Phase I Evaluation of Prolonged-infusion Gemcitabine with Fludarabine for Relapsed or Refractory Acute Myelogenous Leukemia


Duke University Medical Center, Division of Medical Oncology and Transplantation and the Duke Oncology Consortium, Durham, North Carolina 27710

ABSTRACT

Purpose: The purpose of this study was to determine the maximum tolerated duration of infusion of gemcitabine at 10 mg/m²/min in combination with fludarabine at 25 mg/m²/day for 5 days in the treatment of relapsed or refractory acute myelogenous leukemia.

Experimental Design: Eighteen patients with relapsed or refractory acute myelogenous leukemia were enrolled. The median age was 54.5 years (range, 21–80 years). Patients received a 30-min infusion of fludarabine at 25 mg/m²/day for 5 days. i.v. gemcitabine was given as a single infusion at 10 mg/m²/min with the duration adjusted following a modified continuous reassessment method.

Results: After 18 patients, the maximum recommended dose of gemcitabine in combination with fludarabine was selected as a 15-h infusion given at 10 mg/m²/min (9000 mg/m²). Severe stomatitis or esophagitis was the most common nonhematological dose-limiting toxicity. Myelosuppression was universal. Fever or neutropenia, and myelosuppression should be anticipated; however, this regimen may be beneficial in patients with relapsed or refractory leukemia.

Conclusions: Prolonged-infusion gemcitabine at a fixed dose rate of 10 mg/m²/min for 15 h with 25 mg/m²/day fludarabine for 5 days is a tolerable induction regimen for relapsed or refractory leukemia. Stomatitis, esophagitis, febrile neutropenia, and myelosuppression should be anticipated; however, this regimen may be beneficial in patients with relapsed or refractory leukemia.

INTRODUCTION

Despite advances in cancer therapy, the majority of adults with acute leukemia will relapse and die of their disease (1, 2). Alternative treatments should be explored to provide better outcomes for these patients.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a deoxycytidine analogue with multiple mechanisms of action (3, 4). It has shown activity in a wide range of solid tumors including breast (5), pancreatic (6), biliary tree (7), non-small cell lung (8), ovarian (9), and urothelial cancer (10) when given as a 30-min infusion. Various concentrations and dosing strategies have been evaluated in efforts to maximize activity. Infusion rates of 10 mg/m²/min have been shown to produce an extracellular gemcitabine concentration of 15–20 μM, which is the level at which maximal incorporation into DNA occurs, suggesting that this dose rate may optimize cytotoxicity (11, 12). Furthermore, in vitro studies in Chinese hamster ovary cells have postulated that both dose and duration of exposure contribute to the cytotoxicity of gemcitabine (13). Animal studies also support this theory. Studies in euthymic mice with colon carcinoma have shown that a 24-h continuous infusion of gemcitabine was more efficacious than frequent discrete injections (14). A 24-h prolonged infusion of gemcitabine was reported in patients with inoperable non-small cell lung cancer and was generally well tolerated (15).

Fludarabine and gemcitabine have both been shown to have activity in hematological malignancies (16–18) but have not been reported in combination for these diseases. In vitro evidence suggests that the combination of gemcitabine and fludarabine has synergistic effects in leukemic cell lines. A minimally active concentration of either agent combined with a dose range of the other produced a 3–4-fold increase in activity compared with either agent alone. One explanation of this synergism could be that the active metabolite of each compound inhibits ribonucleotide reductase, causing a decrease in the deoxyribonucleotide pool, possibly potentiating each other’s incorporation into DNA (3, 19). This synergism, coupled with data suggesting the unique cytotoxic properties of continuous gemcitabine infusion, formed the basis for our investigation. This Phase I study investigated the toxicity and feasibility of administering a prolonged infusion of fixed dose-rate gemcitabine in
combination with fludarabine in patients with relapsed or refractory AML.\(^4\)

**PATIENTS AND METHODS**

**Patient Eligibility.** Patients were eligible for this trial if they had relapsed or refractory AML. Other eligibility requirements included the following: liver function tests < three times the upper limit of normal, unless related to leukemia; estimated creatinine clearance > 40 ml/min; Karnofsky performance status of 50 or greater; life expectancy of at least 5 weeks; and human immunodeficiency virus negative. Patients with active leukemic central nervous system involvement were eligible if they met all other criteria. Patients could not have received any other chemotherapy for a minimum of 1 week before initiation of therapy. Before starting chemotherapy, patients must have adequately recovered from prior therapy, as judged by the treating physician. This typically resulted in patients not having been exposed to myelotoxic chemotherapy for at least 1 month before enrollment. The protocol was approved by the Duke University Institutional Review Board, and all patients were required to provide written informed consent before initiating therapy.

