Subcutaneous Erythropoietin for Treatment of Refractory Anemia in Hematologic Disorders. Results of a Phase I/II Clinical Trial

By Mario Cazzola, Luisa Ponchio, Yves Beguin, Vittorio Rosti, Gaetano Bergamaschi, Nicola L. Liberato, Vittorio Fregoni, Giulio Nalli, Giovanni Barosi, and Edoardo Ascari

We have used recombinant human erythropoietin (rHuEPO) in a phase I/II clinical trial to evaluate its ability to reverse refractory anemia in hematologic disorders. rHuEPO was administered subcutaneously 5 days per week at escalating doses (50 to 150 U/kg per day). The aim of treatment was a hemoglobin (Hb) level ≥10 g/dL without blood transfusion. Of 25 patients treated, 17 were evaluable, most of them with a regular need for transfusion. Eight of these had lymphoproliferative disorders (three cases of malignant lymphoma and five of monoclonal gammopathy) and were exposed to cytotoxic therapy. The other nine patients had hematopoietic stem cell disorders (four cases of myelodysplastic syndrome, three of idiopathic myelofibrosis, and two of chronic myelogenous leukemia). All patients with lymphoproliferative disorder had serum EPO levels inappropriately low for the degree of anemia, while patients with stem cell disorder showed variable values. Erythroid marrow activity was inadequate in all cases. Seven of eight patients with lymphoproliferative disorder responded to treatment maintaining Hb above 10 g/dL without transfusion. The median dose of rHuEPO required for correction of anemia was 75 U/kg. In four cases response was maintained with 50 U/kg, three times per week. There was no complete response among patients with hematopoietic stem cell disorder, although transfusion requirement was eliminated or reduced in four cases. Four patients developed functional iron deficiency during rHuEPO treatment and required iron supplementation to obtain response. Aggravation of splenomegaly was observed in two cases of myeloproliferative disorder. We conclude that: (1) subcutaneous administration of rHuEPO can be effective and safe in patients with lymphoproliferative disorder exposed to chemotherapy and showing inappropriate EPO response to anemia; (2) this is less likely in hematopoietic stem cell disorders, although favorable responses may be observed in occasional patients; and (3) functional iron deficiency as a cause of nonresponse to rHuEPO is frequent also in nonrenal anemia.

© 1992 by The American Society of Hematology.

Patients and Methods

This study was approved by the ethical committee of the Department of Internal Medicine and Medical Therapy of the University of Pavia. The clinical trial protocol was a nonrandomized, escalating-dose regimen designed to investigate the efficacy and safety of rHuEPO for treatment of anemia in lymphoproliferative disorders and hematopoietic stem cell disorders. Patients were followed at the Clinica Medica 2, Department of Internal Medicine and Medical Therapy, University of Pavia, Pavia, and at the Department of Medicine, Ospedale di Bormio e Sondalo, Sondalo, Italy.

Subjects of the study. The 25 subjects who entered the study fulfilled the following major criteria: (1) age ≥18 years; (2) hemoglobin (Hb) value <10 g/dL, without or with transfusional requirement; (3) no evidence of additional cause for anemia apart from the basic disorder. No restriction was applied with respect to life expectancy, so that also subjects with advanced disease were enrolled. All patients gave written informed consent to participation in the trial.

EPO. The rHuEPO β used in the study was provided by Boehringer Mannheim Italia S.p.A., Monza, Italy.

Treatment with rHuEPO. rHuEPO was administered once daily subcutaneously for 5 days per week (Monday to Friday), on an outpatient basis whenever possible.

The aim of treatment was a Hb level ≥10 g/dL without transfusion, and this was defined as complete response. Elimination of transfusion requirement with Hb levels <10 g/dL, or reduction in transfusion requirement were both defined as partial response.

From the Department of Internal Medicine and Medical Therapy, University of Pavia and IRCCS Policlinico S. Matteo, Pavia; the Department of Medicine, Ospedale di Bormio e Sondalo, Sondalo, Italy; and the Department of Medicine, Division of Hematology, University of Liège, Liège, Belgium.

