; herpes zoster, 1 patient. Eleven of the 20 patients experienced more than one febrile episode.

**Response.** Overall, three patients (11%) achieved CR, two (7%) PR, and one (4%) HI. The overall (CR + PR) response rate was 19% (Table 4). Responses were observed in 4 of 17 patients with AML or acute undifferentiated leukemia (AUL) (24%), and in 1 of 3 patients with CML-BP (33%). Responses by salvage status were: first salvage, 3 of 8 (38%); later salvages, 2 of 19 (11%).

The characteristics of the six responders are detailed in Tables 5 and 6. Patient no. 1 achieved CR after one course of Topotecan at 10 mg/m². She received two maintenance courses at 8 mg/m², and relapsed 4 months later. Patient no. 2 did not respond to the first course of Topotecan at 5.25 mg/m² but achieved CR with a second course at 7.9 mg/m². Disease reappeared 4 weeks later and she achieved a second CR with Topotecan administered at 10 mg/m². She developed progressive leukemia 4 weeks later and died. Patient no. 3 had CML-BP and achieved CR after one course at 11.8 mg/m², which also produced grade 2 mucositis. Cytogenetic

### Table 3. Toxicities Observed With Topotecan Therapy

<table>
<thead>
<tr>
<th>Dose Level (mg/m²)</th>
<th>No. of Patients</th>
<th>Mucositis</th>
<th>Nausea/Vomiting</th>
<th>Diarrhea</th>
<th>Prolonged Myelosuppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>5.25</td>
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<td>0</td>
</tr>
<tr>
<td>7.9</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>10</td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>11.8</td>
<td>5</td>
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<td>0</td>
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<tr>
<td>18</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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analysis in remission showed 100% Ph-positive metaphases. He later developed a second chronic phase CML picture and received maintenance therapy with three courses of Topotecan 8 mg/m² monthly, followed by maintenance with hydroxyurea and interferon α. He relapsed with CML-BP 4 months after achieving CR. Patient no. 4 was treated in first salvage with Topotecan 10 mg/m². She achieved PR after the first course with normalization of the peripheral counts and a reduction of the marrow blasts from 33% to 9%. The first course was associated with grade 2 mucositis. She received two additional induction courses, one at the same dose level and the second at 8 mg/m² but was still in PR. She was then taken out of the study and is receiving salvage combination therapy. Patient no. 5 also achieved PR after one uncomplicated course of Topotecan at 10 mg/m² but had increasing blasts after receiving his second course at the same dose level. Patient no. 6 had a diagnosis of Ph-positive AUL and had hematologic and cytogenetic relapse 3 months following allogeneic bone marrow transplantation, with redocumentation of Ph-positive metaphases. Following one course of Topotecan at 7.9 mg/m² her marrow, white blood cell count, and differential became normal. However, the platelets were only $60 \times 10^9/\mu L$. Repeat cytogenetic analysis at the time of marrow CR showed 100% diploid metaphases. Leukemia recurred 3 months later and did not respond to a second course of Topotecan at 10 mg/m².

**Topoisomerase I-DNA complex measurements.** Figure 1 shows the results of the first clinical application of the new filter-binding method using Alu-probe hybridization to quantify topo I-DNA complexes. Ten patients were tested. Topotecan produced marrow hypoplasia in the three patients shown in open symbols but not the other seven patients. Considering the small number of patients investigated and the different dose schedules used in this phase I study, there was no clear correlation between patient response to therapy and the magnitude of complex formation induced by Topotecan treatment in vitro. However, the interpatient variability in the production of Topotecan-stabilized topo I-DNA complexes is noteworthy and suggests there may be differences in drug sensitivity of the cellular target in this small patient sample.

