Maintenance Treatment of the Anemia of Myelodysplastic Syndromes With Recombinant Human Granulocyte Colony-Stimulating Factor and Erythropoietin: Evidence for In Vivo Synergy

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Patients with myelodysplastic syndromes (MDS) have refractory cytopenias leading to transfusion requirements and infectious complications. In vitro marrow culture data have indicated that granulocyte colony stimulating factor (G-CSF) synergizes with erythropoietin (EPO) for the production of erythroid precursors. In an effort to treat the anemia and neutropenia in this disorder, MDS patients were treated with a combination of recombinant human EPO and recombinant human G-CSF. Fifty-five patients were enrolled in the study of which 53 (96%) had a neutrophil response. Forty-four patients were evaluable for an erythroid response of which 21 (48%) responded. An erythroid response was significantly more likely in those patients with relatively low serum EPO levels, higher absolute basal reticulocyte counts and normal cytogenetics at study entry. Seventeen (81%) of the patients who responded to combined G-CSF plus EPO therapy continued to respond during an 8-week maintenance phase. G-CSF was then discontinued and all patients' neutrophil responses were diminished, whereas 8 continued to have an erythroid response to EPO alone. In 7 of the remaining 9 patients, resumption of G-CSF was required for recurrent erythroid responses. The median duration of erythroid responses to these cytokines was 11 months, with 6 patients having relatively long and durable responses for 15 to 36 months. Our results also indicate that approximately one half of responding patients require both G-CSF and EPO to maintain an effective erythroid response, suggesting that synergy between G-CSF and EPO exists in vivo for the production of red blood cells in MDS.© 1996 by The American Society of Hematology.

MATERIALS AND METHODS

Patients. Fifty-five patients with histologically confirmed MDS were enrolled in this study, 26 at Stanford University Medical Center (Stanford, CA) and 29 at Vanderbilt University Medical Center (Nashville, TN). Twenty-eight patients were reported on previously.29 Central pathological review was performed (by J.V.), and BM aspirates were classified according to the French-American-British (FAB) criteria as previously described.29 Written informed consent was obtained from all patients according to guidelines established by the Human Experimentation Committees at either Stanford University or Vanderbilt University.

Treatment protocol. All patients were evaluated before study entry with complete history and physical examination. Eligibility criteria were identical to those previously reported.29 Patients with iron deficiency or serum creatinine levels greater than 2 mg/dL were ineligible for study until these values were either corrected or resolved. G-CSF was administered as a daily subcutaneous injection with a starting dose of 1 µg/kg/d that was titrated to maintain a normal absolute neutrophil count (ANC) or, if initially normal, was titrated to maintain an ANC twice the baseline value of that particular patient. Two weeks later, r-HuEPO (EPO) was added also as a single daily subcutaneous injection. Initially, the EPO was begun at 100 U/kg/d and was dose-escalated every 4 weeks to 150 U/kg/d and then to 300 U/kg/d to achieve an erythroid response. In only 1 patient was a response noted at the lower dose levels; therefore, for more efficient treatment, the later patients began EPO treatment at 300 U/kg/d.
Treatment was continued for a minimum of 8 weeks at an EPO dose of 300 U/kg/d. As previously reported, erythroid responses were defined as good (GR) if there was an increase in the hemoglobin level of greater than 2 g/dL or a 50% decrease in RBC transfusion requirement, partial (PR) if there was an increase in the hemoglobin level of greater than 1 g/dL and less than 2 g/dL or a 30% decrease in RBC transfusion requirements, or none (NR) if the response was less than a PR. Patients with either GRs or PRs were eligible for maintenance therapy for an additional 8 weeks. At the end of that time, the G-CSF was discontinued, and the patients were treated with EPO alone. After an additional 8 weeks, patients who maintained their erythroid responses were continued on EPO alone, whereas those patients who lost their erythroid responses were restarted on G-CSF and EPO.

G-CSF was supplied by Amgen (Thousand Oaks, CA), and r-HuEPO was supplied by Ortho Biotech Inc (Raritan, NJ) through the Cancer Therapy Evaluation Program (Investigational Drug Branch, National Cancer Institute, Bethesda, MD). Statistical analyses were performed by the Rank-Sum and Student’s t-test. For all patients achieving a response, response duration (as of July 1995) was determined using the Kaplan-Meier life table method. Response duration was measured from the time the patient began treatment until the patient no longer responded to the combination of drugs. For patients who stopped responding when G-CSF was discontinued (for 2 months as per protocol) but who resumed a response when G-CSF was restarted, their time off G-CSF was not included in the response duration. In addition to evaluating all responding patients, individuals who died of causes unrelated to their MDS or cytokine therapy (ie, cardiac disease, n = 3) while responding were considered as being censored at the time of death in evaluating their response duration.