Submitted June 14, 1991; accepted September 3, 1991.

Supported by grants from AIRC (Associazione Italiana per la Ricerca sul Cancro) and CNR (Consiglio Nazionale delle Ricerche) to Mario Cazzola, and by Grants No. 3.4513.88 and 3.4555.91 from FRSB (Fonds de la Recherche Scientifique Médicale, Belgium) to Yves Beguin, and by Boehringer Mannheim Italia.

Address reprint requests to Mario Cazzola, MD, Clinica Medica 2, Policlinico S. Matteo, 27100 Pavia, Italy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1992 by The American Society of Hematology.

0006-4971/92/7901-0014$3.00/0
The initial dose of rHuEPO was 50 units per kilogram of body weight. If complete response was not achieved within 4 weeks, dose was increased to 75 units per kilogram. Using the same criterion, escalations to 100 and 150 units per kilogram were performed at 4-week intervals. If there was no response within 16 weeks, rHuEPO was increased to 75 units per kilogram. Using the same criterion, dose of rHuEPO was adjusted so that Hb concentration was maintained at about 11 g/dL. Adjustment was performed on both dose and number of weekly administrations.

Blood counts. Complete blood counts were performed weekly. Reticulocyte count was corrected to account for anemia.

Body iron status and iron supplementation during the study. Body iron status was evaluated by measuring serum iron, TIBC, and serum ferritin. Oral iron supplements (80 to 100 mg/d) were commenced if at least one of the following events occurred: (1) serum iron <50 μg/dL; (2) transferrin saturation <20%; (3) serum ferritin <100 μg/L.

Serum EPO assay. Circulating EPO levels were measured by a commercially available radioimmunoassay (Incstar Corp, Stillwater, MN), which uses rHuEPO for tracer and standards. To define EPO levels appropriate or inappropriate for a given degree of anemia, the exponential regression of serum EPO versus hematocrit (Hct) was determined in reference subjects (102 normal individuals or patients with iron deficiency anemia, hemolytic anemia, or hypoplastic anemia) and the 95% confidence limits were defined. For Hct values <38%, the regression equation was: log(EPO) = 4.746 - (0.093 × Hct). For Hct values >38%, the regression equation was: log(EPO) = 1.381 - (0.005 × Hct). Based on these equations, the observed/predicted log(EPO) ratio (O/P ratio) was derived for each sample. The O/P ratio averaged 1.00 ± 0.11 in reference subjects (95% confidence interval: 0.80 to 1.19).

Measurement of serum transferrin receptor (TfR). The amount of serum TfR was estimated by an enzyme-linked polyclonal antibody assay, using purified placental receptor-transferrin complexes as a reference standard and rabbit antibodies, as described in detail elsewhere. TfR levels in 165 normal control subjects were 5,000 ± 1,100 μg/L, 95% confidence limits ranging from 2,900 to 7,100 μg/L.

Because serum TfR provides an estimate of erythroid marrow activity, it was used in a functional classification of anemia, whose rationale is described in detail elsewhere. Erythroid activity was determined as the ratio between the patient’s serum TfR and the mean normal value. An erythroid activity equal to or greater than three times normal was considered as adequate marrow response to anemia. Values below the normal range in the presence of anemia were defined as absolute marrow failure, whereas values from normal to three times normal were defined relative marrow failure.

Data analysis and presentation. Data were stored, analyzed, and reported with the packages Exstatix (Select Micro Systems Inc, Yorktown Heights, NY), and DeltaGraph (DeltaPoint Inc, Monterey, CA), both run on a Macintosh SE/30 (Apple Computer Inc, Cupertino, CA) personal computer.

RESULTS

Twenty-five patients entered the clinical trial. Of these, only 17 completed the study, showed adequate compliance, and were considered evaluable. Eight subjects did not complete the study.

