Recombinant Human Erythropoietin in the Anemia Associated With Multiple Myeloma or Non-Hodgkin's Lymphoma: Dose Finding and Identification of Predictors of Response

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Previous phase I-II clinical trials have shown that recombinant human erythropoietin (rHuEpo) can ameliorate anemia in a portion of patients with multiple myeloma (MM) and non-Hodgkin’s lymphoma (NHL). Therefore, we performed a randomized controlled multicenter study to define the optimal initial dosage and to identify predictors of response to rHuEpo. A total of 146 patients who had hemoglobin (Hb) levels ≤11 g/dL and who had no need for transfusion at the time of enrollment entered this trial. Patients were randomized to receive 1,000 U (n = 31), 2,000 U (n = 29), 5,000 U (n = 31), or 10,000 U (n = 26) of rHuEpo daily subcutaneously for 8 weeks or to receive no therapy (n = 29). Of the patients, 84 suffered from MM and 62 from low- to intermediate-grade NHL, including chronic lymphocytic leukemia; 116 of 148 (75%) received chemotherapy during the study. The mean baseline Hb level was 9.4 ± 1.0 g/dL. The median serum Epo level was 32 mU/mL, and endogenous Epo production was found to be defective in 77% of the patients, as judged by a value for the ratio of observed-to-predicted serum Epo levels (O/P ratio) of ≤0.9. An intention-to-treat analysis was performed to evaluate treatment efficacy. The median average increase in Hb levels per week was 0.04 g/dL in the control group and −0.04 (P = .57), 0.22 (P = .05), 0.43 (P = .01), and 0.58 (P = .0001) g/dL in the 1,000 U, 2,000 U, 5,000 U, and 10,000 U groups, respectively (P values versus control). The probability of response (ΔHb ≥ 2 g/dL) increased steadily and, after 8 weeks, reached 31% (2,000 U), 61% (5,000 U), and 62% (10,000 U), respectively. Regression analysis using Cox’s proportional hazard model and classification and regression tree analysis showed that serum Epo levels and the O/P ratio were the most important factors predicting response in patients receiving 5,000 or 10,000 U. Approximately three quarters of patients presenting with Epo levels inappropriately low for the degree of anemia responded to rHuEpo, whereas only one quarter of those with adequate Epo levels did so. Classification and regression tree analysis also showed that doses of 2,000 U daily were effective in patients with an average platelet count greater than 150 × 10^9/L. About 50% of these patients are expected to respond to rHuEpo. Thus, rHuEpo was safe and effective in ameliorating the anemia of MM and NHL patients who showed defective endogenous Epo production. From a practical point of view, we conclude that the decision to use rHuEpo in an individual anemic patient with MM or NHL should be based on serum Epo levels, whereas the choice of the initial dosage should be based on residual marrow function.

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A NEMIA IS THE MOST frequent hematologic abnormality seen in malignancy. Many patients have the so-called anemia of chronic disease, a condition characterized by excessive release of cytokines such as interleukin-1 and tumor necrosis factor. These peptides blunt erythropoietin (Epo) production and inhibit erythroid bone marrow (BM) proliferation.

Additional factors may contribute to anemia in patients with multiple myeloma (MM) and non-Hodgkin’s lymphoma (NHL). BM infiltration by malignant cells is the rule in the former condition and is very common in low-grade NHL. Concomitant renal failure is frequently responsible for defective Epo production in MM. Finally, chemotherapy may inhibit both erythroid BM proliferation and endogenous Epo production.

Phase I-II clinical studies have shown that recombinant human Epo (rHuEpo) can ameliorate the anemia associated with MM and NHL. This has been confirmed in a double-blind, placebo-controlled trial in anemic cancer patients with advanced disease. Overall, about 50 to 60% of patients with MM and NHL appear to respond to rHuEpo, and the duration of treatment required to obtain response varies. As are most biotechnology products, rHuEpo is an expensive drug and cost-containment is a major issue in health care policy today. As recently underlined by Spivak, a reliable means of predicting potential responders and nonresponders would be clinically useful in nonrenal applications. We undertook a randomized controlled multicenter study in anemic patients with MM or NHL to evaluate the safety and efficacy of different doses of subcutaneous (SC) rHuEpo and to identify the optimal initial dosage and predictors of response.