RESULTS

Patients. The clinical characteristics of the 55 patients enrolled in this study are shown in Table 1. This is an extension of our prior study in which the initial 28 patients have previously been reported regarding their short-term responses. There were 41 men and 14 women; the median age was 72 years (range, 32 to 88 years). A total of 20 patients (36%) had refractory anemia (RA), 18 (33%) had RA with excess blasts (RAEB), and 6 (11%) had RAEB in transformation (RAEB-T). Forty-two patients (76%) were dependent on RBC transfusions. A total of 30 patients (55%) were neutropenic, and 25 (45%) were thrombocytopenic. Forty-six patients had evaluable BM cytogenetic studies, of which 18 (39%) were abnormal, primarily involving chromosomes 5 and 7.

Myeloid responses. G-CSF was administered to patients beginning at a dose of 1 μg/kg/d and was modified based on neutrophil response. All patients were evaluable for neutrophil response, and 53 patients (96%) showed a response to G-CSF therapy. The median dose required for a neutrophil response was 0.3 μg/kg/d (range, 0.1 to 5 μg/kg/d).

Erythroid responses. Of the 55 patients, 44 (80%) completed the planned minimum 8-week administration of combined G-CSF and EPO therapy and, therefore, were evaluable for an erythroid response. Twenty-one patients (48%) had an erythroid response, of which 14 (67%) were classified as GRs and 7 (33%) as PRs. Of the 32 evaluable patients who were initially RBC-transfusion-dependent, 12 patients (37%) had responses, of which 7 were no longer dependent on transfusions and 5 had significantly reduced transfusion requirements. Although 51% of the low-risk (RA and RARS) patients had erythroid responses as compared with 33% of the high-risk (RAEB and RAEB-T) MDS patients, this difference was not statistically significant (P = .5). The dose of EPO required was 300 U/kg/d in all cases except in 1 patient who responded at 100 U/kg/d and had the dose eventually reduced to 3 times per week. Figure 1 shows an example of 1 responding patient’s clinical course with the G-CSF and EPO treatment. Of the 55 initial patients, 11 were not evaluable for the evaluation of an erythroid response because both drugs were discontinued early because of either splenomegaly or splenic pain (4 patients), increasing numbers of myeloblasts with G-CSF treatment (3 patients), gastrointestinal bleeding (1 patient), bone pain (1 patient), patient’s request (1 patient), or an unrelated medical illness (1 patient). The clinical characteristics of the 21 patients who had an erythroid response to G-CSF plus EPO treatment were compared with those of the 23 patients who did not respond (Table 2). The responders had significantly lower median serum EPO levels of 120 U/L (range, 6 to 948 U/L) as compared with those of the nonresponders, who had a median serum EPO level of 615 U/L (range, 85 to 3,010 U/L; P = .001; see Fig 2). The response rates of patients with EPO levels greater than 500 U/L versus those of patients with EPO levels less than 500 U/L were 20% versus 65%, respectively (P = .009). In addition, responding patients had higher baseline absolute reticulocyte counts (median, 33.1 × 10^6/L) as compared with those of nonresponding patients (median, 21.3 × 10^6/L; P = .02) and were more likely to have normal cytogenetics (P = .04). As shown in Table 2, no correlation was noted between response and patient age, disease duration, prior RBC transfusion requirements, ANC or absolute platelet count at study entry, or BM cellular maturation patterns (relative erythroid differentiation indice and relative myeloid differentiation indice). The erythroid responses occurred in all FAB classifications (Table 3).
On completion of the initial 8 weeks of G-CSF and EPO treatment or at the time of erythroid response, responding patients were treated for an additional 8 weeks of maintenance therapy. Seventeen patients (81%) who had an initial response continued to respond, whereas 4 patients lost their response. At the completion of the maintenance phase of treatment, the G-CSF was discontinued and patients were treated with EPO alone. Eight patients (47% of the responders) continued to have an erythroid response, whereas 9 did not. The 9 patients who lost their responses to EPO alone were restarted on G-CSF, and 7 of these patients regained an erythroid response (Table 4). One patient did not regain an erythroid response to G-CSF plus EPO, and 1 patient developed osteomyelitis while the G-CSF was discontinued and died from complications of infection.

