

Research article

## Timed sequential chemotherapy with concomitant Granulocyte Colony-Stimulating Factor for high-risk acute myelogenous leukemia: a single arm clinical trial

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### Abstract

**Background:** The timed-sequential chemotherapy regimen consisting of etoposide, mitoxantrone and cytarabine (EMA) is an effective therapy for relapsed or refractory acute myelogenous leukemia (AML). We postulated that granulocyte colony-stimulating factor (G-CSF) might enhance the cytotoxicity of EMA by increasing the proportion of leukemic blasts in S-phase. We added G-CSF to EMA (EMA-G) for therapy of advanced high-risk AML patients.

**Methods:** High-risk AML was defined as refractory, relapsed or secondary to either an antecedent hematologic disorder or exposure to cytotoxic agents. The patients were treated with one course of EMA-G consisting of mitoxantrone and cytarabine on days 1–3, and etoposide and cytarabine on days 8–10. G-CSF was started on day 4 and continued until absolute neutrophil count recovered.

**Results:** Thirty patients were enrolled. The median age was 51 years (range, 25–75). Seventeen (61%) patients had unfavorable cytogenetic karyotypes. Twenty (69%) patients had secondary AML. Ten (34%) had relapsed disease. Four (14%) had refractory AML. Three (10%) patients died from febrile neutropenia and sepsis. Major non-hematologic toxicity included hyperbilirubimemia, renal insufficiency, mucositis, diarrhea, nausea and vomiting, skin rash. A complete remission was achieved in 13 (46%) patients. Median overall survival was 9 months (range, 0.5–66). Median relapse-free survival (RFS) for those who had a CR was 3 months (range, 0.5–63) with RFS censored at the time of allogeneic bone marrow transplantation or peripheral stem cell transplantation for 6 of the patients.

**Conclusions:** EMA-G is a safe and efficacious option for induction chemotherapy in advanced, high-risk AML patients. The activity of EMA may be increased if applied in patients with less advanced disease.

## Background

The promise of improved outcome with the use of hematopoietic growth factors in the treatment of acute myelogenous leukemia (AML) has yet to be realized. In several prospective trials, patients were randomized to either a hematopoietic growth factor or a placebo after induction chemotherapy in an attempt to accelerate neutrophil recovery and improve outcome. These studies consistently show accelerated neutrophil recovery without an increase in leukemic relapse in patients treated with either granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF). [1–4] Unfortunately these studies have failed to consistently show a benefit in terms of remission and survival when administered in such an adjuvant fashion. [5,6]

The hematopoietic growth factors have also been studied for their therapeutic benefits when given before chemotherapy. Both G-CSF and GM-CSF recruit quiescent leukemic blasts into cell cycle thus rendering them more susceptible to the S-phase specific cytotoxicity of cytarabine. [7–13] Several studies suggest a potential therapeutic benefit from administering hematopoietic growth factors before starting cytarabine-based chemotherapy. [14–19] However, the results of larger randomized trials have been disappointing. [20–23] Not only have remission and survival rates not been increased but the direct effect of growth factors on the untreated leukemia has led to severe toxic reactions and death. [24–26]

Chemotherapy also recruits quiescent leukemic blasts into cell cycle. Burke and colleagues demonstrated in an *in vitro* model that following an initial cycle of chemotherapy "humoral factors" are generated which are capable of recruiting quiescent leukemic blasts into S-phase. [27] The maximal recruitment occurred about 4 to 6 days after chemotherapy was administered. To apply these observations clinically, patients were first treated with cytarabine and daunorubicin. This initial cycle of chemotherapy was then followed by either a second course of cytarabine on day 10 or AMSA on days 8–10. This "timed sequential therapy" achieved a complete remission rate of 88% in *de novo* and 70% in secondary AML. [28] Timed sequential therapy has been employed in relapsed and *de novo* acute leukemia and achieves complete remission rates comparable to those achieved with other salvage chemotherapeutic regimens. [29,30]

The combination of timed sequential chemotherapy and hematopoietic growth factors allows for an initial cytoreductive course of chemotherapy before starting growth factors. The growth factor could then work with the other "humoral factors" generated by chemotherapy to maximize recruitment of leukemic blasts into cell cycle. Few studies have tested this approach. We report the results of

a clinical trial assessing the potential benefit of adding G-CSF to a proven timed sequential chemotherapeutic regimen.

