Intensive Sequential Chemotherapy With Mitoxantrone and Continuous Infusion Etoposide and Cytarabine for Previously Treated Acute Myelogenous Leukemia

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Intensive sequential chemotherapy with mitoxantrone, 12 mg/m²/d on days 1 through 3, etoposide, 200 mg/m²/d as a continuous infusion on days 8 through 10, and cytarabine, 500 mg/m²/d as a continuous infusion on days 1 through 3 and 8 through 10 was administered to 72 patients aged less than 60 years with previously treated acute myelogenous leukemia (AML). Forty patients had refractory AML (nonresponse to prior therapy, early first relapse, or multiple relapse) and 32 had late first relapse. Sixty-one percent of patients, with a 95% confidence interval (CI) ranging from 49% to 72%, achieved complete remission (CR), including 45% (CI: 30% to 62%) of refractory patients and 61% (CI: 54% to 93%) of late first relapse patients. Twenty-nine percent of patients (CI: 19% to 41%) did not respond to therapy and 10% (CI: 4% to 19%) died from therapy-related toxicity. Median duration of aplasia was 30 days. Nonhematologic WHO grade 3 or more toxicity included sepsis (57% of patients), vomiting (10%), mucositis (35%), diarrhea (7%), skin rash (6%), and hyperbilirubinemia (11%). Postinduction therapy was attempted in 36 of 44 CR patients: 16 of them received a second course of the same regimen, 7 received maintenance chemotherapy, 4 underwent autologous bone marrow transplantation (BMT), and 9 allogeneic BMT. At a median follow-up of 20 months, 23 of the 44 complete remitters have relapsed, 1 to 14 months after achievement of CR, including 19 of 31 patients not undergoing BMT. Median survival is 7 months with 16% (CI: 4% to 28%) projected survival at 47 months. Median disease-free survival is 6 months with 21% (CI: 3% to 39%) of CR patients projected to remain disease-free at 46 months. Twenty-six percent (CI: 13% to 43%) of the evaluable patients who did not receive transplantation had inversion of CR duration. Among patients younger than 50 years, there was no significant difference in disease-free survival between patients receiving postinduction chemotherapy and those receiving BMT. We conclude that this chemotherapeutic regimen is highly efficient and could be used as first-line therapy in young patients with AML.

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P RIMARY NONRESPONSE to induction chemotherapy occurs in 10% to 25% of adult patients with newly diagnosed acute myelogenous leukemia (AML), while relapse occurs in 50% to 90% of patients achieving complete remission (CR) when they are treated by chemotherapy only. Only 25% to 50% of these patients with previously treated AML can still achieve CR, generally of less than 6 months duration, with a new chemotherapeutic attempt. Chemotherapy alone allows long-term survival in less than 5% of these patients and bone marrow transplantation (BMT) is regarded as the only potentially curative alternative. Most of the chemotherapy regimens currently used for previously treated AML include a single 5- to 10-day continuous sequence of chemotherapy, similar to that usually used in first-line therapy. These regimens generally include one drug not previously administered to the patient, such as new anthracyclines or analogues, etoposide or asparaginase, in association with conventional or high-dose cytarabine.

The interest of timed-sequential therapy for recruiting AML cells into cell cycle by a first sequence of chemotherapy and eventually achieving maximal cell kill using S-phase–dependent drugs in the second sequence has been emphasized 10 years ago, using daunorubicin and cytarabine in newly diagnosed AML patients. Repetition of the same timed-sequential chemotherapy regimen during CR significantly increased CR duration. We used this regimen in 36 patients with previously treated AML who had already received cytarabine and daunorubicin in a previous conventional regimen: 56% of patients achieved CR, with a median duration of CR of 7 months in the absence of postremission therapy, showing little cross-reactivity with the previously received regimen.

The regimen described in this report (EMA regimen) was a modification of the above-mentioned regimen with replacement of daunorubicin in the initial sequence by mitoxantrone, an anthracene-derived intercalating agent with a partially non–cross-reactive spectrum of activity in vitro when compared with daunorubicin. In phase I and II studies, mitoxantrone had promising activity in AML previously treated with anthracyclines with relatively low cardiac toxicity. The second sequence of EMA regimen included etoposide (VP-16), a cycle-active podophyllotoxin derivative that has been shown to induce 9% to 50% CR in early-phase studies in AML. The mechanism of action of etoposide is different from that of cytarabine and the two drugs are synergistic in vitro. Etoposide was administered in continuous rather than intermittent infusion simultaneously with cytarabine based on studies reporting increased efficacy when the duration of drug administration is extended.