PATIENTS AND METHODS

Patients. This study was approved by the Ethical Committees of participating centers, and all patients enrolled gave informed con-
sent. Investigating physicians had to ensure that this clinical trial was conducted in accordance with the Helsinki Declaration (as revised) and with corresponding national rules and regulations.

Patients were eligible for the study if the following inclusion criteria were fulfilled: (1) adult patients (age ≥18 years) with low/intermediate grade NHL or MM with histologically and/or cytologically proven diagnosis; (2) anemia with hemoglobin (Hb) concentration ≤11 g/dL but with no need for transfusion; (3) life expectancy greater than 3 months; and (4) performance status of 0, 1, or 2 according to the World Health Organization scale.

The following exclusion criteria were adopted: (1) acute or chronic bleeding within 2 months of rHuEpo therapy; (2) hypertension unresponsive to treatment; (3) acute infection; (4) acute liver disease; (5) platelet count less than 20 × 10^9/L; (6) remediable causes of anemia (ie, iron deficiency, vitamin B12 or folate deficiency); (7) Coombs’ test-positive hemolytic anemia; (8) epilepsy; (9) pregnancy or lactation; (10) unreliable contraception; and (11) administration of any unregistered drug within 30 days of the first dose of the study drug.

Diagnosis and staging of MM were performed using the criteria of Durie and Salmon.15 Patients were also stratified into three risk groups using C-reactive protein and β2-microglobulin serum levels in accordance with Bataille et al.16

The Working Formulation17 was used to classify NHLs, and the Ann Arbor criteria18 were adopted as the staging system. Subjects with chronic lymphocytic leukemia (CLL) were staged according to the system of Rai et al.19 NHL patients were also stratified into three risk groups using initial serum levels of β2-microglobulin and lactate dehydrogenase in accordance with Swain et al.20

Objectives of the study. The primary objective was to compare the response (Hb increase) to four different dose regimens of rHuEpo (1,000, 2,000, 5,000, and 10,000 U daily SC for 8 weeks) in patients with low/intermediate grade NHL or MM who did not require blood transfusion with that of an untreated control group. The secondary objective was to investigate the safety of SC rHuEpo therapy in these patients.

Study design. The study was implemented as a randomized, controlled, open-dose–finding trial composed of five parallel groups, each with an 8-week treatment period. Results of phase I-II trials suggested that patients with malignant disease might require doses of rHuEpo higher than those of patients with renal failure to correct their anemia.6-11 Effective doses ranged from 100 to over 1,000 U/kg/wk. Therefore, patients were randomized to receive 1,000 U, 2,000 U, 5,000 U, or 10,000 U of rHuEpo daily SC for 8 weeks or to receive no therapy. Assuming a body weight equal to 70 kg, a daily dose of 1,000 U corresponds to 100 U/kg/wk. The rHuEpo was provided as epoetin β by Boehringer Mannheim GmbH (Mannheim, Germany).

The drug was self-administered or was administered by relatives or ambulant nurses. Therefore, the patients or relatives were trained in SC administration during the pretreatment phase. The number of weekly doses was to be reduced from 7 to 3 if an increase in Hb level greater than 2 g/dL occurred or if the Hb level reached 12.5 g/dL at two consecutive check-ups without blood transfusion. For safety reasons, rHuEpo was to be discontinued if the Hb value exceeded 13 g/dL in patients with MM or Waldenström macroglobulinemia or if it increased above 15 g/dL in patients with NHL.

The primary variable, the average weekly increase in Hb in each patient during the 8-week observation phase, was derived by linear regression analysis. Response was defined as an Hb increase ≥2 g/dL between baseline and two consecutive check-ups without blood transfusion in the previous 6 weeks.

Hb, hematoctrit (Hct), red blood cell (RBC) and reticulocyte counts were measured weekly; whereas serum iron, transferrin saturation, and serum ferritin levels were determined every 4 weeks. Oral iron supplements were commenced if serum iron and/or transferrin saturation levels decreased below the lower normal limit. Serum Epo levels (radioimmunassay; Boehringer Mannheim), safety laboratory variables, and anti-Epo antibodies were measured at the baseline and final check-up. All adverse events were documented.

