A Phase I and Pharmacodynamic Study of Fludarabine, Carboplatin, and Topotecan in Patients With Relapsed, Refractory, or High-Risk Acute Leukemia

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ABSTRACT

Purpose: A novel regimen designed to maximize anti-leukemia activity of carboplatin through inhibiting repair of platinum-DNA adducts was conducted in poor prognosis, acute leukemia patients.

Experimental Design: Patients received fludarabine (10 to 15 mg/m² × 5 days), carboplatin (area under the curve 10 to 12 by continuous infusion over 5 days), followed by escalated doses of topotecan infused over 72 hours (fludarabine, carboplatin, topotecan regimen). Twenty-eight patients had acute myelogenous leukemia (7 untreated secondary acute myelogenous leukemia, 11 in first relapse, and 10 in second relapse or refractory), 1 patient had refractory/relapsed acute lymphoblastic leukemia, and 2 patients had untreated chronic myelogenous leukemia blast crisis. Six patients had failed an autologous stem cell transplant. Patients ranged from 19 to 76 (median 54) years. Measurement of platinum-DNA adducts were done in serial bone marrow specimens.

Results: Fifteen of 31 patients achieved bone marrow aplasia. Clinical responses included 2 complete response, 4 complete response with persistent thrombocytopenia, and 2 partial response. Prolonged myelosuppression was observed with median time to blood neutrophils ≥200/µL of 28 (0 to 43) days and time to platelets ≥20,000/µL (untransfused) of 40 (24 to 120) days. Grade 3 or greater infections occurred in all of the patients, and there were 2 infection-related deaths. The nonhematologic toxicity profile was acceptable. Five patients subsequently received allografts without early transplant-related mortality. Maximum tolerated dose of fludarabine, carboplatin, topotecan regimen was fludarabine 15 mg/m² × 5, carboplatin area under the curve 12, and topotecan 2.55 mg/m² over 72 hours. An increase in bone marrow, platinum-DNA adduct formation between the end of carboplatin infusion and 48 hours after the infusion correlated with bone marrow response.

Conclusions: Fludarabine, carboplatin, topotecan regimen is a promising treatment based on potential pharmacodynamic interactions, which merits additional study in poor prognosis, acute leukemia patients.

INTRODUCTION

Treatment options are limited for the majority of patients with acute leukemia developing after pre-existing hematologic conditions or relapsed after initial induction chemotherapy. New promising approaches include combinations of novel agents designed to modulate DNA repair mechanisms and thereby provide synergistic cytotoxicity. In early clinical trials the topoisomerase I inhibitor, topotecan, and the platinum analog, carboplatin, have successfully induced clinical remission in a small, but substantial number of patients with refractory or relapsed acute leukemia (1–8). Moreover, combined administration of cisplatin or carboplatin with topotecan in Phase I studies is associated with profound myelosuppression, suggesting a potential role in the treatment of acute leukemia, where bone marrow aplasia is generally required to achieve remission (9, 10).

DNA cross-linking is believed to be the primary cytotoxic mechanism of platinum-based chemotherapy, and differences in the ability of a cell to repair DNA damage may affect the magnitude of tumor cell kill (11, 12). In tumor cell lines, combinations of cisplatin analogs and topoisomerase I inhibitors are synergistic, in part, because of a decreased ability to repair platinum-DNA adducts (13–17). Concurrent administration of cisplatin and fludarabine has also been shown to enhance formation of platinum-DNA cross-links (18, 19). Therefore, addition of fludarabine and topotecan to carboplatin-based chemotherapy for acute leukemia may provide a way to circumvent...
both intrinsic and acquired platinum resistance through inhibition of platinum-DNA cross-link repair.

Based on these preliminary data, we designed a Phase I pharmacodynamic study of 5 days of concurrent fixed-dose fludarabine and continuous infusion carboplatin followed by a 72-hour infusion of escalating doses of topotecan for patients with relapsed or refractory acute leukemia, secondary acute myelogenous leukemia, or chronic myelogenous leukemia blast crisis. Pharmacodynamic and platinum-DNA adduct companion studies were correlated with bone marrow aplasia and patient outcome.