**Treatment.** Before initiation of therapy, all patients received hydration and were given allopurinol at the discretion of the treating physician. i.v. gemcitabine was given as a single continuous infusion at 10 mg/m\(^2\)/min for varying durations according to the mCRM described below. The first patient received a 15-h-duration gemcitabine infusion (9000 mg/m\(^2\)). Subsequent patients received longer or shorter durations as directed using the mCRM. Fludarabine (25 mg/m\(^2\) i.v. over 30 min) was administered daily for 5 days. Due to the potential synergy of the combination, the first dose was given at the midpoint of the gemcitabine infusion. This allowed the patient to receive single-agent gemcitabine for some period, combination therapy for some period, and single-agent fludarabine with subsequent doses of fludarabine. Patients were given G-CSF (filgrastim) s.c. at 5 \(\mu\)g/kg/day beginning on day 11–14 (exact start date was at the discretion of the treating physician). G-CSF was continued until the ANC was greater than 1.5 \(\times\) 10\(^9\) cells/liter for at least 2 days or until disease progression. All patients received prophylactic antiemetics consisting of ondansetron. Other antiemetics were provided at the treating physician’s discretion.

**Patient Monitoring and Toxicity Assessment.** Within 3 weeks before treatment initiation, each patient underwent assessment including history and physical examination, evaluation of prior treatment toxicity, and performance status. All patients underwent the following tests at initiation: complete blood count with differential; liver function tests; creatinine; electrolytes; and total protein and albumin. Bone marrow examination with aspirate, biopsy, flow cytometry, and cytogenetics was also obtained.

During therapy, patients were examined daily. Complete blood counts were taken daily until ANC was more than 0.5 \(\times\) 10\(^9\) cells/liter and platelets were more than 10 \(\times\) 10\(^9\) cells/liter, then they were taken twice weekly until platelets were more than 20 \(\times\) 10\(^9\) cells/liter, and then they were taken as needed. Serum creatinine and liver function studies were obtained daily while on chemotherapy and at least 2 times/week until the patient recovered from therapy. Platelets were to be maintained at a minimum of 10 \(\times\) 10\(^9\) cells/liter, and the hematocrit was to be maintained above 25%. Bone marrow aspirate and biopsy were repeated at day 11–14 and again when the ANC was above 1 \(\times\) 10\(^9\) cells/liter and evaluated for response to therapy. Cytogenetic analysis was performed if the patient was otherwise determined to be in complete remission.

Toxicities were evaluated using the National Cancer Institute Common Toxicity Criteria, version II (20). DLT was defined as neutropenia (ANC < 1 \(\times\) 10\(^9\) cells/liter) lasting longer than 28 days with < 5% blasts in the bone marrow. Prolonged neutropenia due to persistent disease was not considered a DLT. Other toxicities were not considered dose-limiting unless there was nonhematological grade 4 toxicity lasting 3 days or longer or grade 3 toxicity lasting 7 days or longer. Febrile neutropenia and nausea were assessed but not considered in the evaluation of DLT.

Patient response was evaluated using the guidelines published by the National Cancer Institute (21). CR was defined by the absence of all leukemia for a minimum of 4 weeks based on the laboratory and bone marrow examinations performed at the time of myeloid (ANC > 1.5 \(\times\) 10\(^9\) cells/liter) and platelet (at least 100 \(\times\) 10\(^9\) cells/liter) recovery. These criteria require the marrow to have > 20% cellularity and all lines maturing, no Auer rods detected, < 5% blasts in the bone marrow, and no abnormal blasts in the peripheral blood for at least 1 month after myeloid recovery. A PR was defined as <25% blasts on bone marrow examination performed at the time of myeloid recovery but an insufficient decrease to be classified as a CR. All patients were followed until progression or death.