Sample-size calculation was based on the assumption that a difference between the weekly Hb increase observed in the control group and that observed in each dose group, which has an extent of one standard deviation of Hb increase, could be detected with a power of 80% at a significance level of 5%. For a protocol correct analysis, the resulting sample size aimed for was 17 evaluable patients in each group. To take into account drop-outs and protocol failures, the number of members in each group was increased to 24, bringing the total sample size to 120 patients.

Evaluation of adequacy of serum Epo level. Serum Epo levels should not be quantitated in absolute terms in anemic patients but should be evaluated in relation to the degree of anemia. In fact, if the Epo-generating apparatus in the kidney is efficient, levels should increase exponentially as the Hct level decreases; consequently, serum Epo levels must be expressed in relation to Hct levels.

The definition of ‘‘inadequate Epo response to anemia’’ relies primarily on documentation of a downregulated dependence of serum Epo levels on Hct levels in comparison with reference patients.1) Accordingly, the definition leads to a mathematical comparison between the patient serum Epo value and the reference threshold values for that degree of anemia. Beguin et al4 determined the exponential regression of serum Epo levels versus Hct levels in reference subjects (normal individuals or patients with iron-deficiency anemia, hemolytic anemia, or hypoplastic anemia) and defined the 95% confidence limits. For Hct values <38%, the regression equation was log10 (serum Epo) = 4.746 - (0.093 × Hct). For Hct values greater than 38%, the regression equation was log10 (serum Epo) = 1.381 - (0.005 × Hct).

We derived an observed/predicted log10 (serum Epo) ratio (O/P ratio) for each sample; the predicted level was calculated from the above equations. The O/P ratio is below 1 if the observed value is lower than the predicted one.21 In reference subjects, the 95% confidence interval ranged from 0.80 to 1.20.4 Statistical methods. Randomized patients were evaluated according to an intention-to-treat analysis. The primary variable (average weekly increase in Hb level) was calculated for each patient by linear regression (least squares method). In the case of transfused patients, only the first transfusion-free interval was used. In the case of dose reductions, the subsequent Hb values were withdrawn from regression analysis.

The effect of the four different rHuEpo doses was compared with that of no treatment using a two-way analysis of variance with the factors ‘‘study treatment’’ and ‘‘chemotherapy’’ (yes/no). A sequentially rejecting testing procedure based on F-tests was chosen in the protocol to test the contrasts of each dose group versus that of the control group sequentially from the highest to the lowest dose at a nominal significance level of 5%. This procedure guaranteed an experiment-wise error rate of 5% in a strong sense.

Time to response was analyzed by the Kaplan-Meier method22 and the log-rank test.23 The time to response was censored if patients dropped out prematurely for reasons other than death, adverse event, or nonresponse. In these last three cases, the patient was counted as a nonresponder for the total study time.

Analysis of prognostic factors on response was performed using the classification and regression tree (CART) method.24 In the first step (univariate analysis), an optimal cutoff value was selected for each factor by repeated log-rank tests that divided the patients into a group with high or one with low response rates (no. per group ≥10). Thus, 4 to 5 meaningful cutoff values between approximately the 25%-quantile and the 75%-quantile of each quantitative covariate
were tested. Adjustment of $P$ values was accomplished using the minimum of the two adjusted $P$ values calculated by the method of Miller and Siegmund\(^{(25)}\) and that of Bonferroni.\(^{(26)}\) The two subgroups that resulted from splitting with the lowest $P$ value of all tests were implemented. In the next step, this procedure was repeated with all factors within the subgroups already obtained. If the lowest $P$ value was above .05, the procedure was terminated. If two or more factors were found to significantly split a subpopulation into two subgroups, then these factors were evaluated in multivariate models for the subpopulation using Cox proportional hazard regression.\(^{(21)}\) In the CART analysis of prognostic factors, blood cell counts were tested both as baseline and average values during the study.

