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Phase II Study of Combination Human Recombinant GM-CSF With Intermediate-Dose Cytarabine and Mitoxantrone Chemotherapy in Patients With High-Risk Myelodysplastic Syndromes (RAEB, RAEBT, and CMML): An Eastern Cooperative Oncology Group Study

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A Phase II study of GM-CSF with intermediate-dose cytarabine and mitoxantrone was conducted in patients with high-risk myelodysplastic syndrome. It was designed to evaluate if priming with growth factor could increase the efficiency of chemotherapy. In this older population only two of 10 patients achieved a bone marrow CR, including one patient whose leukemic blasts had an "S" phase increase of 2.55× at 48 hr. Unexpected hepatotoxicity was noted. This regimen cannot be recommended for this elderly population of patients. Am. J. Hematol. 66:23–27, 2001. © 2001 Wiley-Liss, Inc.

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INTRODUCTION

The myelodysplastic syndromes (MDS) represent a heterogeneous group of disorders originating from neoplastic alterations of early hematopoietic precursor cells. Their overall incidence is estimated at 3 in 100,000 persons per year but is significantly higher in ages 60 years and above [1].

The French American British (FAB) working committee has defined five subtypes of MDS according to the percentage of blast cells in the bone marrow and blood, of ringed sideroblasts and monocytes, and the degree of dyshematopoiesis [2–4]. In addition to FAB subtyping, analysis of the percentage of blasts, degrees of cytopenias, and chromosomal studies have resulted in a widely accepted prognostic scoring system (IPSS) [5,6]. These differences provide some guidelines for the management of MDS patients which generally consists of supportive measures for the low-risk group and cytostatic therapy for the high-risk group [7–9].

A potential new approach to the treatment of MDS

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patients has come from the discovery, characterization. and cloning of hematopoietic growth factors, especially of GM-CSF and G-CSF and the evaluation of their action on hematopoietic precursor cells. GM-CSF preferentially stimulates the growth of precursor cells of the myelomonocytic lineage and increases the function of mature granulocytes and monocytes [10]. The action of G-CSF seems to be restricted to more differentiated cells of the myeloid lineage only [11,12]. Both factors were also shown to enhance the growth of AML blasts in in-vitro experiments [13,14].

ECOG's recent Phase III randomized study in patients with Acute Myeloid Leukemia 55–70 years (EST 1490) demonstrated that GM-CSF (sargramostim) given 4 days after completion of induction therapy significantly reduced the duration of neutropenia, increased the CR% from 44 to 60% compared to placebo, and increased overall survival [15].

Innovative therapy is warranted to improve upon the dismal outlook for patients with Refractory Anemia with Excess of Blasts (RAEB) and in Transformation (RAEBT). The current study, therefore, addressed the two main reasons for treatment failures in advanced MDS by trying to increase the efficacy of antileukemic therapy through priming by GM-CSF and by attempting to decrease therapy-associated morbidity and mortality related to uncontrollable infections.

A preliminary randomized trial of high-risk MDS patients utilizing this principle, but with *Escherichia coli*-derived GM-CSF accrued 28 patients [16]. Of these patients 11 (37%) achieved a CR, and an additional 7 patients had a lower stage of MDS after recovery of counts. Unmaintained remission duration was 35% at 3 years.

This pilot study utilized the recombinant GM-CSF [Immunex Corporation (Sargramostim) NSC-6137950] and the identical chemotherapy utilized by the German AML Trial Group in adults with AML [17] and in MDS [16] with intermediate-dose cytarabine (ARA-C) followed by mitoxantrone.

MATERIALS AND METHODS

Patients had to have morphologic proof from bone marrow aspirates of the myelodysplastic syndrome [2], Refractory Anemia with Excess Blasts (RAEB), Refractory Anemia with Excess Blasts in Transformation (RAEB-T), or Chronic Myelomonocytic Leukemia (CMML). At least 11% blasts were required to be present in the marrow, since a competing study for low-risk MDS patients with <10% blasts was active at the same time (a study of erythropoietin ± Granulocytic Colony Stimulating Factor). Prospective morphologic review by one of the co-authors (J.M.B.) was mandatory to assure a uniform diagnosis. In addition to pathology requirements patients had to be at least 18 years of age; have an ECOG performance status less than 3; normal laboratory parameters including a creatinine of <2.0 mg/dl; hepatic enzymes <2.0 × upper limit of normal (SGOT, SGPT, bilirubin), and uncompromised cardiac function as determined by a normal ECG and either a 2-D echo or MUGA scan. Patients could not have received hematopoietic growth factors within 8 weeks of entry and no antineoplastic treatment for MDS. A total leukocyte count of $<30 \times 10^9$/l was required before the administration of GM-CSF Sargramostim, yeast-derived rhu granulocyte-macrophage colony stimulating factor. Both primary (idiopathic) and secondary MDS cases were eligible provided that no chemotherapy or radiation had been received within 3 months. Patients known to be human immunovirus (HIV) positive were excluded as well. Informed consent, according to Institutional Review Board (IRB) requirements, was mandatory.