Withdrawals. Reasons for withdrawals are reported in Table 1. Of the eight withdrawals, five were due to disease progression responsible for the patient’s death (case no. 1 to no. 4) or leukemic transformation (case no. 5). Three other patients were withdrawn by investigators: a patient with idiopathic myelofibrosis showed aggravation of splenomegaly during treatment, with spleen size returning to basal values after discontinuation of rHuEPO; a woman with myelodysplastic syndrome showed elevation of serum transaminases, whose levels returned normal after rHuEPO

Table 1. Reasons for Withdrawals

<table>
<thead>
<tr>
<th>Patient, No., Sex, Age (yr)</th>
<th>Condition</th>
<th>Reason of Withdrawal and Comment on Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, F, 58</td>
<td>Multiple myeloma, clinical stage III, refractory to chemotherapy, high transfusion requirement</td>
<td>Died of progressive disease after 6 wk of treatment. No evidence of response to rHuEPO</td>
</tr>
<tr>
<td>2, F, 75</td>
<td>Multiple myeloma, clinical stage III, high transfusion requirement</td>
<td>Died of renal failure after 3 wk of treatment. Showed reticulocytosis</td>
</tr>
<tr>
<td>3, M, 46</td>
<td>Waldenström’s macroglobulinemia, transfusion requirement</td>
<td>Died of progressive disease after 8 wk of treatment. Showed reticulocytosis</td>
</tr>
<tr>
<td>4, F, 43</td>
<td>Non-Hodgkin lymphoma, refractory to radiotherapy and chemotherapy, transfusion requirement</td>
<td>Died of progressive disease after 5 wk of treatment. No evidence of response</td>
</tr>
<tr>
<td>5, M, 62</td>
<td>Myelodysplastic syndrome (refractory anemia with excess of blasts in transformation), transfusion requirement</td>
<td>Progressed to AML during the second month, and rHuEPO was stopped. No evidence of response</td>
</tr>
<tr>
<td>6, M, 69</td>
<td>Idiopathic myelofibrosis, transfusion requirement</td>
<td>rHuEPO was stopped due to aggravation of splenomegaly after 4 wk of treatment. No evidence of response</td>
</tr>
<tr>
<td>7, M, 63</td>
<td>Multiple myeloma, clinical stage III, pancytopenia and transfusion requirement</td>
<td>rHuEPO was stopped after 3 wk of treatment because of severe epistaxes (platelet count was 13 × 10⁵/L). Showed reticulocytosis</td>
</tr>
<tr>
<td>8, F, 70</td>
<td>Myelodysplastic syndrome (refractory anemia, transfusion requirement</td>
<td>Elevation of serum AST and ALT; levels returned normal after discontinuation of rHuEPO. No evidence of response in 6 wk</td>
</tr>
</tbody>
</table>
was stopped; and in another patient (multiple myeloma),
treatment was discontinued because of the appearance of
severe epistaxes, likely due to the pre-existing thrombocyto-
penia.

**Evaluative patients.** Seventeen patients had data suitable
for analysis. Eight of them had lymphoproliferative disor-
ders (three cases of malignant lymphoma and five of
monoclonal gammapathy, see Table 2). The other nine
patients had hematopoietic stem cell disorders (four cases
of myelodysplastic syndrome, three cases of idiopathic
myelofibrosis, and two cases of chronic myelogenous leuke-
mia, see Table 3).

**Appropriateness of EPO response to anemia and erythroid
marrow activity.** As shown in Fig 1, 10 of 17 patients had
values for serum EPO inappropriately low for the degree of
anemia, and other four patients had borderline values. In
particular, all patient with lymphoma or monoclonal gam-
mapathy showed low EPO levels. The most defective values
were found in the three patients with renal failure (arrowed
symbols in Fig 1; patients no. 7 and 8 in Table 2 and patient
no. 1 in Table 3).

Additional information was provided by the ratio of
observed-to-predicted EPO levels. As shown in Fig 2, all
patients with lymphoma or monoclonal gammapathy had
O/P ratios below the reference range, whereas all but two
patients with hematopoietic stem cell disorder had values
within the reference range.