Toxicity. The injections were well tolerated. Occasional ecchymoses were noted at the injection sites. Two patients developed severe bone pain requiring discontinuation of the G-CSF and withdrawal from the study. One of these patients was subsequently discovered to have metastatic cancer. Five patients had increasing numbers of myeloblasts on peripheral blood smear requiring discontinuation of both study drugs. One additional patient developed thrombocytopenia unrelated to disease progression requiring discontinuation of the study drugs and treatment with corticosteroids.

Long-term follow-up. Of the 21 patients who had an erythroid response, 17 patients maintained their responses over the initial 4 months of treatment. The median duration of response was 11 months, with 35% of responses (6 of 17 responders) persisting from 15 to 36 months (Fig 3). Response duration was also considered separately for patients who responded to EPO alone and for patients who required both G-CSF and EPO. Median response duration was 34 months for the 7 patients who were able to maintain a response on EPO alone as compared with that of 20+ months for the 9 patients who required both G-CSF and EPO ($P = .098$). Three patients died of cardiac events while on the study, all of whom were responding to the treatment. In 2 of these patients the cardiac problem was caused by atherosclerotic heart disease, whereas, in the third patient, the cardiac problem was caused by iron overload. Censoring these 3 patients for analysis of response duration at the time of their deaths had minimal effects on this evaluation, because the median response duration including these patients was 10 months. An additional patient went off study after 19 months because of thrombocytopenia unrelated to disease progression. One patient developed osteomyelitis while on EPO alone and died of infection. Two patients progressed to acute myelogenous leukemia while on maintenance treatment and lost their responses. One patient who developed BM fibrosis did not respond to reinstitution of G-CSF and EPO.
Table 2. Comparison of Clinical Characteristics of Patients With MDS Predictive of Erythroid Responses to Recombinant Human G-CSF plus EPO Treatment

<table>
<thead>
<tr>
<th></th>
<th>Responders (N = 21)</th>
<th>Nonresponders (N = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (yr)</td>
<td>73 (32-88)</td>
<td>72 (47-84)</td>
</tr>
<tr>
<td>Disease duration in months</td>
<td>11 (1-120)</td>
<td>21 (2-108)</td>
</tr>
<tr>
<td>FAB subtypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA/RARS</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>RAEB/RAEB-T</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Prior RBC transfusion requirements in U/mo</td>
<td>1.3 (0-6)</td>
<td>2.5 (0-6)</td>
</tr>
<tr>
<td>Reticulocytes x 10^9/L</td>
<td>33.1 (2.8-131.2)</td>
<td>21.3 (2.5-66)*</td>
</tr>
<tr>
<td>Neutrophils x 10^9/L</td>
<td>2,162 (136-6,110)</td>
<td>1,449 (200-7,885)</td>
</tr>
<tr>
<td>Platelets x 10^9/L</td>
<td>250 (22-740)</td>
<td>176 (24-566)</td>
</tr>
<tr>
<td>Serum EPO in U/L</td>
<td>120 (6-948)</td>
<td>615 (85-3,010)†</td>
</tr>
<tr>
<td>REDI</td>
<td>3.3 (0.7-28.7)</td>
<td>3.2 (0.2-11.7)</td>
</tr>
<tr>
<td>RMDI</td>
<td>10.1 (1-17.4)</td>
<td>4.8 (0.8-62.3)</td>
</tr>
</tbody>
</table>

Cytogenetics (abnormal/normal) 4/15 10/9*

*Unless otherwise indicated, ranges are shown in parentheses.

* P < .05.
† P = .001.
‡ Orthochromatic + polychromatic normoblasts/basophilic + pro-normoblasts.
§ Neutrophils + bands + metamyelocytes/promyelocytes + myeloblasts.

DISCUSSION

Patients with MDS often have chronic cytopenias resulting in frequent infections and the need for transfusion products. Prior studies have shown that the majority of patients who are treated with G-CSF or granulocyte-macrophage CSF have neutrophil response.49 This response can be maintained over many months.12

r-HuEPO has also been widely studied for the treatment of the anemia associated with MDS. In a series of studies with a variety of doses and schedules, response rates have varied between 10% to 28%.18-26 The association of responsiveness to EPO treatment with serum EPO levels and FAB subtypes were shown.25,26 In our previous studies, G-CSF treatment led to partial erythroid responses in a small subset of patients,11,12 although none of those patients achieved responses that would be categorized as a GR by the current criteria. Other studies with G-CSF in MDS patients have not shown erythroid responses.13 In vitro synergy has been shown for the stimulation of erythroid progenitors using both G-CSF and EPO,27,28 which led to the current study design. In our previous report, the proportion of erythroid responses appeared to be increased in MDS patients who were treated with G-CSF and EPO28 as compared with that reported in patients treated with EPO alone.18-26 In the current study, we have extended this study and included a larger number of patients to determine whether both G-CSF and EPO are required for maintenance of an erythroid response.