PATIENTS AND METHODS

Research Participants

Adult patients (≥18 years) with relapsed or refractory acute myelogenous leukemia or acute lymphoblastic leukemia were eligible for treatment. In addition, patients with acute leukemia secondary to pre-existing hematologic disorders or chemotherapy, high-grade myelodysplastic syndromes, or chronic myelogenous leukemia in blast crisis were eligible for treatment at diagnosis or after no more than 2 prior induction regimens. Patients who had failed autologous transplantation were eligible provided that they had recovered from acute toxicities of treatment. Adequate cardiac, pulmonary, hepatic, and renal function (defined as calculated creatinine clearance ≥50 ml/minutes, total serum bilirubin ≤2.0 mg/dl, and blood transaminase ≤3 times the upper limit of normal), Eastern Cooperative Oncology Group performance status ≤3, and a life expectancy of at least 1 month without definitive treatment were required. Prior chemotherapy including camptothecin analogs was allowed, and a minimum of 2 weeks must have elapsed since the last cytotoxic therapy (excluding hydroxyurea and corticosteroids). Exclusion criteria included previous allogeneic transplant, uncontrolled infections, or central nervous system toxicity. The clinical protocol was approved by the Institutional Review Board at University Hospitals (Case Western Reserve University), and all of the patients gave written informed consent.

Treatment Plan and Dose Escalation Schema

Treatement schema, schedule of correlative laboratory studies, and dose escalation schedule are described in Fig. 1 and Table 1. Drug dosages were calculated according to body surface area calculation with the lesser of actual or corrected body weight [ideal body weight + 25% (actual-ideal body weight)]. For dose levels 1 to 3, carboplatin was administered by continuous infusion over 120 hours for a total dose calculated to achieve an area under the curve of 10 according to the Calvert formula (20), and fludarabine at a dose of 10 mg/m² was administered over 30 minutes daily. For dose levels 4 and above, carboplatin was given to achieve an area under the curve of 12, and the daily fludarabine dose was raised to 15 mg/m²/day. Topotecan at the assigned dose (see schema in Fig. 1) was administered by continuous infusion for 72 hours beginning immediately after the carboplatin infusion was completed. An early treatment assessment bone marrow aspirate and biopsy were obtained 7 days after the completion of topotecan (day 15 to 18 after the beginning of treatment). Patients with residual leukemia at this evaluation were eligible for a second induction cycle to begin no sooner than day 16 and no later than day 35 after the beginning of induction cycle 1. Treatment with hematopoietic growth factors was initiated in patients who achieved bone marrow aplasia, defined as marrow cellularity ≥5% and/or ≥5% blasts 15 to 18 days from the initiation of treatment. Patients who achieved a complete or partial response could receive one cycle of consolidation therapy with fludarabine, carboplatin, and topotecan within 4 to 8 weeks of hospital discharge or recovery of peripheral blood counts to neutrophils ≥1200/µl and platelets ≥75,000/µl. Dose modifications for consolidation therapy were made according to toxicities encountered during the initial induction therapy. No dose escalations were allowed within individual patients.

Toxicities were graded by the NCI common toxicity criteria (version 1.0). Dose-limiting toxicity was defined as a grade 3 or worse nonhematologic toxicity. Grade 3 or greater stomatitis, diarrhea, or infectious complications (unless greater than 7 days duration) were not considered dose limiting because these are commonly observed during leukemia induction therapy. Dose-limiting hepatoxicity was defined as ≥grade 4 hyperbilirubinemia or ≥grade 3 transaminase elevation, which did not resolve to <3× the upper limit of normal by day 35 of treatment. Dose-limiting hematologic toxicity was defined as an absolute neutrophil count ≤200/µl or an unsupported platelet count ≤20,000/µl with a ≤5% cellular bone marrow without evidence of residual leukemia lasting 35 days or greater from the beginning of the most recent cycle of chemotherapy. Deaths occurring within 6 weeks of the beginning of protocol treatment were classified into 3 categories: (1) disease related, for example, because of leukostasis; (2) cytopenia-related, such as infection or bleeding (providing cytopenia was <5 weeks duration);
and (3) treatment-related, for example, because of organ toxicities such as mucositis, hepatotoxicity, and cytopenia ≥35 days or unexpected toxicities not related to bleeding and infection. The dose escalation schema was modified for category 3 deaths only. If no patient at an individual dose level developed a dose-limiting toxicity, dose escalation proceeded. If 1 of 3 patients developed a dose-limiting toxicity as defined above, 3 additional patients were enrolled at the same dose level. If <2 of 6 patients developed dose-limiting toxicity, dose escalation continued. If 2 or greater dose-limiting toxicity developed, the dose of topotecan was reduced either a full step for moderate or severe toxicities or half step for mild toxicities observed at the next lowest dose level. Moderate toxicity was defined as grade 2 or worse nonhematologic toxicity, grade 3 or worse stomatitis, diarrhea, or hepatotoxicity. Infectious complications were excluded unless caused by prolonged aplasia unrelated to persistent leukemia. Each individual in a cohort was followed for a minimum of 6 weeks before the next cohort was entered. Maximum tolerated dose was defined as the dose level at which no more than 1 of 6 patients experienced dose-limiting toxicity.