In all the patients studied, erythroid marrow activity
ranged from 0.3 to 2 times normal, without any significant
difference between lymphoproliferative disorders and hema-
topoietic stem cell disorders (Fig 3). Thus, erythroid activity
was inappropriately low for the degree of anemia, indicat-
ing that the marrow response to long-lasting anemia was
inadequate in all cases.11,12

**Erythroid response to rHuEPO and body iron status.** As
reported in Table 2, seven of eight patients with lymphoma
or monoclonal gammapathy showed a complete response to
rHuEPO, eliminating the need for transfusion and maintain-
ing Hb levels greater than 10 g/dL. The median rHuEPO
dose required for correcting anemia was 75 U/kg. The only
patient who did not respond was a woman with refractory
IgM multiple myeloma: she had been heavily exposed to
cytotoxic therapy and needed several plasmaphereses dur-
ing rHuEPO administration for control of a severe hypervis-
cosity syndrome. It is interesting to note that erythroid
response was associated with an improvement in the basic
disorder in only one case (no. 3 in Table 2); the remaining
patients showed an erythropoietic response despite a stable
or even worsening basic condition.

Three of the seven responders developed functional or
relative iron deficiency, ie, a combination of low serum iron
(<50 µg/dL), low transferrin saturation (<20%), and
normal to high serum ferritin. In these three cases oral iron
supplementation resulted in a further increase in Hb
concentration, as shown by the example reported in Fig 4.

In four cases, response was maintained with a rHuEPO
dose of 50 U/kg, three times weekly, for periods ranging
from 4+ to 9+ months.

None of the nine patients with hematopoietic stem cell
disorder showed a complete response to treatment (Table
3). Partial responses were observed in cases no. 3, 6, 8, and
9: this latter case is reported in Fig 5. With the only
exception of case no. 6, responses were transient. Patient
no. 6 (idiopathic myelofibrosis) developed functional iron
deficiency and required iron supplementation to obtain
response. This case was peculiar also in that the partial
response was maintained after rHuEPO was stopped;
likely, chemotherapy was responsible for worsening of
anemia before treatment with rHuEPO.

**Response predictive factors and response indicators.** All
patients showing a complete response to rHuEPO had serum EPO levels inappropriately low for the degree of
anemia and O/P ratios below the reference range. There
was no relationship between response and erythroid mar-
row activity (Spearman R .12, P > .05).

All responding patients showed an increase in reticulo-
cyte count and serum transferrin receptor, and a decrease
in serum iron and serum ferritin (examples are reported in
Figs 4 and 5). Circulating transferrin receptor appeared to
be the earliest and most reliable predictor of response. In
the group of lymphoproliferative disorders, all seven re-
sponders showed a consistent increase in its level after
2 weeks of treatment. The maximum increase in circulating
transferrin receptor ranged from 35% to 200% (median
value: 133%). Despite these percentage changes, maximum
values ranged from normal to about three times basal, so
that in no case erythroid response increased into the
“adequate range” of Fig 3.

Nonresponders showed no significant increase in serum
transferrin receptor level both at 2 weeks and later in the
course of treatment. However, there were two exceptions: a
patient with idiopathic refractory anemia and a patient with
idiopathic myelofibrosis (cases no. 1 and no. 5 in Table 3).
In these two cases, there was a consistent increase in
circulating transferrin receptor with marginal elevations in
reticulocyte count and no change in transfusion require-
ment, suggesting that rHuEPO essentially stimulated inef-
fective erythropoiesis.

**Adverse effects.** Two patients were withdrawn from study
because of possible adverse effects: aggravation of spleno-
megaly in a patient with idiopathic myelofibrosis (case no. 6
in Table 1) and elevation of serum transaminases in a
patient with myelodysplastic syndrome (case no. 8 in Table
1). We also noticed a splenic infarction in a patient with
chronic myelogenous leukemia (case no. 8 in Table 3):
however, this woman had had a splenic infarction a few
months before, during progression from chronic to acceler-
ated phase of chronic myelogenous leukemia (CML). rHuEPO
was discontinued for 1 week, and no adverse
effect was observed after restarting it with the patient’s
consent.