During the first 2 years of activation of this protocol, 34 patients were treated with an overall 53% CR rate. However, four of six patients aged more than 60 years in this series died from toxicity of therapy. Subsequently, only...
patients aged less than 60 years were considered for therapy. The six initial patients aged over 60 are not analyzed in this report.

**MATERIALS AND METHODS**

**Patient Eligibility**

Patients were eligible for the study if they were younger than 60 years, with a diagnosis of primary AML or AML following transformation of a previously known myelodysplastic syndrome (MDS) or following chemotherapy for a previous malignancy, nonresponsive to chemotherapy, or in first or subsequent relapse. AML was diagnosed according to the revised French-American-British (FAB) Group criteria. Nonresponse to chemotherapy was defined as the absence of CR after at least two courses of intensive anthracycline and cytarabine containing chemotherapy in case of primary AML at diagnosis and one course of intensive chemotherapy in a case of secondary AML or primary AML in relapse. Patients with AML following a previous myelodysplastic syndrome were also regarded as nonresponders if they failed to achieve CR after two courses of low-dose cytarabine. Refractoriness was defined, according to Hiddemann et al., as (1) nonresponse; (2) early first relapse, occurring after a first CR of less than 6 months duration or while the patient is still on therapy; and (3) second and subsequent relapses. Late first relapse was defined as first relapse occurring while off-therapy after a first CR of 6 months duration or more. Only patients with a performance status of 2 or less and no grade greater than 2 organ failure according to the World Health Organization (WHO) grading system could enter the study. All patients had to give informed consent according to institutional policy.

**EMA Chemotherapy Regimen**

The induction phase included two 3-day sequences of chemotherapy separated by a 4-day chemotherapy-free interval. The first sequence associated mitoxantrone, 12 mg/m²/d as a 30-minute intravenous infusion from day 1 to day 3 and cytarabine, 500 mg/m²/d as a continuous infusion on the same days. The second sequence included etoposide, 200 mg/m²/d as a continuous infusion from day 8 to day 10 and cytarabine, 500 mg/m²/d as a continuous infusion on the same days. During induction, 53 patients were monitored in conventional reverse isolation rooms and 19 in sterile laminar air-flow rooms. All patients received gastrointestinal decontamination and prophylactic red blood cells and platelet transfusions. Broad spectrum empirical antibiotherapy was initiated as soon as the patient became febrile.

Postinduction chemotherapy was scheduled to include a second course of the same chemotherapy regimen administered 8 to 12 weeks after initiation of induction chemotherapy. Patients aged less than 50 who had a suitable donor received allogeneic BMT. Some patients aged less than 50 received autologous BMT based on institutional policy to autograft all eligible AML patients in relapse or nonresponsive to previous chemotherapy and 58 patients had relapsed AML, including 55 patients in first relapse with a median duration of first CR of 9 months (2 to 41 months) and three patients in subsequent relapse (Table 2).

**Evaluation of Therapy**

CR and relapse were defined according to the Cancer and Leukemia Group B (CALGB) criteria. Treatment failures were classified, according to Preisler, as nonresponse (NR), including all patients with proven blastic regrowth after chemotherapy even if they died before blood count recovery, and other failures (OF) corresponding to patients who died while nonblastic from presumably chemotherapy-related toxicity. Severity of treatment-related toxicity was graded according to the WHO criteria.

**Statistical Analysis**

The following parameters were analyzed for potential prognostic significance for CR achievement, disease-free survival (DFS) and overall survival: age, sex, previous therapy, indication for EMA regimen (refractoriness, late first relapse, and primary AML or secondary AML and AML following a previous MDS), WHO performance status, initial hepatosplenomegaly or other extramedullary involvement, fever and hemorrhages, initial blood counts, percent of bone marrow blasts, FAB morphologic subtype, bilirubinemia, serum liver enzymes, and lactate dehydrogenase (LDH) levels.