All $P$ values are two-tailed. Analyses were performed with the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS

Study population. From March 1992 until June 1993, 146 patients were randomized to receive 1,000 U ($n = 31$), 2,000 U ($n = 29$), 5,000 U ($n = 31$), or 10,000 U ($n = 26$) of rHuEpo daily SC or to receive no therapy ($n = 29$). Patient characteristics are described in Table 1. A total of 84 patients suffered from MM and 62 from low-intermediate grade NHL. Most patients with MM (52%) were at stage 3 according to the Durie and Salmon staging system\(^{(15)}\); most NHL patients had low-grade lymphoma (73%), and 55% of subjects were in an advanced stage of disease (stage IV of the Ann Arbor system\(^{(7)}\) and stages III-IV of the Rai system\(^{(1)}\)). There was no imbalance in the five treatment groups according to any of the following staging systems: Durie and Salmon,\(^{(15)}\) C-reactive protein and $\beta_2$-microglobulin,\(^{(16)}\) Ann Arbor,\(^{(18)}\) Rai,\(^{(19)}\) and $\beta_2$-microglobulin and lactate dehydrogenase.\(^{(20)}\)

Most subjects (116 of 146 [79%]) received chemotherapy during the study. About one fourth of the patients experienced transient severe neutropenia, and one third underwent episodes of severe thrombocytopenia. On the average, there were no relevant changes in neutrophil or platelet counts between baseline, treatment phase, and last value in the five treatment groups, indicating that the myelosuppression caused by both chemotherapy and underlying disease was practically unchanged during the study.

Of the 146 patients, 14 dropped out of the study prematurely for the following reasons: death ($n = 3$), adverse events ($n = 9$), and personal reasons ($n = 2$).

Baseline hematological parameters and endogenous Epo production. Hematologic parameters are reported in Table 1. The mean baseline Hb level was 9.4 ± 0.9 g/dL. Twenty-one patients (14%) had received blood transfusions within the 8 weeks before randomization. Serum Epo level was assayed in 110 patients; the median baseline level was 32 mIU/mL, and endogenous Epo production was found to be defective in 77% of patients as judged by an O/P ratio ≤0.9 (Table 1).

Efficacy of rHuEpo treatment. An intention-to-treat analysis was performed to evaluate treatment efficacy. The primary variable was the individual average increase in Hb level per week as determined by linear regression for each patient. The median average increase in Hb level per week was 0.04 g/dL in the control group and −0.04 ($P = .57$ in comparison with control), 0.22 ($P = .055$), 0.43 ($P = .014$), and 0.58 ($P = .0001$) g/dL in the 1,000 U, 2,000 U, 5,000 U, and 10,000 U groups, respectively (Table 2). Thus, an rHuEpo dose of 1,000 U daily did not show any effect, whereas clearly dose-dependent stimulation of erythropoiesis could be observed in the doses ranging from 2,000 to 10,000 U.

When considering patients who fulfilled the planned protocol-correct criteria, no relevant differences in the Hb increase could be observed in comparison with those of the intention-to-treat population (data not shown). The efficacy was similar between patients with MM and NHL and between the patients with and without chemotherapy.

Time to first response. The time required for response to treatment was analyzed by survival time methods. For this analysis, response was defined as an increase in Hb level of at least 2 g/dL between baseline and two consecutive check-ups in the treatment phase without blood transfusion