**DISCUSSION**

The potency of rHuEPO has not gone unnoticed outside
nephrology.15 Preliminary reports indicate that EPO can
correct the anemia associated with malignant lymphoma or
multiple myeloma,16,17 whereas it is probably less effective in
Table 2. Clinical Data and Response to rHuEPO in the Eight Patients With Malignant Lymphoma or Monoclonal Gammapathy

<table>
<thead>
<tr>
<th>Patient No., Sex, Age (yr)</th>
<th>Condition</th>
<th>Hb (g/dL)</th>
<th>Transfusion Requirement (U/mo)</th>
<th>Response Pattern</th>
<th>Management Issues, Adverse Effects, and Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M, 45</td>
<td>Malignant lymphoma (WF:E, diffuse, small cleaved), clinical stage IV (heavy marrow involvement), at clinical onset and under chemotherapy</td>
<td>7.8</td>
<td>Occasional</td>
<td>Complete response, obtained with 150 U/kg of rHuEPO. Peak Hb: 12.8 g/dL</td>
<td>Treatment induced functional iron deficiency and response required iron supplementation (see Fig 4). Hb level is now maintained between 11 and 12 g/dL with 3,000 U of rHuEPO (50 U/kg) administered 3 times weekly (duration of response: 8+ mo)</td>
</tr>
<tr>
<td>2, M, 66</td>
<td>Malignant lymphoma (WF:H, large cell immunoblastic) in relapse. No bone marrow involvement; worsening of anemia with chemotherapy</td>
<td>8.8</td>
<td>2</td>
<td>Complete response, obtained with 50 U/kg of rHuEPO. Peak Hb: 12.1 g/dL</td>
<td>Hb level was maintained above 10 g/dL with 3,000 U of rHuEPO 3 times weekly. Did not respond to chemotherapy and died of progressive disease: response was maintained for 3 mo</td>
</tr>
<tr>
<td>3, F, 33</td>
<td>Malignant lymphoma (large cell, B with sclerosis) clinical stage IV (no marrow involvement). Worsening of anemia with chemotherapy</td>
<td>8.0</td>
<td>0</td>
<td>Complete response, obtained with 75 U/kg of rHuEPO. Peak Hb: 15.5 g/dL</td>
<td>Treatment induced functional iron deficiency, and response required oral iron supplementation. Complete remission was obtained with MACOP-B, and Hb level remained normal after rHuEPO was discontinued</td>
</tr>
<tr>
<td>4, M, 52</td>
<td>Multiple myeloma in relapse. Worsening of anemia with chemotherapy</td>
<td>8.1</td>
<td>6</td>
<td>Complete response, obtained with 75 U/kg of rHuEPO. Peak Hb: 11.0 g/dL</td>
<td>Hb was maintained between 10 and 11 g/dL with the same dose of rHuEPO. Patient died of progressive disease; response was maintained for 4 mo</td>
</tr>
<tr>
<td>5, M, 55</td>
<td>Multiple myeloma in relapse, under chemotherapy</td>
<td>7.7</td>
<td>2</td>
<td>Complete response, obtained with 100 U/kg of rHuEPO. Peak Hb: 14.7 g/dL</td>
<td>At the peak Hb level of 14.7 g/dL rHuEPO was discontinued. Hb fell to 10.4 g/dL; rHuEPO was restarted and a new response has now been obtained (duration of response: 9+ mo)</td>
</tr>
<tr>
<td>6, F, 66</td>
<td>Multiple myeloma in relapse, under chemotherapy</td>
<td>7.2</td>
<td>Occasional</td>
<td>No response</td>
<td>rHuEPO induced a sustained reticulocytosis but there was no stable increase in Hb. This patient underwent frequent plasmaphereses due to hyperviscosity syndrome</td>
</tr>
<tr>
<td>7, F, 69</td>
<td>Multiple myeloma in relapse, under chemotherapy</td>
<td>7.5</td>
<td>4</td>
<td>Complete response, obtained with 50 U/kg of rHuEPO. Peak Hb: 11.7 g/dL</td>
<td>This patient had renal failure (creatinine: 3.50 mg/dL). Response maintained for 4+ mo with 3 weekly administrations (50 U/kg)</td>
</tr>
<tr>
<td>8, M, 57</td>
<td>AL amyloidosis, under chemotherapy</td>
<td>8.9</td>
<td>3</td>
<td>Complete response, obtained with 75 U/kg of rHuEPO. Peak Hb: 12.8 g/dL</td>
<td>This patient had renal failure (creatinine: 2.30 mg/dL). Response required iron supplementation due to the appearance of functional iron deficiency, and is maintained with 3 week administrations (50 U/kg) (duration of response: 7+ mo)</td>
</tr>
</tbody>
</table>
Table 3. Clinical Data and Response to rHuEPO in the Nine Patients With Hematopoietic Stem Cell Disorders