Clinical Evaluation
Complete response was defined as neutrophil count ≥12,000/µl, platelet count ≥100,000/µl, without circulating leukemic blasts, and bone marrow cellularity of ≥20% with ≤5% blasts. A partial response included all of the criteria listed above except that the bone marrow contained 5 to 25% blasts. There was also a category of a complete response with the exception of persistent thrombocytopenia. Other responses of interest included achievement of an early aplastic bone marrow defined as bone marrow cellularity on day 16 to 18 of treatment to <5% and/or blast percentage to <5%. All clinical responses were reviewed by a clinical hematopathologist (H.J.M.).

Laboratory Studies
Topoisomerase I Activity Assays. Topoisomerase I activity levels were assayed from bone marrow aspirates before treatment, at the end of the carboplatin infusion, and ~48 hours after the start of the topotecan infusion. Five milliliters of bone marrow or 10 ml of peripheral blood were collected in an EDTA tube and diluted 1:1 with PBS. Marrow mononuclear cells were separated by a Ficoll-Hypaque discontinuous gradient and then washed in PBS at 4°C (21). Contaminating red blood cells were lysed with RBC lysis buffer (Sigma, St. Louis, MO). Cell pellets were flash frozen in liquid nitrogen and stored at −80°C. Topoisomerase I was quantitated from specimens containing a minimum of 1 × 10⁷ mononuclear cells by measuring enzymatic activity as ATP-independent relaxation of supercoiled qX174 DNA according to previously described methods (22). Gels were stained with ethidium bromide, photographed under short wave UV light, and densitometric profiles of the negatives were quantitated. One unit of topoisomerase activity was defined as the amount of enzyme needed to relax 0.6 nmoles/g of supercoiled DNA in 30 minutes at 37°C (22–24). Topoisomerase I activity was expressed relative to DNA and protein content of cell samples. All samples were run against a standard control colon cancer cell line. Because of the limited amount of DNA, most samples were analyzed once. Variability of the assay was within 5% in samples run in duplicate or triplicate.

Topotecan Total and Lactone Concentration
Samples of blood for topotecan total and lactone concentration were obtained before the start of the topotecan infusion and at 24, 48, and 68 hours after the start of the topotecan infusion (where possible). Whole blood (3 ml) was collected in a heparinized syringe, transferred to a 5-ml red top tube, centrifuged at 4°C for 5 minutes at 3,000 rpm, and transferred into a glass tube. Exactly 1 ml of plasma was immediately pipetted into a borosilicate glass tube containing 5 ml of methanol (from −20°C freezer), vortex mixed for 10 seconds, and spun in 4°C centrifuge at 3,000 rpm for 5 minutes. The resulting supernatant was stored at −70°C until analysis. Topotecan lactone and total topotecan were determined with high-pressure liquid chromatography (model 1050 pump, 1046A fluorescence detector, Hewlett Packard, Wilmington, DE) with minor modifications of sample quantification and reconstitution and volumes of previously published procedures (25). Quality control samples of high- and low-concentration plasma total topotecan and topotecan lactone were determined with each set of patient samples. The control limits (mean ± SD) for the high and low total topotecan concentration were 7.4 ± 0.7 and 1.1 ± 0.1 ng/ml, respectively. For topotecan lactone the control limits were 5.8 ± 0.8 ng/ml and 1.0 ± 0.2 ng/ml for the high and low concentrations, respectively.