In this study, 48% of evaluable MDS patients had erythroid responses to the combination of G-CSF and EPO, the majority of whom had this response persist for over at least the initial 2-month period. In all patients except 1 the dose of EPO that was required for an erythroid response was high (300 U/kg/d) as compared with that required for erythroid

Fig 2. Comparison of pretreatment serum EPO levels in patients who had erythroid responses (responders) or who lacked such responses (non-responders).

Table 3. Erythroid Responses to Recombinant Human G-CSF Plus EPO Treatment in MDS

<table>
<thead>
<tr>
<th>FAB Subgroup</th>
<th>GR</th>
<th>PR</th>
<th>NR</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>RAARS</td>
<td>6</td>
<td>4</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>RAEB</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>RAEB-T</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>23</td>
<td>44 (48)</td>
</tr>
</tbody>
</table>

Abbreviation: NR, no response.
responses in patients with renal failure. The relatively high dose of EPO required for a response in MDS patients has implications with respect to cost when used in this clinical setting outside a clinical trial. Although in this study we have used daily dosing of both cytokines, the optimal schedule is not known because other schedules have not been systematically investigated. By study design, the G-CSF was discontinued and approximately half of the patients responded to EPO alone, whereas the majority of the other patients required both G-CSF and EPO to maintain an erythroid response. This was particularly evident in the RARS subtype of patients, as previously suggested by a study reviewing EPO responses in MDS. These findings and the resumption of erythroid responses when G-CSF was readministered in 7 of 8 evaluable patients who lost their erythroid responses while receiving EPO alone supports the concept of in vivo synergy between these agents for RBC production in MDS patients.

Previous studies with EPO alone generally did not determine the response duration but were predominantly concerned with the response rate. However, the clinical utility of growth factor therapy in MDS depends on the response duration as well as the response rate. In our study the median duration of maintenance of the erythroid responses (beyond the initial 2-month acute response period) was 11 months, with a substantial percentage of patients (35%; ie, 6 of the 17 initial erythroid responders) having relatively prolonged and durable responses for 15 to 36 months. The patients who required both growth factors tended to have a shorter median duration of response compared with that of the patients who responded to EPO alone, although this difference was not statistically significant.

A number of clinical criteria were evaluated in an effort to predict which patients would respond to this form of therapy. Patients responding to G-CSF and EPO had significantly lower serum EPO levels (particularly, <500 U/L), higher absolute reticulocyte counts, and normal cytogenetics at study entry. It is likely that the increased reticulocyte count reflects more effective erythropoiesis because no patients had evidence for hemolysis. Although these clinical features may help predict possible responses, these criteria were not exclusive because some degree of overlap was found among responding and nonresponding patients. Because the cost of combined G-CSF plus EPO therapy outside of a clinical trial is substantial, the identification of clinical characteristics that will predict an erythroid response to such combined therapy is clearly relevant.

The combined therapy was generally well tolerated, with bone or splenic pain reported in a minority of patients. Patients were able to be treated on an outpatient basis with minimal side effects. These results indicated that a significant proportion of MDS patients had both myeloid and erythroid responses to combined G-CSF and EPO treatment that persisted for relatively prolonged periods, and approximately half of these patients required both growth factors for maintaining an erythroid response. However, the definitive role of the combined use of G-CSF and EPO treatment will require a randomized clinical trial comparing this combination with EPO alone.

ACKNOWLEDGMENT

The secretarial assistance of Vibeke Cleaver and Sara Clark and the assistance of Dr Ruby Wong (Stanford University), who performed some of the statistical analysis, are greatly appreciated.

REFERENCES


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Table 4. Erythroid Responses at Different Stages of Treatment With Recombinant Human G-CSF Plus EPO

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Erythroid Responders (%)</th>
<th>Erythroid Nonresponders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF plus EPO</td>
<td>21 (48)</td>
<td>23 (52)</td>
</tr>
<tr>
<td>Maintenance phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF plus EPO</td>
<td>17 (81)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>EPO only</td>
<td>8</td>
<td>9*</td>
</tr>
<tr>
<td>G-CSF plus EPO</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

* One patient not evaluable.

![Fig 3. Response duration for patients having erythroid responses to initial administration of recombinant human G-CSF plus EPO. Tick marks indicate patients continuing to respond.](image-url)


Maintenance treatment of the anemia of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor and erythropoietin: evidence for in vivo synergy

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