<table>
<thead>
<tr>
<th>Patient No., Sex, Age</th>
<th>Condition</th>
<th>Hb (g/dL)</th>
<th>Transfusion Requirement (U/mo)</th>
<th>Response Pattern</th>
<th>Management Issues, Adverse Effects, and Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M, 80</td>
<td>Myelodysplastic syndrome (refractory anemia with ring sideroblasts). Transfusional hemochromatosis</td>
<td>6.2</td>
<td>8</td>
<td>No response</td>
<td>This patient had mild renal failure (creatinine: 2.7 mg/dL)</td>
</tr>
<tr>
<td>2, M, 58</td>
<td>Myelodysplastic syndrome (refractory anemia with excess of blasts)</td>
<td>8.4</td>
<td>4</td>
<td>No response</td>
<td>Percentage of bone marrow blasts was stable</td>
</tr>
<tr>
<td>3, F, 68</td>
<td>Myelodysplastic syndrome (refractory anemia)</td>
<td>7.5</td>
<td>2</td>
<td>Partial response (Hb maintained between 9 and 10 g/dL without transfusion for 3 mo)</td>
<td>Reappearance of transfusion requirement after rHuEPO was discontinued</td>
</tr>
<tr>
<td>4, M, 44</td>
<td>Myelodysplastic syndrome (refractory anemia)</td>
<td>6.4</td>
<td>8</td>
<td>No response</td>
<td>Stable disease</td>
</tr>
<tr>
<td>5, M, 56</td>
<td>Idiopathic myelofibrosis</td>
<td>8.2</td>
<td>6</td>
<td>No response</td>
<td>No change in spleen size Treatment included functional iron deficiency and response required oral iron supplementation. rHuEPO was tapered off, and Hb is stable between 9 and 10 g/dL 5 mo after rHuEPO was stopped. Hydroxyurea had been stopped one month before initiation of rHuEPO treatment</td>
</tr>
<tr>
<td>6, F, 57</td>
<td>Idiopathic myelofibrosis, treated with hydroxyurea for thrombocytosis (this produced prolonged myelosuppression)</td>
<td>6.5</td>
<td>2</td>
<td>Partial response (elimination of transfusion requirement) Peak Hb: 9.6 g/dL</td>
<td>Stable disease</td>
</tr>
<tr>
<td>7, M, 65</td>
<td>Idiopathic myelofibrosis</td>
<td>7.0</td>
<td>6</td>
<td>No response</td>
<td>No change in spleen size Spleenic infarction during the second week of treatment (there had been a previous one before treatment). rHuEPO was continued and spleen size returned to basal. There was no apparent effect on peripheral and marrow blast counts</td>
</tr>
<tr>
<td>8, F, 63</td>
<td>Chronic myelogenous leukemia in accelerated phase, treated with hydroxyurea and 6-mercaptopurine</td>
<td>8.8</td>
<td>6</td>
<td>Reduction in transfusion requirement (from 6 to 2 U/mo) for 2 mo</td>
<td>Stable disease</td>
</tr>
<tr>
<td>9, F, 73</td>
<td>Chronic myelogenous leukemia with worsening anemia</td>
<td>7.6</td>
<td>5</td>
<td>Elimination of transfusion requirement for 3 mo (peak Hb 9.8 g/dL, see Fig 5)</td>
<td>Stable disease</td>
</tr>
</tbody>
</table>

the treatment of the anemia associated with hematopoietic stem cell disorders.16-22