**Statistics and Gemcitabine Duration Modification.** The end point of the study was the estimate of the MTD of gemcitabine infusion at a fixed dose rate of 10 mg/m\(^2\)/min in combination with fludarabine daily for 5 days. This study determined patient dosing using a mCRM (22–24). The mCRM is a Bayesian statistical method that allows rapid dose modification. Further details of the proper use of this method are referenced above. The MTD was defined as the duration of gemcitabine that caused a DLT in approximately one-third of the patients. The initial duration-toxicity curve was derived by three medical oncologists’ estimated prediction of DLT risk at different treatment durations. The estimates were averaged and used as initial assumptions of the relationship between gemcitabine infusion duration and the risk of DLT in the logistic function log \(\left[p/(1 - p)\right]\), where \(p\) is DLT risk. The logistic function with constant term equal to 3 approximated the underlying duration toxicity curve. These estimates provided the pretrial predicted duration-toxicity curve that was then recalculated based on the results of each patient.

The mCRM allows treatment to commence at the dose with

---

\(^4\) The abbreviations used are: AML, acute myelogenous leukemia; mCRM, modified continuous reassessment method; G-CSF, granulocyte colony-stimulating factor; ANC, absolute neutrophil count; DLT, dose-limiting toxicity; CR, complete response; PR, partial response; MTD, maximum tolerated dose.
a prior probability of DLT closest to the target DLT, and this may not necessarily be the lowest dose. Thus, the first patient received a 15-h gemcitabine infusion because its prior probability of DLT (0.28) was closest to our target DLT (0.33). After observing the toxicity outcome (DLT/no DLT) for a patient, a new estimate of the duration-toxicity curve was obtained and used to assign the next patient to the dose level whose associated mean posterior probability of toxicity was closest to 0.33. Dose escalation/de-escalation was restricted to one level (3-h difference in duration) at a time, and skipping a level was not permitted. One patient per cohort was treated before switching to the look ahead option was permitted, meaning that at the time the last patient in the current cohort was accrued, the program precalculated the recommended dose level for the next cohort. This option was only used if the outcomes for patients who had been accrued, but whose toxicity outcomes were not yet observed, would not change the next recommended dose by the mCRM.

RESULTS
Patient Characteristics. Eighteen patients with AML were treated in this trial (Table 1). This group consisted of 10 men and 8 women. There were 3 African Americans, 1 Hispanic, and 14 Caucasians. Five patients had AML arising from myeloproliferative disorders or myelodysplasia. One patient had AML that likely resulted from prior therapy for Ewing’s sarcoma. The median age was 54.5 years (range, 21–80 years). The median number of prior regimens and cycles was 2.0 (range, 1–4 regimens) and 3.5 (range, 1–10 cycles), respectively. All had failed prior therapy with cytarabine, including six patients who received high-dose cytarabine (2–3 g/m² every 12 h for 6–10 doses) as part of prior consolidation and/or induction. One patient had progressed following a matched unrelated donor allogeneic stem cell transplant. Twelve of 18 patients (67%) had achieved a prior CR and received a median of 3.0 (range, 0–9) cycles of consolidation therapy. Three patients received consolidation with high-dose cytarabine. Of this group, the average duration of first CR was 5.8 months (range, 1–12 months), and 6 of these 12 patients had a first CR of 4 months or less. Among the patients with relapsed disease, 4 of 12 (25%) had received at least one course of salvage chemotherapy before fludarabine and gemcitabine. Of the six patients with primary refractory disease, two had failed one course, and four had failed two or more courses of induction chemotherapy, which included high-dose cytarabine in two patients. One patient failed two courses of the same chemotherapy regimen. Two patients failed two consecutive courses of different regimens, and one patient failed three consecutive courses of different regimens. One patient had a favorable karyotype (inv 16) but had relapsed disease, six patients had an intermediate karyotype (normal karyotype), and seven patients had a poor prognosis karyotype (any other clonal abnormality). Four patients did not have cytogenetics performed.

Toxicity. Severe stomatitis/esophagitis was the most common nonhematological adverse event and was a cause of DLT in two patients (Table 2). Five of 18 patients (28%) had grade 3–4 stomatitis/esophagitis. However, these symptoms re-
solved within a median of 7 days (range, 2–15 days), and the duration of symptoms met our criteria for DLT in three patients (one patient also had DLT from pulmonary complications). Four of 18 patients (22%) developed grade 2–3 nausea or vomiting, and 3 of 18 patients (17%) developed grade 2–3 diarrhea. One of 18 patients (5.5%) had bilirubin elevated up to 1.5 times normal, and 3 of 18 patients (17%) had liver transaminases elevated up to 10 times normal. Elevations in liver transaminases were transient and resolved in 5 days or less. There was one case of dose-limiting anorexia that lasted 14 days. Four patients developed grade 1–2 transient rashes; however, given the patients’ multiple drug exposures, we were unable to determine the precise cause of the rashes. Biopsies from two patients revealed no evidence of malignancy and negative bacterial and fungal stains. One patient developed grade 2 dyspnea, and one patient developed grade 2 pulmonary edema. A patient with a history of congenital heart disease acquired a grade 3 cardiac dysrhythmia that resolved with diltiazem. Another patient developed grade 2 confusion. Her mental status cleared upon discontinuation of several drugs, including prochlorperazine.