CR rates were compared using Yate's corrected chi-square, and 95% confidence intervals (CIs) on proportions of CR, NR, and OF patients were calculated using the exact binomial formula. Survival and DFS probabilities were calculated using the Kaplan and Meier product-limit estimate method and their 95% symmetrical CI limit was calculated according to Greenwood's method. Survival curves were compared using the logrank test. For analysis of survival and DFS, patients undergoing autologous or allogeneic BMT while in CR were conventionally censored at the time of transplantation, unless otherwise indicated. Prognostic factors for CR were studied using multiple logistic regression and prognostic factors for DFS and overall survival were studied using Cox's proportional hazard model. All computations were made using BMDP software (BMDP Statistical Software, Los Angeles, CA).

**RESULTS**

**Patient Population**

Between April 1986 and April 1990, 72 patients from the eight participating centers entered the study. Accrual by center is indicated in Table 1. All eligible patients seen at the various participating centers since the date of initial participation of each center in the study were included. All entered patients were eligible and subsequently analyzed. Sixty-six patients had primary AML, three patients had AML secondary to chemotherapy for a previous malignancy, and three patients had AML following transformation of a previously known MDS. Overall, 14 patients were nonresponsive to previous chemotherapy and 58 patients had relapsed AML, including 55 patients in first relapse with a median duration of first CR of 9 months (2 to 41 months) and three patients in subsequent relapse (Table 2).
After a previous MDS, had previously received two courses with amacrine and high-dose cytarabine as described above. Patients had previously received at least two courses of low-dose cytarabine. Seven nonresponsive patients treated in first relapse had previously received one intensive consolidation course described above. Following achievement of first CR, 37 patients had received one intensive consolidation course with amasacrine and high-dose cytarabine as described above followed by a second consolidation course with daunorubicin and conventional-dose cytarabine similar to the induction course or, on a randomized basis, one timed sequential course of daunorubicin and intermediate-dose cytarabine. Among the 44 complete remitters, 16 received a second course of intensive chemotherapy using the same regimen as for consolidation administered at a median of 9 weeks after initiation of induction (range 8–18 weeks) and 4 weeks after achievement of CR. Eight patients received no postinduction therapy because of early relapse (four patients), prolonged aplastic period after the first induction course (one patient), patient refusal of any further chemotherapy (one patient), or insufficient follow-up (two patients). Seven patients received maintenance chemotherapy using low-dose cytarabine (five patients) or monthly courses of chemotherapy using the same drugs as during induction at reduced dosages (two patients) because of severe toxicity following induction course (four patients) or practical reasons (three patients). Thirteen patients younger than 50 years received BMT within a median of 2 months in CR after conditioning with busulfan and cytoxan or total body irradiation and cytoxan. Of these patients received autologous BMT according to institutional policy. The remaining nine patients received allogeneic BMT from a family-related donor including six fully matched transplants and three transplants with a one-HLA antigen mismatch.

### Efficacy of Therapy

Results of induction regimen according to the stage of AML at the beginning of therapy are reported in Table 3. Overall, 44 patients or 61% (95% CI ranging from 49% to 72%) achieved CR. One of them reached CR after two courses of chemotherapy while he only had a partial remission after the first induction course. CR rate of nonresponse patients, multiply relapsed patients, and patients in first relapse after a previous CR of less than 6 months duration or while still on therapy were 36%, 33%, and 52%, respectively. Overall, 18 of the 40 patients considered as refractory (45%, CI ranging from 30% to 62%) achieved CR, compared with 26 of the 32 patients in late first relapse (81%, CI ranging from 64% to 93%, \( P = .004 \)). This difference in CR rate was explained by a.
higher percentage of NR in refractory patients than in late first-relapse patients (45% v 10%). Overall, 21 patients (29%, CI ranging from 19% to 41%) were nonresponsive and seven patients (10%, CI ranging from 4% to 19%) died of direct toxicity of induction. Four (67%) of the six patients with secondary AML or AML after a previous MDS had more early relapses than late first-relapse patients, with median DFS of 3.5 months and 8 months, respectively, long-term disease-free survivors were observed in both groups of patients (Fig 1) and the poor prognosis of refractoriness for DFS did not reach statistical significance (P > .1). Poor prognosis factors for survival included elevated serum LDH level (P = .004) and refractoriness (P = .05), with median survival of 4 months and 8 months and long-term survival of 7% and 30%, respectively, in refractory and late first-relapse patients (Fig 2). Only elevated LDH level remained prognostically significant in the multivariate analysis (P = .02). There was no statistically significant difference in CR rate, DFS, and survival between patients treated in the coordinating center and satellite centers nor between patients having received different chemotherapy protocols before entering the study. Projected DFS at 24 months for patients younger than 50
years receiving postinduction therapy with chemotherapy alone, whatever the chemotherapy protocol used, autologous BMT, or allogeneic transplantation are of 34%, 25%, and 39%, respectively. No statistically significant difference is observed at this time between the three groups of patients; however, the numbers of patients in each group are limited.