**DISCUSSION**

In our search for effective antileukemic agents with novel mechanisms of action, the camptothecin derivatives, such as Topotecan and camptothecin-11 (CPT-11), are of interest in the therapy of acute leukemia. In this phase I study, Topotecan was administered by a 5-day continuous infusion every 3 to 4 weeks to patients with refractory or relapsed acute leukemia. The MTD was 10 mg/m² per course, a fivefold higher dose than the MTD in patients with solid tumors, which was limited by myelosuppression. At this dose schedule, mucositis was the dose-limiting toxicity, whereas other side effects, such as nausea and vomiting, diarrhea, and prolonged myelosuppression, were unusual. Objective responses were observed in 5 of 27 patients treated (19%), including 4
of 17 patients with AML or AUL (24%). One additional patient achieved an HI with a marrow morphologic and cytogenetic CR and normalization of counts except for persistent thrombocytopenia.

Camptothecins have produced objective responses in a broad spectrum of tumors. In the phase I study of Topotecan in patients with solid neoplasms, responses were reported in patients with non-small cell lung cancer and ovarian carcinoma. Similarly, the phase I and II studies of CPT-11 conducted in Japan demonstrated a response rate of 32% in patients with untreated non-small cell lung cancer, 46% in colorectal lung cancer, 21% in ovarian carcinoma, and 24% in cervical cancer.

In an early phase II study conducted in lymphoma and leukemia, Ohno et al reported a response rate of 24% among 29 patients with lymphoma. Among 26 patients treated for leukemia, 3 patients (12%) responded (1 CR and 2 PR). All 3 responders were among 12 patients treated with a schedule using CPT-11 in a 1-hour infusion twice daily for 7 days every 3 to 4 weeks, whereas none of 14 patients receiving shorter CPT-11 infusion or exposure schedules responded. This is in agreement with in vitro studies demonstrating superior antileukemic efficacy with more frequent dosing schedules, and with our phase I study of Topotecan using a 5-day continuous infusion schedule that produced a 19% response rate.

Unlike CPT-11, Topotecan does not seem to produce hemorrhagic cystitis. Gastrointestinal side effects such as nausea, vomiting, and diarrhea, which were observed in 70% to 75% of patients treated with CPT-11, were unusual with this schedule of Topotecan. On the other hand, mucositis was a serious problem with the 5-day continuous infusion of Topotecan and was dose-limiting at levels above 10 mg/m².

The antileukemic efficacy of Topotecan is potentially quantifiable in vitro at the cellular target level. In our study, the quantity of topo I-DNA complex was measured following in vitro exposure of the patients' leukemic cells to Topotecan. Heterogeneity in the amount of drug-stabilized topo I-DNA complex was observed. This amount may potentially parallel clinical response to Topotecan therapy. Correlations between in vitro and in vivo response may yield a predictive test for individual responsiveness to Topotecan and possibly to other topo I-reactive agents, and provide a rationale for strategies aimed at enhancing topo I-DNA complex formation.

Once the antileukemic efficacy and optimal dose-schedule of Topotecan are defined, combination studies with Topotecan and other antileukemic agents need to be explored. These may include ara-C or its analogues, topo II-reactive agents, or platinum analogues (cisplatin, carboplatin). In vitro studies have shown: (1) topo II-reactive agents to be effective against leukemic cell lines resistant to topo I-reactive drugs; and (2) increased topo II levels in cell lines resistant to topo I-reactive drugs. These observations would encourage the evaluation of combination regimens containing topo I-reactive (Topotecan, CPT-11) and topo II-reactive drugs (anthracyclines, amsacrine, etoposide). On the other hand, antagonistic effects have been observed under certain experimental conditions between topo I- and topo II-reactive agents. These antagonistic in vitro interactions may be schedule dependent. Similar contradictory preclinical data exist to support or caution against the use of topo I-reactive agents with ara-C or deoxyazacytidine or with platinum analogues. This suggests that Topotecan-based combination regimens require further evaluation in preclinical animal models, and cautious exploration in the clinical trials.

In summary, this phase I study of Topotecan in patients with refractory or relapsed leukemia has defined the MTD of a 5-day continuous infusion schedule to be 10 mg/m² per course. Severe mucositis was the dose-limiting toxicity. Antileukemic activity was observed. The antileukemic efficacy was also potentially quantifiable at the target level via measurements of the topo I-DNA complex formation, and such correlations will be evaluated more extensively in postphase I studies.

ACKNOWLEDGMENT

We thank Billy Nowak for her excellent technical help.

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