Two of 18 patients (11%) died during the course of treatment, and their deaths were considered DLTs. After the gemcitabine infusion and one dose of fludarabine, one patient treated with the 18-h gemcitabine infusion had laboratory studies consistent with tumor lysis syndrome. His electrolyte abnormalities did not resolve with i.v. fluids and bicarbonate, and his renal function began deteriorating. The patient refused hemodialysis and died of cardiorespiratory arrest 12 days after beginning treatment. Another patient treated with the 15-h gemcitabine infusion developed respiratory distress and cough 3 days after beginning treatment. Chest X-ray showed bibasilar air space disease consistent with hemorrhage or aspiration. Her respiratory status continued to decline, and she died 9 days after initiating therapy.

Myelosuppression was noted in all patients, with thrombocytopenia more pronounced than neutropenia. Responders required a median of 16 days (range, 12–22 days) and 27 days (range, 25–48 days) before ANC > 1 × 10^9 cells/liter and platelets > 20 × 10^9 cells/liter (untransfused), respectively. Febrile neutropenia was common, and 5 of 18 patients (28%) developed infectious complications (Clostridium difficile colitis, Acinetobacter and Enterococcus bacteremia, Pantoaea agglomerans and coagulase-negative Staphylococcus bacteremia, Streptococcus viridans bacteremia, and a genital herpes reactivation). There were no cases of sepsis syndrome.

Three dose levels were evaluated before concluding this Phase I trial (Table 3). Overall, five patients developed DLT. None of the patients treated with a 12-h infusion of gemcitabine developed DLT. 2 of 13 patients (15%) treated with a 15-h infusion developed DLT (pulmonary complications and stomatitis, stomatitis), and 3 of 3 patients (100%) treated with an 18-h infusion developed DLT (renal insufficiency, anorexia, and stomatitis). One patient received a second cycle of chemotherapy (15-h infusion) and did not experience DLT with either cycle. His second cycle was not used in determining the MTD from the mCRM. Based on the mCRM, we consider the 15-h gemcitabine infusion to be the MTD when given with a 5-day course of fludarabine. According to the mCRM, the mean posterior probability of DLT with 15 h of gemcitabine was 20% (90% prediction interval, 9–43%). The posterior probability that the 15-h infusion represented the MTD was 91%.

**Response.** Given the two early deaths, 16 patients were evaluable at their nadir for an antileukemic effect. At the day 11–14 bone marrow evaluation, 6 of 16 patients (37%) had a marrow cellularity of 20% or less, and 4 of 16 (25%) had a cellularity of 5% or less. Three patients achieved a CR at count recovery. This indicates a significant antileukemic effect, although it was limited in most patients.

Three of the 18 patients (17%) treated in this trial achieved a CR, and 2 of 18 patients (11%) achieved a PR, for an overall response rate of 5 of 18 patients (28%). One patient treated with the 15-h infusion experienced a PR and received a second cycle of therapy but did not respond to the second cycle. The other patient who experienced a PR with the 18-h infusion demonstrated a CR in the bone marrow but had persistent skin disease. Thirteen patients were treated with 15 h of gemcitabine, and 3 of 13 achieved a CR (23%; 95% confidence interval, 0–46%). Of the patients who achieved a CR, one had primary refractory disease after failing one cycle of high-dose cytarabine induction chemotherapy. She received one cycle of fludarabine and gemcitabine as initial salvage. After obtaining a CR with this regimen, she declined further treatment and relapsed 4 months after therapy. Another patient had a first CR of 9 months and received three cycles of high-dose cytarabine and two cycles of gemtuzumab ozogamicin as consolidation. She then relapsed 3 months after salvage therapy with mitoxantrone and etoposide but subsequently attained a CR with one cycle of fludarabine and gemcitabine. This patient then proceeded to consolidation with nonmyeloablative allologeneic transplantation, relapsing 6 months after treatment with fludarabine and gemcitabine. The third patient had a first CR from cytarabine, daunorubicin, etoposide, and valspodar induction followed by one consolidation cycle with the same agents minus valspodar. She relapsed 1 year after treatment and received one cycle of fludarabine and gemcitabine as initial salvage therapy. She declined further treatment and remained in CR for 8 months. Among the patients who did not achieve a CR, three had a hypocellular (<5%) marrow at the day 11–14 bone marrow evaluation. The median percentage of marrow blasts was 50% at this time, a decrease from the 60% median blast count at study entry.