DNA synthesis was assessed by BRDU incorporation "in vitro" at baseline and after 48 hr of in vivo stimulation by GM-CSF. Blood and/or marrow cells were incubated with BRDU at 1:20 dilution in RPMI + 10% FBS) for 60 min at 37°C to label cells in S phase of the cell cycle. Excess BRDU was washed with 2 washes in PBS. Cells were then fixed in cold 70% ethanol and stored at 4°C until staining was continued. Cells were subsequently washed with PBS twice and incubated with a solution of 0.2 mg/ml pepsin in 2 N HCL for 20 min at 37°C. The cells were then washed in PBS twice and then resuspended in PBS + 0.5% Tween-20 for permeabilization. Cells were then stained for 30 min at 4°C with 5 μl of FITC-conjugated anti-BRDU. After a PBS wash, cells were incubated for 30 min and the fluorescence intensity was collected on a Coulter Epics flow cytometer.

Overall survival was defined from the time of entry into the study to the date of death from any cause. The survival distribution for overall survival was estimated by the method of Kaplan and Meier [18]. The 90% confidence interval was calculated for the proportions of responders [19]. Accrual was stopped for initial evaluation of response for the first 10 eligible patients. The study was designed to reopen if at least three complete remissions were seen.

Standard response criteria were utilized [20]. Briefly a complete remission (CR) required that the neutrophil count $>1.0 \times 10^9$/l, platelet count $>100{,}000 \times 10^9$/l, lack of leukemic blasts in the peripheral blood films, and no evidence of morphologic dysplasia. In the bone marrow aspirate the percentage of blasts had to be <5%, cellularity (on bone marrow biopsy) at least 20% with maturation of all cell lines.

A partial remission (PR) required all of the above criteria for CR except that the percentage of blasts could range from 5% to 25%. The presence of Auer rods with

less than 5% blasts or morphologic dysplasia assigned cases to the PR category.

Following registration all patients received GM-CSF for 2 days (48 hr) followed by cytosine arabinoside (1 g/m^2 over 3 hr every 12 hr for 4 consecutive doses and again 1 week later for an additional 4 doses). Mitoxantrone, 10 mg/m^2 over 30 min, was administered following the completion of cytosine arabinoside for two consecutive days for a total of four doses. GM-CSF was continued if the day 14 bone marrow revealed less than 5% blasts until the absolute neutrophil count reached and was maintained at $>1.5 \times 10^9$/l for 3 consecutive days or the patient was taken off study.

RESULTS

The study opened on February 20, 1997, and closed on January 23, 1998.

Nineteen potential cases were prospectively screened for study entry. Of these 7 were diagnosed as AML (including 4 diagnosed as acute erythroleukemia [FAB M6]) and were excluded. Twelve cases were registered on this study. Two cases were canceled just prior to entry because of rapid progression to AML. Ten patients were eligible and evaluable. The median age was 71 years (range was 56–78 years). Eight were male, and two were female. By FAB subtype 5 patients had RAEB, 3 were RAEB-T (20–30% blasts), and 2 had CMML. Cytogenetic data was reviewed centrally by GD. Six patients had abnormal karyotypes including one with t(1;17)(p36;q21); one with del(5)(q22-35); one with +8; one with −7; and one with +11. Three of the 6 had additional anomalies. The International Prognostic Scoring System (IPSS) was high risk in all 10 patients.

All 10 evaluable patients were treated at 100% calculated doses of therapy with full courses of drugs, including GM-CSF, and achieved bone marrow aplasia.

Expected hematologic toxicity occurred in all 10 patients with marrow aplasia achieved in all patients by day 14. Unexpected hepatic toxicity was observed in 7 patients (1 patient had Grade 1; 3 had Grade 2; 1 had Grade 3; and 2 had Grade 4). The major abnormality was bilirubinemia (median 6.3; range 2.2–22.4 mg/dl). In only one case was there a Grade 2 elevation of SGOT (Table I). In two patients the hepatic toxicity was felt to contribute to their demise. In one of these autopsy revealed marked autolysis with ischemic change.

Among the 10 eligible patients two patients achieved bone marrow criteria for CR except that platelet counts remained below 10×10^9/l. The 90% confidence interval for "bone marrow CR" was 4–51%: the two bone marrow CRs survived 221 and 834+ days. The overall median survival was 36 days (range of 13–834+ days) (Fig. 1). Seven of the 10 evaluable patients died as a result of infectious complications during the period of marrow aplasia.