**Toxicity of EMA 86 Regimen**

Hematologic toxicity of induction included cytopenia with granulocyte count below $0.5 \times 10^9/L$ during a median of 30 days (range 14 to 59 days) and median platelet transfusion requirement duration of 30 days (range 12 to 85 days). Median time to platelet recovery $>100 \times 10^9/L$ was 47 days (range 25 to 178 days). Extra-hematologic toxicity of induction graded according to the WHO system is shown in Table 4. Major toxicity was infection with severe (grade 3 or more) infectious episodes in 40 patients (57%), including infectious death in nine patients. Four of these patients were blastic at the time of death. Most patients had vomiting, and 25 patients (35%) had severe oral mucositis preventing any food intake for several days. Four patients (6%) had grade 3 or more diarrhea. Eight patients (11%) had marked hyperbilirubinemia without clinical manifestations. Severe bleeding occurred in six patients (8%): four of them died, with persistent blastosis in three. One patient died of a gastric hemorrhage while in CR on the day after his discharge from the hospital and has been considered as a toxic death for analysis. Other toxicities were less frequent; however, one case of severe cerebellar toxicity and two cases of conjunctivitis probably related to cytarabine were observed. The patient who experienced cerebellar toxicity had no cerebellar dysfunction noted during a previous course of high-dose cytarabine. Miscellaneous toxicities included metabolic disorders of unclear origin responsible for the death of one patient, reversible cardiac failure in one patient, and activation of acquired immunodeficiency syndrome in a previously seropositive patient. Overall, 14 patients died during induction, with persistent blastosis as a contributing factor of death in seven of them (Table 4).

Toxicity of the second course of intensive chemotherapy in the selected group of patients who received it was similar to that of the induction course. Median duration of granulocytes below $0.5 \times 10^9/L$ was 33 days (range 19 to 44 days), median platelet transfusion requirement duration was 33 days (19 to 75 days), and median time of platelet recovery above $100 \times 10^9/L$ was 71 days (27 to 212 days). Two patients died of infection and one of cerebral hemorrhage.

**DISCUSSION**

Retreatment of patients with previously treated AML with conventional chemotherapy regimens used as first-line therapy induces less than 30% CR in refractory patients and less than 60% CR in late first-relapse patients. Recent approaches to the treatment of these patients generally involved the use of cytarabine in intermediate or high-dose alone or in association with new intercalating agents, such as asparaginase, mitoxantrone or idarubicin, etoposide, or asparaginase. However, reported results with these new regimens widely vary, with CR rates ranging between 0% and 70% because of the limited number of patients included in many reports and because of differences in eligibility criteria and previous therapy received by the patients between the different series. These regimens induce only occasional prolonged CR, and median DFS is generally less than 6 months. Although a direct comparison with already-reported regimens is not possible, our results seem promising with 45% of refractory patients and 81% of late first-relapse patients achieving CR in a series including many patients having previously received high-dose cytarabine. The probability of long-term remission in patients receiving postinduction chemotherapy is above 20% in refractory as well as late first-relapse AML patients. These results seem equivalent to those achieved with autologous and allogeneic BMT in our series as well as in recently reported studies of autologous or allogeneic BMT in