Evaluation of Platinum-DNA Adducts
Bone marrow aspirates were taken before the beginning of treatment, at the completion of the carboplatin infusion on day 5, and at 48 hours after the start of the topotecan infusion. DNA was isolated from these aspirates with a Qiagen DNA Mini kit (Qiagen, Valencia, CA), and the concentration of DNA in each sample was quantified by UV absorption (A₂₆₀). DNA samples were diluted in 3.5% nitric acid and hydrolyzed overnight at 70°C. Platinum-DNA adduct levels were determined with a ThermoFinnigan Neptune plasma ionization multicollector mass spectrometer as described previously (26). This inductively coupled plasma mass spectrometry approach allows a substantial improvement in sensitivity to that observed with previous methods, such as atomic absorption spectrophotometry and ELISA, with detection limits in the single femtogram range being obtainable. Because of the labor-intensive nature of running these samples on the inductively coupled plasma mass spectrometry and, in some cases, limitations in the amount of DNA sample available, most samples were analyzed once. However, at the beginning and end of the analytical run, a number of samples were run in duplicate, with replicates within 5% in all of the cases. Final platinum-DNA adduct levels were calculated as nmoles/g DNA.

Statistical Analyses
The nonparametric, one-way ANOVA Kruskal-Wallis test was used to test for differences in topotecan plasma concentration medians across the topotecan dose groups. This test was also used to compare clinical and early bone marrow response groups with median absolute platinum adduct levels at 120 hours. Linear regression was used to test the correlation between the mean plasma topotecan concentration and the time in days to recover to an absolute neutrophil count >200/µl. Fisher’s exact
test was used to test the dependency on bone marrow response and clinical response of the proportion of patients with increases in adduct levels between 120 and 168 hours.

RESULTS

Patient Characteristics

Thirty-one patients were enrolled between September 1998 and November 2001 (Table 2). Fourteen men and seventeen women were treated of whom 27 were Caucasian and 4 were African American. Median age was 54 (range, 19 to 76) years. Twenty-eight patients had acute myelogenous leukemia as follows: 7 untreated, 11 in first relapse, and 10 in second relapse or refractory. All untreated patients had acute myelogenous leukemia secondary to myelodysplastic syndrome or previous cytotoxic chemotherapy for other malignancies. Two patients had chronic myelogenous leukemia in untreated blast crisis and 1 patient had refractory/relapsed acute lymphoblastic leukemia. Ten patients had antecedent myelodysplastic syndromes, and 6 patients had failed autologous stem cell transplant. Median duration of remission of the patients treated in first relapse was 5 (range, 2 to 10) months.

Clinical Response

Two patients at each of the dose levels 3 and higher achieved a clinical response. Four patients achieved a complete response, 2 achieved a complete response with the exception of persistent thrombocytopenia, and 2 patients achieved a partial response. No responses were seen at dose levels 1 and 2 of treatment. Five of 9 previously untreated patients achieved a clinical response compared with 2 of 11 patients treated in first relapse and 1 of 11 patients with later stage disease. Patient characteristics and description of treatment response are included in Table 3. Nine additional patients achieved an early aplastic bone marrow response without subsequent remission. Of these patients, 1 died of infection on day 35 of the second cycle of induction treatment without any evidence of hematopoietic recovery, 1 received alternate salvage treatment, 2 recovered only circulating blasts, 3 achieved transient recovery of neutrophils with persistent grade 4 thrombocytopenia, and 2 patients had transient recovery of absolute neutrophil count ≥1500/µL and platelets ≥100,000/µL with persistent circulating blasts.

Five patients (3 with clinical responses and 2 with persistent disease after fludarabine, carboplatin, topotecan regimen treatment) underwent allogeneic transplantation shortly after receiving protocol therapy. Two of these patients remain alive in complete remission at 46 months after receiving an allogeneic transplant from an HLA identical sibling in first partial response and at 36 months after an umbilical cord blood donor in the setting of persistent disease. One patient with chronic myelogenous leukemia blast crisis died of extensive chronic graft versus host disease without evidence of disease recurrence ~12 months after receiving an allogeneic transplant from a matched unrelated donor. Two additional patients died from recurrent leukemia. There were no early (first 100 days) transplant-related deaths or excessive end-organ toxicities observed in the patients who underwent allogeneic stem cell transplantation after receiving the fludarabine, carboplatin, topotecan regimen.

Toxicity Evaluation

Two of 31 patients died within the first 6 weeks of receiving induction chemotherapy. One patient treated at dose level 5 died of overwhelming pseudomonas pneumonia on day 35 after a second cycle of induction fludarabine, carboplatin, topotecan regimen without evidence of hematopoietic recovery. This was considered a dose-limiting hematologic and pulmonary toxicity and category 3 death (see Patients and Methods). A patient with relapsed/refractory acute lymphoblastic leukemia treated at dose level 5A also died. This death was coded as a category 2 death as it was caused by overwhelming infection on day 22.