TABLE I. Hepatic Toxicity

Grade[a]	Bilirubin	SGOT	Alkaline phosphatase
0	3	9	7
1	1	0	3
2	3	1	0
3	1	0	0
4	2	0	0
Total	10	10	10

[a]Common Toxicity Criteria, Version 2.0, National Cancer Institute.

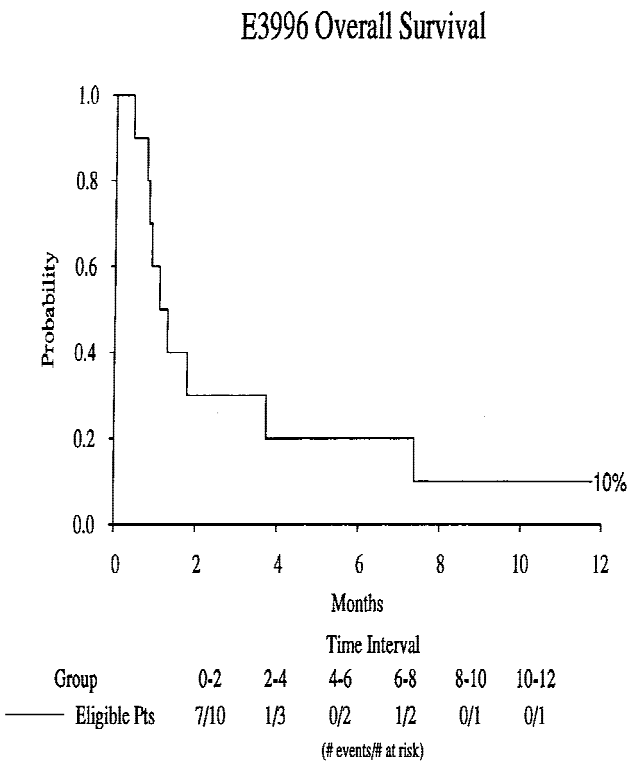

Fig. 1. Overall survival.

Although day "0" marrows were submitted on all 10 patients for "S"-phase analysis, only 6 samples were received after 48 hr of GM-CSF administration. Of these, 2 patients had a significant increase in the percentage of cells in S phase (one patient had an increase from 14.7% to 37.5%; one patient had an increase from 6.5% to 16.6%). Of interest is that the longest surviving patient, who achieved one of the two bone marrow CRs, had a 2.55× increase in the percentage of cells in S phase at 48 hr.

DISCUSSION

This Phase II pilot study was activated to confirm a preliminary CR% of 35% (10/29) obtained in a randomized trial of *E. coli*-derived GM-CSF [25] and the iden-

tical chemotherapy regimen. In this study 35% of patients died within the first 6 months of treatment, similar to the present study. However, there were two important differences in the current study. First the median age was 71 years versus 57 years, and there was an unexpected hepatic toxicity not witnessed previously in other similar chemotherapy programs that combine cytosine arabinoside and mitoxantrone [21].

The 2.55× increase in the percent of cells in S phase at 48 hr [22] is consistent with the in vitro studies on leukemic stem cells in patients with AML, indicating a significant increase in cell kill by cytarabine after preadministration of GM-CSF for 24–48 hr [23].

Because of the extreme degree of heterogeneity in MDS, the clinician is faced with a broad menu of treatment options. These range from supportive care (transfusion, antibiotics), erythropoietin, and cytokines for the majority of patients. Chemotherapy, ranging from single agent to multiple drugs, and finally bone marrow transplant are offered to the minority of cases, usually those under 60 years of age. With advanced age, co-morbidity factors, and, often, chemo-resistant disease, response rates have been quite variable and long-term survival uncommon.

Low-dose cytosine arabinoside has achieved response rates of 30%, including 10% CRs [24], but median survival is 8–10 months. A ring analogue of cytosine, 5-azacytidine, achieves a similar response rate but appears to delay blastic progression and is well tolerated [25]. Combination chemotherapy [9] with standard AML reduction rates achieves a higher CR% with a corresponding increase in early deaths and is often employed prior to auto BMT in selected patients, usually under age 65 years. A recent study [6] of 65 patients with Intermediate-2 and high-risk MDS received daunorubicin and cytarabine + G-CSF. The median age was 62 years. Sixty-three percent achieved CR: 61% were alive at 1 year, and 22% were projected to be alive at 2 years. Recent experience with topotecan and high-dose cytosine arabinoside [26] has achieved CR rates of 67% with median survival exceeding 1 year.

This Phase II study was stopped prematurely because the initial goal was to proceed only if 3/10 CRs were obtained and also because of unexpected hepatotoxicity (essentially hyperbilirubinemia). The regimen produced 100% aplasia but with only two CRs and a very high early death rate. Although the small number of patients makes interpretation difficult and also because the advanced age could have contributed to the poor outcome, nonetheless this regimen cannot be recommended for further study.

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