**Table 4. Extra-Hematologic Toxicity of Induction Chemotherapy**

<table>
<thead>
<tr>
<th>WHO Grade (no. of patients)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5*</th>
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<tr>
<td><strong>Fever/infection:</strong></td>
<td>1</td>
<td>21</td>
<td>10</td>
<td>26</td>
<td>6</td>
<td>9 (4)</td>
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<tr>
<td><strong>Nausea/vomiting:</strong></td>
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<td>13</td>
<td>45</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Diarrhea:</strong></td>
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<td>12</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Mucositis:</strong></td>
<td>31</td>
<td>1</td>
<td>15</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cutaneous rash:</strong></td>
<td>54</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Hyperbilirubinemia:</strong></td>
<td>29</td>
<td>32</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Clinical bleeding:</strong></td>
<td>60</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>4 (3)</td>
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<td><strong>Conjunctivitis:</strong></td>
<td>70</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Cerebellar syndrome:</strong></td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cardiac failure:</strong></td>
<td>71</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Metabolic disorders:</strong></td>
<td>69</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1†</td>
</tr>
</tbody>
</table>

*Overall, 14 patients died during induction chemotherapy. Persisting leukemia was a contributing factor of death in seven of these patients, numbers of patients with persisting leukemia at the time of death are indicated in parentheses.

†Coma of unclear cause.
this indication. However, our results regarding long-term DFS and overall survival need to be interpreted cautiously given the limited number of patients with adequate follow-up and the wide CI on the terminal part of the curves.

These encouraging results could be explained by both the choice of drugs and the sequential timing of their administration in EMA regimen. Mitoxantrone is partially non-cross-reactive with daunorubicin and doxorubicin previously administered in our patients. It has shown superiority over daunorubicin for induction of CR in newly diagnosed AML patients. Etoside is among the drugs inducing the highest CR rates in phase II trials in AML, following intercalating agents and cytarabine. Furthermore, etoside and cytarabine have been found synergistic and non-cross-reactive in vitro. Etoside administered as a continuous infusion in addition to cyclophosphamide induced CR in AML patients nonresponsive to high-dose cytarabine. It has also been shown to increase CR duration when added to a conventional anthracycline-cytarabine regimen in newly diagnosed AML patients. Since the initiation of our study, the association of etoside and mitoxantrone has demonstrated efficacy in two large series of patients, including one cooperative trial from the Eastern Cooperative Oncology Group (ECOG). Most patients in one of these series were refractory according to the definition used in our study and overall CR rate was 43%; however, no CR lasted longer than 14 months. Association of mitoxantrone, etoside, and cytarabine in a nonsequential regimen has recently been administered to 36 patients and led to a 58% CR rate, with no CR exceeding 12 months. The usefulness of the sequential design used in this study has been demonstrated by cell-cycle studies showing that the percentage of cells recruited into cycle by the first sequence of chemotherapy was a prognostic factor for achievement of CR. Whether these biologic findings are expressed in the overall clinical results of induction cannot be affirmed in the absence of a randomized study. However, the use of such a sequential therapy has been associated with long CR duration in newly diagnosed patients and could therefore explain the unexpectedly high long-term DFS in our series. The usefulness of the repetition of such a sequential regimen to prolong DFS cannot be evaluated in our series because only a few patients are evaluable for long-term survival. One patient who received maintenance with low-dose cytarabine is alive disease free after more than 3 years in second CR.

Toxicity of EMA regimen remained manageable, with an aplasia duration and death rate similar to those reported in the literature for patients treated with mitoxantrone and etoside. Higher death rates have been observed in patients treated with high-dose cytarabine in association to mitoxantrone or etoside, but series are difficult to compare because of the different previous therapies. The limiting extra-hematologic toxicity in our patients was mucositis, as observed in other regimens using etoside. We observed no unexpected extra-hematologic toxicity in our patients. Initial toxicity did not hinder subsequent autologous or allogeneic BMT: all patients aged less than 50 with a suitable family donor have received allogeneic BMT, and no adverse effect possibly related to the toxicity of previous chemotherapy, such as veno-occlusive disease, was observed after BMT. Repetition of the initial chemotherapy regimen appeared tolerable; however, only 16 patients effectively received the second course of intensive chemotherapy and a bias toward treatment of better-risk patients cannot be ruled out.

Overall, results achieved with this regimen are encouraging for both refractory and late first-relapse AML patients. If recruitment is to play a role in the efficacy of this regimen, association of newly available stimulating factors to chemotherapy might even increase CR rate in refractory patients. Because the CR rate of 81% achieved in late first relapse patients with one course of EMA regimen is at least equal to that achieved in newly diagnosed patients with two courses of conventional chemotherapy, this regimen could probably find a place in the first-line treatment of AML.

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