Nonhematologic toxicities of grade 3 or greater are summarized in Table 4. Grade 3 or greater infections were observed in all of the patients. Two episodes of atrial fibrillation were observed at dose levels 2 and 4 and were not considered dose-limiting because they occurred after amphotericin administration in the setting of a prior cardiac history in 1 patient and multiple electrolyte abnormalities and infections in the second patient. Two episodes of grade 3 neurologic toxicity (l ethargy that was possibly therapy-related) lasting 3 days at dose level 3 and grade 3 headache at dose level 5 prompted treatment of an additional 3 patients on each of these dose levels. Another patient treated at dose level 4 had a malignant pleural effusion (grade 3) before starting protocol treatment. Because of an error in topotecan administration (1 of 3 doses given as a bolus rather than infusion), seven patients were treated at dose level 5. Grade 3 to 4 hypocalcemia was common. Hepatic and gastrointestinal toxicities, which were not considered dose-limiting according to protocol guidelines, included grade 3 elevation of alanine aminotransferase for 3 days, 3 episodes of grade 3 hyperbilirubinemia of 1 day’s duration, and 1 episode of grade 3 neutropenic enterocolitis.

Hematologic recovery after induction therapy in patients who achieved clinical response is described in Table 5A. The time to recover neutrophils ≥2000/µL ranged from 20 to 43 days (median 28), and time to platelet transfusion independence ranged from 24 to 120 days (median 40) from the first day of the most recent cycle of induction chemotherapy. One of 6 patients

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**Table 2** Patient characteristics (N = 31)

| Male:Female | 14:17 |
| Median age (range) | 54 (19–76) years |
| Race | |
| Caucasian | 27 |
| African American | 4 |
| Diagnosis | |
| AML | 28 |
| Untreated | 7 |
| First relapse | 11 |
| Second relapse | 2 |
| Refractory | 8 |
| ALL | 1 |
| CML blast crisis | 2 |
| Prior myelodysplasia | 10 |
| Prior autotransplant | 6 |
| Duration of first CR | 5 (2–10) months |

Abbreviations: AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; CR, complete response.
at dose level 3, none of the first 3 patients who received dose level 4, and 2 of 6 patients treated at dose level 5 experienced dose-limiting hematologic toxicity. Dose level 5A, which included a half-step reduction of topotecan between dose 4 and 5 was added, and 2 of 6 patients developed dose-limiting hematologic toxicity. Three additional patients were then accrued to dose level 4, and a total of 1 of 6 patients treated at this dose experienced prolonged platelet recovery. Therefore, the maximum tolerated dose of the regimen was defined as dose level 4.

Four patients received fludarabine, carboplatin, topotecan regimen as consolidation therapy (Table 5B). One of these patients died with an aplastic bone marrow 81 days after receiving fludarabine, carboplatin, topotecan regimen chemotherapy without evidence of leukemia.

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### Laboratory Studies

#### Pharmacokinetics

Topotecan and lactone plasma concentration were available for 21 patients. The mean ratio of total topotecan to plasma lactone was 2.33 (SD, 0.51). The average of topotecan and lactone plasma determinations at 24, 48, and 72 hours was used for pharmacokinetic correlations. There was no significant relationship between dose of topotecan administered and plasma concentration of topotecan ($P = 0.19$). There was, however, a significant correlation between topotecan plasma concentration and recovery of neutrophils ($n = 9; P = 0.016$; Fig. 2).

#### Pharmacodynamic Studies

**Topoisomerase I Activity.** Topoisomerase I activity from mononuclear cell fractions of bone marrow aspirates was analyzed as shown in the schema (Fig. 1). For these studies the blast percentage of the mononuclear cell fractions was not determined. A change in topoisomerase I activity was defined as

<table>
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<th>Toxicities</th>
<th>Cardiac</th>
<th>Gastrointestinal</th>
<th>Hepatic</th>
<th>Hypocalcemia</th>
<th>Infection</th>
<th>Neurologic</th>
<th>Pulmonary</th>
<th>Patients/cycles</th>
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<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
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<td></td>
<td>3 (3)</td>
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</tr>
<tr>
<td></td>
<td>*NCI common toxicity criteria (version 1.0).†</td>
<td>Dose level 5A was added after dose-limiting hematologic and neurologic toxicity in an individual patient and 1 patient death because of prolonged aplasia was observed at dose level 5 and includes a half-step reduction of topotecan.‡</td>
<td>Number of patients (grade of toxicity).§</td>
<td>Dose-limiting toxicity.</td>
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Table 5  Hematologic recovery in patients achieving clinical response