## Materials and Methods

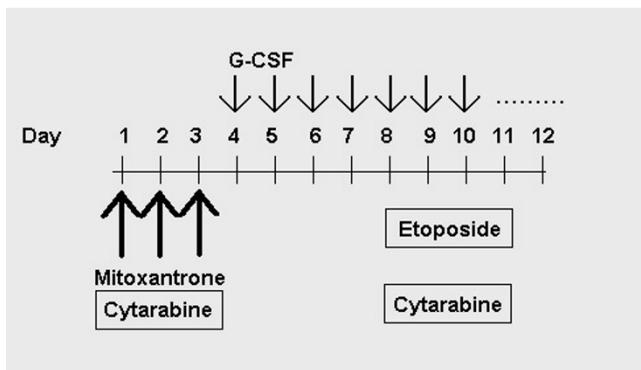
### Patients

All patients had AML defined according to the standard French-American-British (FAB) cytologic and cytochemical criteria [47]. Patients were considered to have good-risk cytogenetics if their karyotype revealed either t(8;21), inv(16), t(16;16) or t(15;17). A normal karyotype defined intermediate-risk cytogenetics. All other clonal abnormalities were considered poor-risk. Patients were eligible for this protocol if their leukemia was: 1) refractory to previous induction chemotherapy, 2) in first or subsequent relapse after initial complete remission following induction chemotherapy, 3) secondary to transformation from an antecedent hematologic disorder, or 4) secondary to previous exposure to cytotoxic agents. All patients were over 18 years of age. Only patients with a performance status of 2 or less and no organ failure [less than grade 2 according to the World Health Organization (WHO) grading system] could enter the study, except if organ failure was related to the leukemia itself. Patients with leukemic meningitis were excluded. All patients gave signed informed consent and were treated on clinical protocols approved by the Cleveland Clinic Foundation's Institutional Review Board.

### EMA-G Regimen

The EMA regimen contains etoposide, mitoxantrone and cytarabine administered in a timed-sequential fashion. [32] We added G-CSF to the EMA regimen, thus EMA-G. EMA-G consists of mitoxantrone 12 mg/m<sup>2</sup> per day intravenous (IV) bolus for 3 days (day 1 to 3) with cytarabine 500 mg/m<sup>2</sup> per day IV continuous infusion (CI) for 72 hours (day 1 to 3). Etoposide 200 mg/m<sup>2</sup> per day IVCI for 72 hours and cytarabine 500 mg/m<sup>2</sup> per day IVCI for 72 hours were administered on day 8 to 10. G-CSF 5 mg/kg per day subcutaneously was started on day 4 and continued until the absolute neutrophil count (ANC) rose above  $0.5 \times 10^9/L$  for two consecutive days (Fig. 1). Only one course of EMA-G induction was given. Post remission therapy included chemotherapy, allogeneic bone marrow transplantation (BMT) or peripheral stem cell transplantation (PSCT) at the treating physician's discretion and availability of a transplant donor.

All patients were cared for in semi-private rooms supplied with HEPA filters on a regular oncology unit. All patients received standard supportive care including blood component support for severe cytopenias as previously described. [31] Acyclovir 250 mg/m<sup>2</sup> per day IV or 400–800 mg PO bid, allopurinol 300 mg PO qd and fluconazole 400 mg PO qd were administered prophylactically.



**Figure 1**  
Schema of EMA-G regimen.

**Evaluation of Therapy**

Bone marrow aspirates and biopsies were obtained on day 8 of chemotherapy and then at the discretion of the treating physicians. When a bone marrow aspirate or biopsy obtained on or after Day 14 contained 5% or more blast cells, G-CSF was stopped.

Complete remission (CR) was achieved when the following parameters were met: 1) bone marrow was normocellular, containing <5% blasts, >15% erythroid cells, and >25% normal granulocytes; 2) peripheral blood had neutrophils > 1.5 × 10<sup>9</sup>/L, hemoglobin >11 gm/dl, and platelets > 1 × 10<sup>11</sup>/L; 3) no evidence of extramedullary leukemia, and 4) all of the above fulfilled for at least 4 weeks. Nonresponse to EMA-G induction was defined as the absence of CR after one course of induction therapy. Relapse was defined by the appearance of circulating blasts or bone marrow blasts >5% in a patient previously in complete remission.

**Evaluation of Toxicity**

Severity of treatment-related toxicity was graded according to the WHO criteria (World Health Organization Handbook for Reporting Results of Cancer Treatment, 1979.)

**Statistical Analysis**

The method of Kaplan and Meier was used to analyze overall survival, relapse-free survival, time to neutrophil recovery and time to platelet recovery. Exact 95% confidence intervals (CI) based on the binomial distribution were calculated for proportions such as the complete response rate.