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Control</th>
<th>1,000 U</th>
<th>2,000 U</th>
<th>5,000 U</th>
<th>10,000 U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>15/14</td>
<td>16/15</td>
<td>16/13</td>
<td>17/14</td>
<td>8/18</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>68 (28-82)</td>
<td>67 (48-82)</td>
<td>65 (40-82)</td>
<td>68 (42-85)</td>
<td>63 (28-80)</td>
</tr>
<tr>
<td>MM/NHL</td>
<td>16/13</td>
<td>19/12</td>
<td>19/10</td>
<td>14/17</td>
<td>16/10</td>
</tr>
<tr>
<td>Hb level (g/dL)</td>
<td>9.5 ± 1.1</td>
<td>9.3 ± 0.9</td>
<td>9.4 ± 0.9</td>
<td>9.4 ± 1.2</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>Serum iron level (μmol/L)</td>
<td>13.8 (10.0-15.3)</td>
<td>14.6 (11.6-19.6)</td>
<td>11.8 (9.4-21.5)</td>
<td>13.5 (9.8-20.8)</td>
<td>15.1 (12.1-17.4)</td>
</tr>
<tr>
<td>Serum ferritin level (μg/L)</td>
<td>317 (138-633)</td>
<td>326 (153-933)</td>
<td>250 (129-366)</td>
<td>218 (96-465)</td>
<td>248 (166-519)</td>
</tr>
<tr>
<td>Serum Epo level (mU/mL)</td>
<td>32 (19-66)</td>
<td>43 (22-69)</td>
<td>30 (21-64)</td>
<td>24 (11-47)</td>
<td>38 (16-70)</td>
</tr>
<tr>
<td>O/P ratio</td>
<td>0.82 (0.61-1.00)</td>
<td>0.77 (0.64-0.85)</td>
<td>0.73 (0.59-0.84)</td>
<td>0.66 (0.55-0.88)</td>
<td>0.74 (0.62-0.91)</td>
</tr>
<tr>
<td>O/P ratio ≤0.9 (% of patients)</td>
<td>65</td>
<td>78</td>
<td>91</td>
<td>76</td>
<td>74</td>
</tr>
<tr>
<td>Patients undergoing chemotherapy (%)</td>
<td>83</td>
<td>81</td>
<td>83</td>
<td>76</td>
<td>81</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (interquartile range), unless otherwise stated.

*Within the last 8 weeks before starting rHuEpo treatment.
The average weekly increase in Hb level was calculated for each patient by linear regression (least squares method). In the case of transfused patients, only the first transfusion-free interval was used. In the case of dose reductions, the subsequent Hb values were withdrawn from regression analysis.

in the last 6 weeks. Table 3 shows the probability of response for the different groups at given times during the 8 weeks of treatment. Probability of response was significantly dose-dependent, and after 8 weeks the cumulative responses were 31%, 61%, and 62% in the 2,000 U, 5,000 U, and 10,000 U groups, respectively.

The time course of Hb levels in different groups is reported in Fig 1. During the treatment phase, 8 (28%), 7 (23%), 5 (17%), 6 (19%), and 4 (15%) patients received blood transfusions in the control, 1,000 U, 2,000 U, 5,000 U, and 10,000 U groups, respectively. The total number of red blood cell units transfused in each group was as follows: 26 (control), 22 (1,000 U), 19 (2,000 U), 16 (5,000 U), and 6 (10,000 U). There was a trend towards a reduced transfusion need in the highest dose groups (5,000 and 10,000 U).

Analysis of prognostic factors for time to response.

The CART method was used to investigate whether there were confounding or prognostic factors for time to response; Fig 2 shows the resulting regression tree. In the univariate analysis (see Table 4; first step of the CART analysis), the rHuEpo dose had the most significant effect on response if the whole study population was split into the following two subgroups: up to 2,000 U and from 5,000 to 10,000 U. Hb level, platelet count, O/P ratio, serum Epo level, and sex of the patient also correlated significantly with response in the univariate analysis. No significant effects were found for age, underlying disease (MM or NHL), neutrophil count, serum creatinine level, transferrin saturation level, or chemotherapy (yes, no).

In patients treated with 5,000 or 10,000 U of rHuEpo, the second step of the CART procedure showed that serum Epo level or, alternatively, the O/P ratio was the most important factor predicting response (Fig 2). As shown in Table 5, the best discriminating cutoff points were a serum Epo level of 50 mU/mL or an O/P ratio of 0.8; 70 mU/mL for O/P ratio less than 0.8; 70 mU/mL and 0.9, respectively, were alternative cutoff points that significantly discriminated patients and showed a slightly higher specificity. Approximately 70% to 80% of patients presenting with a serum Epo level ≤50 (or 70) mU/mL and an O/P ratio ≤0.8 (or 0.9) are expected to respond to rHuEpo with a Hb increase of ≥2 g/dL within 8 weeks. On the contrary, the probability of response is only 20% to 30% in patients having a serum Epo level greater than 50 mU/mL and an O/P ratio greater than 0.8. A cutoff point of 0.9 for O/P ratio was chosen in the regression tree of Fig 2. Further significant discrimination of these subgroups was not possible. However, a multivariate analysis using Cox’s proportional hazard regression method showed that serum Epo level (or O/P ratio) and neutrophil count at baseline were independent significant factors in patients treated with 5,000 or 10,000 U/d. Patients with serum Epo levels ≤50 mU/mL (or O/P ratios ≤0.8) and those with neutrophil counts greater than 1.6 × 10^9/L were more likely to respond to rHuEpo.