In the present clinical study, we tried to define the abnormalities in erythron function and to correlate them with the effects of rHuEPO. EPO production was quantitated by serum levels and erythropoiesis was evaluated by the determination of serum transferrin receptor and reticulocyte count. Serum EPO level is useful in investigating whether defective EPO production contributes to anemia.4,13 Circulating transferrin receptor is derived primarily from erythroid precursors in the bone marrow, and its level provides a reliable measurement of total erythroid activity.14,23 The corrected reticulocyte count is an index of RBC production, ie, of effective erythropoiesis.10

Instead of only quantitating serum EPO levels in absolute terms, we evaluated them in relation to the degree of anemia. Serum EPO levels were inappropriately low for the degree of anemia in more than half of the patients studied (Fig 1). All patients with malignant lymphoma or multiple myeloma showed a markedly defective production as indicated by the low O/P ratios (Fig 2). These patients had been exposed previously, and were exposed during the study to cytotoxic therapy. This is a factor potentially responsible for defective EPO production. It has been shown, in fact, that chemotherapy can blunt the normal exponential increase in circulating EPO that occurs with anemia.25 In this respect, it is notable that borderline O/P ratios (0.78 and 0.82) were found in the two patients with chronic myelogenous leukemia under chemotherapy. As already mentioned, EPO production may be suppressed also by an excessive release of cytokines such as IL-1 and TNF. The blunted EPO response, however, is unlikely to be the only factor responsible for anemia in the above conditions. Erythroid marrow can be suppressed directly by inhibitory cytokines, and by chemotherapy or radiotherapy themselves.

Patients with hematopoietic stem cell disorders showed variable EPO responses, more often within the normal
Fig 1. Relationship between serum EPO levels and Hct in the patients studied. Reference subjects are represented by their 95% confidence limits (dashed lines). The three patients arrowed had mild degrees of renal failure (see Tables 2 and 3). The two patients with high EPO levels were studied at the Sondalo Hospital (1,200 m above sea level).

range (Fig 2). This agrees with results of a study on myelodysplastic syndromes. Despite of an appropriate EPO production, however, erythroid marrow activity was inadequate in all subjects, and this is due to the intrinsic stem cell defect.

While planning this study, the subcutaneous route for rHuEPO administration was chosen for convenience. In addition, a number of studies have shown that subcutaneous injections provide more sustained plasma levels, suggesting that this route could be also more effective.

There was a substantial difference in response to rHuEPO between patients with malignant lymphoma or monoclonal gammapathy and those with hematopoietic stem cell disorders. The first ones responded to treatment and maintained Hb values above 10 g/dL, whereas the second ones showed only partial or transient responses. Such a difference was evident also when considering the three patients with mild degrees of renal failure: the two patients with monoclonal gammapathy (nos. 7 and 8 in Table 2) showed prompt responses with low doses of rHuEPO, whereas the patient with sideroblastic anemia (no. 1 in Table 3) did not respond.

One reason for the above difference is undoubtedly the different degree of EPO response to anemia. According to Erslev, patients with values < 100 mU/mL are very likely to respond to rHuEPO, those with levels from 100 to 500 mU/mL would require higher doses, and those with values > 500 mU/mL are very unlikely to have any beneficial effect. All responding patients in this study had values lower than 100 mU/mL, so that defective EPO production likely contributed to their impaired RBC production.

However, additional factors must influence the response to rHuEPO, because patients with hematopoietic stem cell disorder and defective EPO production did not respond to treatment. Subjects with malignant lymphoma or monoclonal gammapathy have residual normal stem cells that sustain erythropoiesis, and erythroid progenitors can be stimulated by pharmacologic doses of EPO. In contrast, hematopoiesis is clonal in patients with stem cell disorders, and the erythroid cells belong to the abnormal clone. The proliferative potential of the most immature erythroid progenitors and their sensitivity to growth factors are reduced in myelodysplastic syndromes and cannot be