**Table 1: Characteristics of 29 patients with AML treated with EMA-G.**

Patients	29
Median Age (years)	51
Range	(25–75)
Male: Female	16:13
Karyotype (n = 28)	
Favorable	1
Intermediate	10
Unfavorable	17
Etiology of AML	
Primary	9
Secondary	20
Stage of the disease	
Untreated (all secondary)	15
Refractory	4
Relapsed < 1 year	5
Relapsed > 1 year	5

**Results**

**Patients**

From August 1994 to November 1996, 30 patients were enrolled onto this study. One patient received high-dose cytarabine instead of EMA-G for induction therapy after enrollment and was therefore excluded from further analysis. Thus, 29 patients received EMA-G induction therapy and are evaluable for treatment-related toxicity. Only 28 patients are evaluable for induction response since 1 patient was lost to follow-up shortly after receiving induction therapy (no evidence of persisting leukemia by the post-induction bone marrow biopsy). The characteristics of the evaluable patients are listed in Table 1. Twenty-eight patients had cytogenetic studies of bone marrow aspirates. Most patients (61%) had unfavorable karyotypes. The majority of patients (69%) had secondary AML transformed from either MDS (n = 10), CML (n = 5) or other myeloproliferative diseases (n = 4), or secondary to cytotoxic agents (n = 1). Overall, 15 patients with secondary, but untreated, AML were enrolled. Four patients were enrolled with AML refractory to previous induction therapy, and 10 were enrolled with relapsed AML.

**Recovery of neutrophil and platelet counts**

The median time from the start of chemotherapy to recovery of an absolute neutrophil count exceeding 0.5 × 10<sup>9</sup>/L was 30 days (range, 19 to 47 days). Three patients died of febrile neutropenia and sepsis during induction therapy. The median duration of platelet transfusion dependence was 30 days (range, 22 to 48 days).

**Efficacy of Therapy**

Twenty-eight patients are evaluable for treatment response. Thirteen patients (46%, 95% CI 27%–66%)

achieved a complete remission. Seven of those 13 patients with CR had secondary leukemia. Twelve patients (43%) failed to achieve CR following induction treatment. One of the non-responders had no evidence of leukemia after induction treatment and proceeded to bone marrow transplant before meeting all of the criteria for complete remission. Three patients (10%) died during induction treatment.

Of the 13 patients who achieved CR, 6 patients received further consolidation chemotherapy (3 with high dose cytarabine, and 3 with intermediate dose cytarabine combined with daunorubicin, etoposide, or interleukin-2), and 6 patients received either allogeneic BMT or allogeneic PSCT. One patient relapsed before any post-remission therapy was given. Of the 13 patients who did not achieve CR, 1 received salvage chemotherapy, 2 received allogeneic BMT, and 10 died due to rapid disease progression.

### Survival

The median overall survival (OS) for all evaluable patients is 9 months (range, 0.5–66 months). Median relapse free survival (RFS) for those who achieved CR is 3 months (range, 0.5–63 months) with 6 patients censored at allogeneic BMT or PSCT. At five-years of follow-up, 3 patients (11%) were still alive. Two of them were in CR, one of whom had allogeneic BMT and the other had high dose cytarabine as post-remission therapy. The third patient is alive with recurrent leukemia.

### Treatment-related Toxicity

All patients experienced fever in the setting of severe neutropenia. Fifteen (15%) patients had documented infections. Three patients (10%) died from febrile neutropenia and sepsis. Other WHO grades III-IV toxicity included mucositis (17%), diarrhea (17%), skin rash (14%), nausea and vomiting (14%), bleeding (3%). Ten (34%) patients had reversible hyperbilirubinemia and 5 (17%) had WHO grades III-IV renal insufficiency. Three (10%) patients experienced severe neurological toxicity (1 with seizure, 2 with severe vertigo) but those complications resolved shortly after the completion of induction therapy. G-CSF was stopped in 1 patient due to persisting peripheral blasts. When G-CSF was discontinued the peripheral blasts cleared and the patient achieved a complete remission.