The control and lower dose groups were split into control, 1,000 U and 2,000 U subgroup at the second step of the CART procedure (Fig 2). The most relevant prognostic factor in the 2,000 U subgroup was neither serum Epo nor the O/P ratio (probably as a consequence of the low O/P ratio values in this group; maximum, 0.91), but was the average platelet count during treatment. No patient with an average platelet count less than 150 × 10^9/L reached the defined response with 2,000 U of rHuEpo. On the contrary, the probability of response was 50% for patients with a platelet count greater than 150 × 10^9/L and an O/P ratio of less than 0.9 who received an rHuEpo dose of 2,000 U/d.

Table 2. Average Increase in Hb Level per Week (g/dL) Based on an Intention-to-Treat Analysis

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 29)</th>
<th>1,000 U (n = 31)</th>
<th>2,000 U (n = 29)</th>
<th>5,000 U (n = 31)</th>
<th>10,000 U (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>−0.04</td>
<td>−0.10</td>
<td>0.7</td>
<td>0.30</td>
<td>0.44</td>
</tr>
<tr>
<td>SD</td>
<td>0.38</td>
<td>0.40</td>
<td>0.32</td>
<td>0.52</td>
<td>0.45</td>
</tr>
<tr>
<td>Median</td>
<td>0.04</td>
<td>−0.04</td>
<td>0.22</td>
<td>0.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>−0.08−0.10</td>
<td>−0.18−0.13</td>
<td>−0.01−0.31</td>
<td>0.12−0.63</td>
<td>0.35−0.67</td>
</tr>
<tr>
<td>P value v control</td>
<td>—</td>
<td>0.5702</td>
<td>0.0563</td>
<td>0.0140</td>
<td>0.0001</td>
</tr>
<tr>
<td>P value v next lower dose</td>
<td>—</td>
<td>0.0124</td>
<td>0.1966</td>
<td>0.2514</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Probability of Response for the Different Treatment Groups During the 8 Weeks of Treatment

<table>
<thead>
<tr>
<th>Probability of response</th>
<th>Control (n = 29)</th>
<th>1,000 U (n = 31)</th>
<th>2,000 U (n = 29)</th>
<th>5,000 U (n = 31)</th>
<th>10,000 U (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 2 wk</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>After 4 wk</td>
<td>0.00</td>
<td>0.00</td>
<td>3.5</td>
<td>16.1</td>
<td>15.4</td>
</tr>
<tr>
<td>After 6 wk</td>
<td>3.7</td>
<td>6.5</td>
<td>20.7</td>
<td>48.4</td>
<td>57.7</td>
</tr>
<tr>
<td>After 8 wk</td>
<td>7.4</td>
<td>6.5</td>
<td>31.0</td>
<td>61.3</td>
<td>61.5</td>
</tr>
<tr>
<td>Log rank test</td>
<td>P &lt; .0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Response was defined as an Hb increment of at least 2 g/dL between baseline and two consecutive check-ups without blood transfusions in the previous 6 weeks.
Algorithm for predicting response to rHuEpo treatment. Ludwig et al. found that, after 2 weeks of therapy, serum Epo level (<100 mU/mL) and change in Hb level (≥0.5 g/dL) taken together were a powerful predictor of responsiveness to rHuEpo treatment. Because serum Epo was not measured during rHuEpo treatment in the present study, we developed an alternative algorithm for predicting response by using the baseline serum Epo level and O/P ratio, taken as indicators of adequacy of endogenous Epo production, and variation in Hb level after 2 weeks, taken as an early indicator of response (Fig 3). For this purpose, we considered only patients treated with 5,000 U or 10,000 U, because the others might have been undertreated.

Step 1 involved the adequacy of endogenous Epo production. Patients having a serum Epo level ≤50 mU/mL or an O/P ratio ≤0.9 were considered to have a blunted Epo response (Fig 3). Only 1 of 8 patients with presumably adequate Epo production responded to rHuEpo treatment, whereas 30 of 40 patients fulfilling at least one of the two criteria for inadequate production showed a definite response. Step-1 accuracy was 77%; specificity, 88%; and sensitivity, 75%.