Fig 2. Ratio of observed-to-predicted EPO levels in patients with malignant lymphoma or monoclonal gammapathy, and those with hematopoietic stem cell disorders. The upper and lower limit of the reference range (95% confidence range) are indicated by dashed lines. The three patients arrowed had mild degrees of renal failure. Analysis of variance showed that there was a significant difference between the two groups (P = .039).
with rHuEPO may be of limited benefit in these subjects. In fact, clinical trials have shown that only about 10% of patients with myelodysplastic syndrome respond to rHuEPO with a stable increase in Hb, while another 10% show just a reduction in transfusion requirement. Responding patients have lower EPO levels and, probably, erythroid progenitors still responsive to the growth factor; therefore, determining serum EPO and assaying circulating erythroid progenitors might be useful in selecting patients for rHuEPO treatment.

**Fig 3.** Erythroid marrow activity in the patients studied. Erythroid activity was determined as the ratio between the patient's serum transferrin receptor and the mean normal value. An erythroid activity equal to or greater than three times normal was considered as adequate marrow response to anemia. Values below the normal range (ie, below the lower 95% confidence limit) were defined as absolute marrow failure, whereas values from normal to three times normal were defined relative marrow failure. The rationale of this functional classification of anemia has been described in detail elsewhere.

Corrected by saturating rHuEPO concentrations in vitro. Moreover, RBC production is variably ineffective in both myelodysplastic syndromes and myeloproliferative disorders. These observations would suggest that treatment...
Few adverse effects were observed in this study. However, aggravation of splenomegaly in myeloproliferative disorders needs to be underlined because two similar cases have been recently reported.31 Stimulation of extramedullary erythropoiesis32 likely plays a role.

A management issue to remark is the high frequency of functional iron deficiency associated with rHuEPO treatment. Erythropoietic response can be blunted by an impaired iron supply to the erythroid marrow.32 This condition is characterized by a reduced serum iron (<50 μg/dL) and transferrin saturation (<20%). Serum ferritin is less reliable for this purpose since it primarily reflects storage iron.32 Functional iron deficiency might be detected also by the measurement of serum transferrin receptor34: an increase in serum transferrin receptor with a stable Hb may indicate that the iron demand exceeds the ability of the reticuloendothelial system to release iron to transferrin. The elevation in serum transferrin receptor in patients with iron deficiency is related to an increased transferrin receptor number on individual erythroblasts rather than to an increase in erythroid cellularity.35 As a practical conclusion, we suggest to give oral iron supplementation routinely to all patients treated with rHuEPO who do not have iron overload. It can be anticipated that some patients who do not respond to oral iron might have beneficial effects from intravenous iron.36

The final question is cost. In Italy, the price of one unit of RBCs is about $75, but the cost for the community may be as high as $200 if one accounts for all the costs related to blood donation and transfusion. The price of 1,000 U of rHuEPO is about $14 for hospital pharmacies. A transfusion requirement of 4 U of blood per month would cost the community $9,000 to $10,000 per annum, excluding transfusion complications. Should such a patient respond to rHuEPO, 75 U/kg five times weekly, and be maintained with 50 U/kg three times weekly, the cost would be $8,500 per annum. Under these circumstances rHuEPO would be also cost effective.

In conclusion, subcutaneous administration of rHuEPO can be effective and safe in patients with lymphoproliferative disorder exposed to chemotherapy and showing inappropriate EPO response to anemia. Phase III clinical trials are required to better define the impact of this treatment on the clinical course of these conditions.

ACKNOWLEDGMENT

The authors thank Boehringer Mannheim Italia for provisions of the rHuEPO and for support; Dr Vittorio Battistel and Dr Lorella Colombi of Boehringer Mannheim Italia S.p.A. for helpful liaison throughout the study; and all patients who volunteered for the study.

REFERENCES

2. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW: Correction of the anemia of end-stage renal disease with...
Subcutaneous erythropoietin for treatment of refractory anemia in hematologic disorders. Results of a phase I/II clinical trial [see comments]

M Cazzola, L Ponchio, Y Beguin, V Rosti, G Bergamaschi, NL Liberato, V Fregoni, G Nalli, G Barosi and E Ascari

Updated information and services can be found at:
http://www.bloodjournal.org/content/79/1/29.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml