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

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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 blast 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 5 had mild-to-moderate mucositis. Nausea, vomiting, diarrhea, and prolonged myelosuppression were uncommon. Three patients (11%) achieved a complete response.

Only two types of drugs have demonstrated significant activity in acute myelogenous leukemia (AML): (1) cytosine analogues, particularly cytosine arabinoside (ara-C), and (2) topoisomerase II (topo II)-reactive agents, such as anthracyclines, amsacrine, or etoposide. Combinations of ara-C and topo II-reactive drugs induce complete remissions in 60% to 80% of adults with AML, but only 15% to 25% are cured. New effective antileukemic agents may ameliorate patient outcome.

By analogy to testicular cancer in which the incorporation of cisplatinum into velban-bleomycin regimens has improved the long-term disease-free survival from 30% to 40% up to 70% to 80%, the discovery of antileukemic agents with novel mechanisms of action and their subsequent incorporation into combination regimens should improve outcome in AML.

Topotecan (tiazofuramine, SKF104864), a semisynthetic water-soluble analogue of the alkaloid camptothecin, is representative of a new class of topo I-targeting agents. Topo I is an enzyme intimately involved in DNA replication. Topotecan and other topo I-targeting drugs stabilize the topo I-DNA complex formed transiently during the interaction of the enzyme and DNA, thus inhibiting topo I function or initiating events that lead to cell death. This drug-stabilized complex can be detected as a DNA single-strand break in a number of biochemical assays. Data suggest that intracellular levels of topo I may be higher in malignant than normal cells. Consequently, topo I may be a therapeutically exploitable target with topo I-reactive drugs exerting selective antitumor activity.

Topotecan has shown activity in a broad spectrum of murine tumor models, including P388 and L1210. It was most active in schedules that correspond to infusion regimens. Sublines of P388 resistant to topo II-reactive agents such as doxorubicin, daunorubicin, mitoxantrone, and amsacrine were not cross-resistant to Topotecan. In phase I clinical trials, myelosuppression was the dose-limiting toxicity, and responses were observed in lung and ovarian carcinoma. Based on this preclinical and clinical experience, we conducted a phase I study using a 5-day continuous infusion schedule in patients with refractory or relapsed leukemia. Our study defines mucositis as the dose-limiting toxicity and demonstrates antileukemic efficacy, with objective responses observed. We also report the first clinical application of a new assay to quantify drug-induced topoisomerase-DNA complexes that can potentially serve as a method to individualize antileukemia drug treatment.

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 on Zubrod scale, and (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-
**Table 1. Characteristics of the 27 Patients Treated in the Phase I Topotecan Study**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AML/AUL</th>
<th>ALL</th>
<th>CML-BP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salvage status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Second</td>
<td>7</td>
<td>3</td>
<td>—</td>
<td>10</td>
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<tr>
<td>Third or more</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>9</td>
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<tr>
<td><strong>Karyotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>6</td>
<td>1</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Chromosome 5 or 7</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Abnormality</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ph+ positive</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Translocation 15;17</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Other abnormalities</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Insufficient metaphases</td>
<td>1</td>
<td>2</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Not available</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>3</td>
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</table>

**Table 2. Dose Escalation Schema**

<table>
<thead>
<tr>
<th>Topotecan Dose (mg/m² continuous infusion over 5 d)</th>
<th>No. of Patients</th>
<th>Subsequent Course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Same Dose</td>
<td>Increased</td>
</tr>
<tr>
<td>3.5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5.25</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>7.9</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>10.0</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>11.8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>18.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27</td>
<td>5</td>
</tr>
</tbody>
</table>
in 8 patients. Nineteen of the 27 patients (70%) received To-
treated with IO mg/m² of Topotecan by continuous infusion
over 5 days, the dose level was increased by
quent at 11.8 mg/m², one had grade
chrome (Ph)-positive acute lymphocytic leukemia
patients; and grade
edema, or ulcers in the oral mucosa, the patient still
a dose level of
mg/m². Of the two additional patients treated subse-
CML-BP, one had Philadelphia
mucositis was described
1.8 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, de-
volved delayed grade 3 mucositis as did the patient treated at
8 mg/m². Of the two additional patients treated subse-
quently 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. Moder-
grade 2 mucositis was described as painful er-
ethema, 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. Se-
vere mucositis was observed in two of five patients admin-
istered 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. Se-
vere 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

### 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>
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<tbody>
<tr>
<td>3.5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>5.25</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.9</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11.8</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

the Betascope (Betagen 603). Topotecan-induced DNA-protein cross-
linking was the ratio of the average counts collected by Betascope of
samples treated with Topotecan versus samples treated with vehicle.

### 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 To-
potecan 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 chro-
mosome 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 con-
tinuous 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, de-
volved delayed grade 3 mucositis as did the patient treated at
18 mg/m². Of the two additional patients treated subse-
quently at 11.8 mg/m², one had grade 3 mucositis and the
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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

### Table 4. Treatment Results in 27 Patients

<table>
<thead>
<tr>
<th>Response</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>3</td>
<td>(11)</td>
</tr>
<tr>
<td>PR</td>
<td>2</td>
<td>(7)</td>
</tr>
<tr>
<td>HI</td>
<td>1</td>
<td>(4)</td>
</tr>
<tr>
<td>Aplastic death</td>
<td>4</td>
<td>(15)</td>
</tr>
<tr>
<td>Secondary resistance</td>
<td>4</td>
<td>(15)</td>
</tr>
<tr>
<td>Primary resistance</td>
<td>13</td>
<td>(48)</td>
</tr>
</tbody>
</table>
Table 5. Characteristics of Responding Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Diagnosis</th>
<th>Karyotype</th>
<th>Duration of First CR (mos)</th>
<th>Salvage Status</th>
<th>Prior Therapy</th>
<th>Topotecan Dose Level (mg/m²)</th>
<th>Response/Duration (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>AML</td>
<td>Trisomy 4</td>
<td>17</td>
<td>1</td>
<td>Daunorubicin + ara-C</td>
<td>10</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>AML</td>
<td>Diploid</td>
<td>0</td>
<td>2</td>
<td>Daunorubicin + ara-C, Carboplatin + VP16</td>
<td>7.8, 10</td>
<td>CR1, CR2</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>CML-BP</td>
<td>Ph</td>
<td>—</td>
<td>1</td>
<td>Hydroxyurea</td>
<td>11.8</td>
<td>CR2, CR→</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>AML</td>
<td>IM</td>
<td>20</td>
<td>1</td>
<td>Daunorubicin + ara-C</td>
<td>10</td>
<td>PR</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>AML</td>
<td>IM</td>
<td>27</td>
<td>2</td>
<td>Mitoxantrone + high-dose ara-C, Daunorubicin + ara-C</td>
<td>10, 10</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>AUL</td>
<td>Ph</td>
<td>0</td>
<td>2</td>
<td>Idfurubicin + ara-C, VAD, allogeneic BMT</td>
<td>7.9</td>
<td>HI</td>
</tr>
</tbody>
</table>

Abbreviations: IM, insufficient metaphases; VAD, vincristine, Adriamycin, dexamethasone; BMT, bone marrow transplantation; CP, chronic phase.

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 at 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 at 10 mg/m². She achieved PR after the first course with normalization of the peripheral counts and a reduction of the marrow blasts from 35% 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 $60 \times 10^9/μL$. 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

Table 6. Outcome of Responding Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC ($\times 10^9/μL$)</th>
<th>% Granulocytes</th>
<th>% Blasts</th>
<th>Platelets ($\times 10^9/μL$)</th>
<th>Marrow Cellularity (%)</th>
<th>% Blasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4/2.2</td>
<td>51/48</td>
<td>0/0</td>
<td>122/193</td>
<td>30/35</td>
<td>45/4</td>
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<tr>
<td>2</td>
<td>3.5/5.3</td>
<td>25/61</td>
<td>0/0</td>
<td>17/142</td>
<td>100/90</td>
<td>44/2</td>
</tr>
<tr>
<td>3</td>
<td>112.4/13.6</td>
<td>26/68</td>
<td>30/0</td>
<td>503/720</td>
<td>100/85</td>
<td>56/1</td>
</tr>
<tr>
<td>4</td>
<td>0.9/2.3</td>
<td>33/66</td>
<td>0/0</td>
<td>33/104</td>
<td>—/25</td>
<td>35/9</td>
</tr>
<tr>
<td>5</td>
<td>0.6/2.2</td>
<td>10/60</td>
<td>0/0</td>
<td>16/147</td>
<td>15/40</td>
<td>71/9</td>
</tr>
<tr>
<td>6</td>
<td>4.4/2.9</td>
<td>89/85</td>
<td>0/0</td>
<td>85/60</td>
<td>60/50</td>
<td>34/0</td>
</tr>
</tbody>
</table>
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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Phase I study of Topotecan, a new topoisomerase I inhibitor, in patients with refractory or relapsed acute leukemia

HM Kantarjian, M Beran, A Ellis, L Zwelling, S O'Brien, L Cazenave, C Koller, MB Rios, W Plunkett and MJ Keating