<table>
<thead>
<tr>
<th>A. Days to hematopoietic recovery* (induction)</th>
<th>Dose level</th>
<th>No. of cycles</th>
<th>ANC ≥ 200</th>
<th>Platelets ≥ 20,000</th>
<th>ANC ≥ 1,200</th>
<th>Platelets ≥ 75,000</th>
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<td>3</td>
<td>2</td>
<td>40†</td>
<td>120†</td>
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</tr>
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<td>1</td>
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<td>40†</td>
<td>70†</td>
<td>48</td>
<td>NR (50,000/μl on day 83)</td>
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<table>
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<th>B. Days to hematopoietic recovery (consolidation)</th>
<th>Patients ID</th>
<th>Cycle no.</th>
<th>Dose</th>
<th>ANC ≥ 200</th>
<th>Platelets ≥ 20,000</th>
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<td>2</td>
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<td>2</td>
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<td>5A‡</td>
<td>81+</td>
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Abbreviations: ANC, absolute neutrophil count; NR, not reached.
* Recovery in days from most recent course of induction.
† Dose-limiting hematologic toxicity defined as ANC ≤ 200/μl or an unsupported platelet count ≤ 20,000/μl lasting 35 or more days from the beginning of the most recent cycle of chemotherapy.
‡ Given at 25% dose reduction, patient died without evidence of leukemia or marrow recovery.

Platinum-DNA Adducts

Mononuclear cells isolated from bone marrow aspirates taken before, on completion of, and 48 hours after the end of the carboplatin infusion were analyzed for determination of in vivo platinum-DNA adduct levels in 19 patients (Table 6). One 48-hour sample was not available for analysis. A large interpatient variation in platinum-DNA adduct levels was observed, with ~130- and 225-fold variations in adduct levels determined at the end of carboplatin infusion on day 5 and 48 hours after completion of carboplatin infusion, respectively (range, 0.22 to 28.61 nmol/g DNA at end of carboplatin infusion; 0.53 – 120.1 nmol/L/g DNA at 48 hours post infusion; Fig. 3). A mean pretreatment platinum-DNA adduct level of 0.10 ± 0.15 moles/g DNA was determined from mononuclear cells isolated from bone marrow samples obtained before carboplatin administration in these patients. Platinum-DNA adduct levels, either determined at the end of carboplatin infusion or 48 hours after completion of infusion, did not show a clear relationship with the actual dose of carboplatin administered. No significant correlations were observed between the extent of platinum-DNA adduct formation and either bone marrow response or clinical response. Furthermore, time to hematopoetic recovery (neutrophils ≥200/μl or platelets ≥20,000/μl) was not influenced by the platinum-DNA adduct level attained at the end of the carboplatin infusion or the 48 hours post-infusion sample. An increase or decrease in adduct level at 48 hours post infusion was defined as a change of >15% from the value determined at the end of carboplatin infusion. In those patients in whom adduct concentration increased between the end of carboplatin infusion and the 48 hours post-infusion sample, 7 of 8 patients achieved an aplastic day 16 bone marrow response compared with only 4 of 10 patients in whom the adduct levels decreased (P = 0.065, Fisher’s exact test). Furthermore, 4 of 7 patients whose platinum-adduct levels increased showed a clinical response compared with 1 of 9 patients in whom platinum-DNA adducts decreased between those two time points (P = 0.106).

![Fig. 2 Correlation between topotecan plasma concentration and neutrophil recovery (n = 9). P = 0.016 for the plotted linear regression. (ANC, absolute neutrophil count)](https://clincancerres.aacrjournals.org/article/content/18/5/1355/DC1/Figure2.large.jpg)
Fludarabine, Carboplatin, and Topotecan in Leukemia

Table 6  Platinum-DNA adduct levels, bone marrow response, and clinical response in 19 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>End carboplatin 48 h after infusion</th>
<th>Bone marrow response</th>
<th>Clinical response</th>
<th>Topotecan dose level</th>
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<td>5</td>
</tr>
<tr>
<td>27</td>
<td>7.36</td>
<td>73.0</td>
<td>N</td>
<td>5A</td>
</tr>
<tr>
<td>28</td>
<td>2.34</td>
<td>0.84</td>
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<td>4</td>
</tr>
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<td>29</td>
<td>1.74</td>
<td>1.43</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td>31</td>
<td>3.16</td>
<td>3.61</td>
<td>Y</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not determined; N, no response; Y, response.