### Discussion

Hematopoietic growth factors clearly increase the cytotoxicity of cytarabine to leukemia cells *in vitro*. [33] Clinically, however, they fail to increase remission rates when started from 24 to 48 hours before any chemotherapy. [20–23] Potential explanations for the failure of growth factors to improve results when administered in this fashion

may reflect either their adverse effect on a large leukemic burden or the necessary delay to the onset of chemotherapy. [34] In our study we started growth factors after the initial leukemic burden was reduced and then optimized the timing of a second course of chemotherapy to take advantage of known leukemic blast cell-cycle kinetics. [27] Our results are comparable to those achieved with other salvage chemotherapeutic regimens that employ high-dose cytarabine. [35–39]

Rather than use a standard high-dose cytarabine regimen we employed the EMA chemotherapy protocol devised by Archimbaud and colleagues. [40] This regimen is effective in patients with relapsed and refractory AML with a reported CR rate of 60%. In addition EMA avoids the cerebellar neurotoxicity of high-dose cytarabine. Recognizing the potential value of hematopoietic growth factors, Archimbaud and colleagues added GM-CSF to the EMA regimen beginning on Day 4 of treatment. Unlike the current report, however, GM-CSF was discontinued on Day 10 of treatment. Unfortunately the addition of GM-CSF did not increase the CR rate when it was compared to placebo in a prospective randomized study in patients with relapsed and refractory AML. [41] The results with EMA and EMA plus GM-CSF are compared with our own results in Table 2.

Clearly the patient population in our study was different than the population studied in the other two studies employing the EMA regimen. Our population is older and a majority of our patients had secondary AML. These two characteristics are among the most predictive of a poor outcome. Although 15 of our patients were untreated at the time of study enrollment all 15 had secondary AML and 5 of them had CML in blast crisis unresponsive to hydroxyurea therapy. The poor-risk features of our patient population probably explain our relatively low CR rate compared to other EMA studies but also confirm the efficacy of our treatment protocol since remissions would only be expected in 40–50% of untreated patients with secondary AML receiving initial induction chemotherapy. [42–44]

Our study did not demonstrate a significant advantage to the addition of G-CSF to the EMA regimen. Potential reasons include the possibility that G-CSF failed to increase the population of leukemic stem cells in S-phase over that achieved with chemotherapy alone. Alternatively recruitment of blasts into S-phase may not increase cytotoxicity to a clinically significant degree. Finally even if S-phase increases cytotoxicity and G-CSF is effective in increasing the proportion of blasts in S-phase these effects may not be sufficient for patients with advanced leukemia. In the few studies exploring the role of timed-sequential chemotherapy as primary treatment for *de novo* untreated AML results

**Table 2: Comparison of three clinical trials of EMA with and without the use of hematopoietic growth factors.**

	EMA[40]	EMA-Placebo[41]	EMA-GM[41]	EMA-G
Patients	133	97	95	29
Median Age	43 (mean)	46	47	51
Status of AML				
Refractory or relapsed <1 yr	124 (93%)	61 (64%)*	59 (61%)*	11 (38%)
Relapsed >1 yr	9 (7%)	36 (36%)	36 (38%)	3 (10%)
Secondary AML	16 (12%)	16 (16%)	13 (14%)	20 (69%)
ANC > 0.5 × 10 <sup>9</sup> /L	31 days	37	38	30
CR Rate	60%	59%	65%	46%
Toxic Death	11%	8%	5%	10%

\* = Refractory AML plus Relapse AML within 6 months ANC = Time to recover a sustained absolute neutrophil count CR = Complete Remission

have been more encouraging with high remission rates and prolonged survival. [45,46]

An initial concern with EMA-G was the effect the concurrent administration of G-CSF and chemotherapy might have on bone marrow recovery. However, the concern was unfounded since the median time to neutrophil and platelet recovery is not different than that noted with EMA (Table 2). This observation suggests that G-CSF may be administered concurrently with chemotherapy without an increase in toxicity to normal residual bone marrow. Other studies utilizing hematopoietic growth factors administered before and concurrently with chemotherapy also demonstrate no adverse effect on bone marrow recovery. [20–22]

### Conclusions

The treatment of advanced high-risk AML patients remains challenging. The use of EMA-G in the current study failed to demonstrate a better CR rate than published data of EMA alone or EMA with GM-CSF (EMA-GM). However, the 46% CR rate achieved in our cohort of patients with a higher proportion of secondary AML and older median age suggests that the EMA-G regimen deserves further study in a population of patients with less advanced disease.

### Competing interests

None declared

### Author's Contributions

X-YH collected and compiled the data and reviewed the manuscript with MK. PE participated in the design of the study, performed the statistical analysis and wrote the statistical section. MH, AL, BP, and SA conducted the study, contributed patients, and commented on the data analysis and manuscript. MK conceived of the study, reviewed the data collection and analytical processes, and wrote the

body of the manuscript. All authors read and approved the final manuscript.

### Abbreviations

AML, acute myelogenous leukemia. G-CSF, granulocyte colony-stimulating Factor. GM-CSF, granulocyte-macrophage colony-stimulating Factor. EMA, etoposide, mitoxantrone and cytarabine. EMA-G, EMA and G-CSF.

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