---

**Table 3** DLT and clinical response by duration of gemcitabine infusion

<table>
<thead>
<tr>
<th>Infusion duration</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h (7,200 mg/m²)</td>
<td>N = 2</td>
</tr>
<tr>
<td>DLT</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CR</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PR</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>15 h (9,000 mg/m²)</td>
<td>N = 13</td>
</tr>
<tr>
<td>DLT</td>
<td>2 (15%)</td>
</tr>
<tr>
<td>CR</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>PR</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>18 h (10,800 mg/m²)</td>
<td>N = 3</td>
</tr>
<tr>
<td>DLT</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>CR</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PR</td>
<td>1 (33%)</td>
</tr>
</tbody>
</table>
**DISCUSSION**

This trial demonstrates that in patients with acute leukemia receiving five daily doses of fludarabine at 25 mg/m², a 15-h infusion of gemcitabine given at 10 mg/m²/min (9000 mg/m²) is the maximum recommended duration of infusion. Stomatitis/esophagitis was the most common dose-limiting effect, and myelosuppression should be anticipated. Nausea, vomiting, and diarrhea were occasionally reported.

This study also demonstrates the usefulness of the mCRM in Phase I trials. Only three infusion durations and 18 patients were needed to determine the MTD with a 91% posterior probability (Table 4). This Bayesian dose-finding method may have the potential to reduce the number of patients treated at inappropriately high or low doses (25). In this study, 13 of 18 patients (72%) were treated at the MTD, whereas only 3 of 18 patients (17%) and 2 of 18 patients (11%) were treated at doses above and below the MTD, respectively. However, the main advantage of this design is that it allows for a more accurate determination of the MTD by incorporating the evaluation of each individual patient’s toxicity into the destination of the posterior probability.

Although this is a Phase I study, the clinical responses are encouraging, given this heavily pretreated and refractory group, including a patient who had failed ablative transplantation. Three of 18 patients (17%) experienced a CR, and 2 of 18 patients (11%) experienced a PR using the National Cancer Institute response criteria (21), for a combined response rate of 3 of 18 patients (28%). Only three patients had a first remission of ≥9 months, and two of these three patients obtained a CR. The duration of CR is also promising in the two patients declining further therapy. However, these results should be interpreted cautiously because the patients who achieved a CR probably had more sensitive disease. Results of this Phase I study are comparable with early reports of the FLAG (fludarabine, cytarabine, and G-CSF) salvage regimen. In one series, the CR rate in patients with relapsed AML was 11% (17), although it has been as high as 68% in others (26, 27). The toxicity of this regimen is acceptable, with stomatitis being the major DLT.

**Table 4** Statistical results of the mCRM

<table>
<thead>
<tr>
<th>Hours of gemcitabine</th>
<th>Prior probability of toxicity</th>
<th>Posterior probability of toxicity</th>
<th>Posterior probability dose is MTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.1170</td>
<td>0.0003</td>
<td>0.0003</td>
</tr>
<tr>
<td>15</td>
<td>0.2830</td>
<td>0.1961</td>
<td>0.0963</td>
</tr>
<tr>
<td>18</td>
<td>0.4830</td>
<td>0.6936</td>
<td>0.0934</td>
</tr>
</tbody>
</table>

---

**REFERENCES**


Phase I Evaluation of Prolonged-infusion Gemcitabine with Fludarabine for Relapsed or Refractory Acute Myelogenous Leukemia


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/9/2/663

Cited articles  This article cites 24 articles, 14 of which you can access for free at: http://clincancerres.aacrjournals.org/content/9/2/663.full.html#ref-list-1

Citing articles  This article has been cited by 2 HighWire-hosted articles. Access the articles at: /content/9/2/663.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.