In addition, we did not observe any correlation between the dose of topotecan administered and the change in platinum-DNA adduct levels determined between the end of the carboplatin infusion and 48 hours after infusion.

DISCUSSION

The combination of a topoisomerase I inhibitor and DNA damaging agent such as carboplatin has been shown to possess synergistic antitumor activity in multiple in vitro and in vivo studies (13–17). We designed a Phase I and translational study of fludarabine, carboplatin, and escalating doses of topotecan (fludarabine, carboplatin, topotecan regimen) given in a sequential fashion for patients with acute leukemia. Pharmacodynamic studies of topoisomerase I activity and platinum-DNA adduct formation were included to explore potential mechanisms of synergy between carboplatin and topotecan.

In comparison to other salvage chemotherapy regimens for acute leukemia, fludarabine, carboplatin, topotecan regimen was well tolerated with acceptable nonhematologic toxicity. Hematologic toxicity was dose-limiting and, in fact, defined the maximum tolerated dose and recommended dose for Phase II studies as fludarabine 15 mg/m² for 5 consecutive days, carboplatin area under the curve of 12 over 120 hours, followed by topotecan 0.85 mg/m²/day by continuous infusion over 72 hours. The median time to achieve an absolute neutrophil count of ≥200/µL was 28 (range, 20 to 43) days, and the median time to platelet transfusion independence was 40 (range, 29 to 120) days. The most commonly observed nonhematologic toxicities included infections, hypocalcemia, and hypomagnesemia. The observed treatment-related mortality of 6% (2 to 31) during induction chemotherapy in this heavily treated group of patients compares favorably to other leukemia induction or salvage regimens (27). One patient who received fludarabine, carboplatin, topotecan regimen with a 25% dose reduction as consolidation therapy died of bone marrow aplasia 81 days after receiving treatment, raising a concern that hematologic toxicity of the regimen may be cumulative. The maximum tolerated dose of topotecan and carboplatin in the fludarabine, carboplatin, topotecan regimen for acute leukemia appears to be approximately twice that observed for solid tumors (10).

At the dose range studied, we found significant interpatient variation of steady-state lactone and total topotecan plasma concentrations within dose levels and did not observe a significant relationship between dose and plasma concentration of topotecan. The consistent ratio of steady-state total topotecan to plasma lactone concentration suggests that sample processing and analysis were accurate. However, the number of patients available for analysis may not have been sufficient to determine any significant correlation between steady-state plasma topotecan concentration and early treatment bone marrow or clinical response. On the other hand, it is of interest that all of the clinical responses were observed at dose level 3 or greater, suggesting that topotecan added significantly to the antileukemic activity of the regimen. In our study, increased plasma concentrations of topotecan correlated significantly with days to neutrophil recovery. Other investigators have observed up to a 10-fold interpatient variability between dose administered and topotecan pharmacokinetics (28–30). Moreover, topotecan plasma concentrations rather than dose administered have been shown to correlate with the development of hematologic and gastrointestinal toxicities (28, 31–34), suggesting that future studies may warrant consideration of dosing of topotecan based on pharmacokinetics (35).

Enhanced cytotoxicity between carboplatin and topotecan in the fludarabine, carboplatin, topotecan regimen may be related to the potential for DNA damaging agents such as carboplatin to increase topoisomerase I activity. However, in the current study we did not observe a correlation between up-regulation of topoisomerase I activity and tumor response among the 13 paired patient samples analyzed. Kanzawa et al. (36) found that the topoisomerase I inhibitory effect of irinote-

![Fig. 3 Change in platinum-DNA adduct levels obtained from bone marrow mononuclear cells on day 5 of carboplatin infusion and 48 hours after the carboplatin infusion was completed. Platinum-DNA adduct levels increased in 8 of the 18 patients.](image-url)
can in small cell lung cancer cell lines was enhanced 10-fold in the presence of the cisplatin analog nedaplatin, potentially explaining the marked synergy of the two agents. In contrast, Ma et al. (37) were unable to correlate synergistic cytotoxicity between cisplatin and topoisomerase I inhibitors in a panel of solid-tumor cell lines with up to a 4-fold difference in topoisomerase I activity. These data suggest that the amount of the target topoisomerase I/DNA complex is not a major contributor to the enhanced cytotoxic activity of these combinations.

As a secondary objective of this Phase I study, bone marrow aspirates were obtained during and after treatment with carboplatin to investigate the potential correlation between platinum-DNA adduct formation as well as previously reported DNA-based synergistic interactions between topotecan and platinum agents (15, 16) and clinical response. Platinum-DNA adduct levels determined in our study were comparable with data from 6 patients receiving high-dose carboplatin chemotherapy, with a similar inductively coupled plasma mass spectrometry method (38). A large, interpatient variation in DNA adduct levels was observed, although carboplatin was dosed to achieve standardized carboplatin exposures (area under the curve of 10 or 12 depending on the dose level). This suggests that platinum-DNA adduct formation is not closely related to drug pharmacokinetics, a finding also previously reported for cisplatin (39). In contrast to the study by Veal et al. (39), in which single-agent cisplatin was administered, we did not observe any correlation between the degree of myelosuppression and extent of platinum-DNA adduct formation. No statistically significant correlations were observed between the concentration of adduct formation and either an aplastic early bone marrow response or clinical response. However, in this relatively limited study group, there appeared to be an association between achievement of early bone marrow aplasia and clinical responses in the patients whose adduct levels increased between the end of carboplatin infusion and 48 hours after the end of infusion. This may suggest an important role for DNA adduct-repair processes in this group of patients. Because our studies were obtained on mononuclear cell fractions of the bone marrow aspirates, changes in platinum-DNA adduct formation observed could be influenced, in part, by variations in blast cell content of the bone marrow during treatment. Furthermore, we did not observe correlations between platinum-DNA adduct formation and either topotecan dose or topotecan plasma concentration in the patients studied. These data are in agreement with a recently published Phase I study showing that sequence of topotecan given in combination with cisplatin did not influence peak platinum-DNA adduct formation in peripheral blood leukocytes of patients (9).

In contrast to our study, Welters et al. (40) found significant correlations between platinum-DNA adducts and tumor response in xenografted tumor tissues and in cultured tumor cells of head and neck squamous cell cancer. Other investigators have explored the relationship between platinum-DNA adduct formation in peripheral blood leukocytes and clinical response in patients undergoing chemotherapy. Reed et al. (41) found a highly statistically significant correlation between platinum-DNA adduct levels in peripheral blood leukocytes during cycle 1 of chemotherapy and disease response in patients with a variety of solid tumors. No such correlation was found by other investigators in similar studies (38, 42). It should be noted that in many of these studies, including the present report, platinum agents are being administered as one of a combination of different anticancer agents.

The apparently anomalous relationship we observed between clinical response and level of platinum-DNA adduct formation has been reported by others. Johnson et al. (12) found 20- to 40-fold higher DNA platination levels in cisplatin-resistant compared with cisplatin-sensitive human ovarian cancer cell lines. Increased adduct levels in more resistant cell lines, compared with sensitive cell lines, have also been observed in other tumor cell types, including colon, bladder, testicular, and leukemia, after exposure to cisplatin (42–47). This may suggest that resistant tumor cell lines are able to tolerate higher levels of DNA damage. On the other hand, as in our study, deficiencies in the ability to repair platinum-DNA adducts have been associated with sensitivity to cisplatin in multiple tumor cell lines (12, 48–50).

We are encouraged by the clinical activity of fludarabine, carboplatin, topotecan regimen in this group of patients with high-risk leukemia. Eight of 25 patients treated at dose level 3 or higher achieved clinical response. This included 5 of 9 patients who had leukemia secondary to antecedent hematologic conditions or cytotoxic chemotherapy and had not previously received leukemia induction therapy, 2 of 11 patients in first relapse, and 1 patient in second relapse. The duration of response was limited in these high-risk patients, and prolonged thrombocytopenia was observed. In this small study, the response rate we observed, particularly in previously untreated patients, compares favorably to cytarabine-based regimens. Furthermore, there were no early transplant-related deaths among the 5 patients who underwent allogeneic stem cell transplantation shortly after receiving protocol treatment, which we believe was due in large part to the favorable toxicity profile of the fludarabine, carboplatin, topotecan regimen. Phase II studies are planned to additionally define the antileukemia activity and toxicity of this regimen.

REFERENCES


A Phase I and Pharmacodynamic Study of Fludarabine, Carboplatin, and Topotecan in Patients With Relapsed, Refractory, or High-Risk Acute Leukemia

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