Step 2 involved the variation in Hb level after 2 weeks. A ΔHb level ≥0.3 g/dL proved to be the best cutoff point (Fig 3). Of the 34 patients with an increase of at least 0.3 g/dL, 88% showed a response. The use of this algorithm in patients treated with 5,000 or 10,000 U of rHuEpo would have reached a sensitivity of 88%, a specificity of 93%, and an overall accuracy of 90%.

Fig 1. Time course of Hb level during treatment phase (intention-to-treat population). Treatment groups: □, control; △, 1,000 U; ○, 2,000 U; ▲, 5,000 U; ○, 10,000 U. The late Hb decreases in the 5,000 and 10,000 U groups reflect dose reduction (accomplished according to protocol rules).

Fig 2. Regression tree derived from analysis of prognostic factors for time to first response using the CART method (intention-to-treat population). Each box contains the most important factors predicting response at that particular step, the cutoff value, and the resulting two subgroups with percentage of responders.
Table 4. Results of Univariate Analysis of the Prognostic Importance of Various Covariates on Time to Response and Cumulative Response Rates After 8 Weeks of Treatment

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Cutoff Level or Subgroups</th>
<th>Response (%)</th>
<th>P Value</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>rHuEpo dose</td>
<td>≤2,000 U</td>
<td>&lt; Cutoff Level &gt; Cutoff Level</td>
<td>&lt;.1 × 10⁻⁷</td>
<td>.0065</td>
<td>.0227</td>
</tr>
<tr>
<td>Baseline Hb</td>
<td>10 g/dL</td>
<td>26</td>
<td>53</td>
<td>.0070</td>
<td>.0238</td>
</tr>
<tr>
<td>Average PLT count</td>
<td>150 × 10⁹/L</td>
<td>20</td>
<td>39</td>
<td>.0144</td>
<td>.0530</td>
</tr>
<tr>
<td>O/P ratio</td>
<td>0.9</td>
<td>38</td>
<td>12</td>
<td>.0102</td>
<td>.0344</td>
</tr>
<tr>
<td>Serum Epo</td>
<td>30 mU/mL</td>
<td>45</td>
<td>21</td>
<td>.0110</td>
<td>.0374</td>
</tr>
<tr>
<td>Baseline PLT count</td>
<td>100 × 10⁹/L</td>
<td>13</td>
<td>38</td>
<td>.0630</td>
<td>.0630</td>
</tr>
<tr>
<td>Sex</td>
<td>M/F</td>
<td>27</td>
<td>39</td>
<td>.1203</td>
<td>.3522</td>
</tr>
<tr>
<td>Average neutrophils</td>
<td>1.8 × 10⁹/L</td>
<td>23</td>
<td>38</td>
<td>.1587</td>
<td>.4482</td>
</tr>
<tr>
<td>Age</td>
<td>60 yr</td>
<td>41</td>
<td>30</td>
<td>.2244</td>
<td>.5720</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>40%</td>
<td>37</td>
<td>27</td>
<td>.3071</td>
<td>.7190</td>
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<tr>
<td>Serum creatinine</td>
<td>0.9 mg/dL</td>
<td>43</td>
<td>29</td>
<td>.3939</td>
<td>.3939</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>NHL/MM</td>
<td>39</td>
<td>29</td>
<td>.4215</td>
<td>1.0</td>
</tr>
<tr>
<td>Baseline neutrophils</td>
<td>2.0 × 10⁹/L</td>
<td>26</td>
<td>37</td>
<td>.6773</td>
<td>.5773</td>
</tr>
</tbody>
</table>

Abbreviation: PLT, platelet.

* For quantitative variables, two subgroups were generated, ≤cutoff and >cutoff.

**Safety.** rHuEpo was found to be safe and well tolerated both systemically and locally. None of the deaths (n = 3 for control and n = 4 for rHuEpo; 10.3% v 3.4%, respectively) or other serious adverse events (n = 4 for control and n = 15 for rHuEpo; 13.8% v 12.8%, respectively) were classified as being causally related to the study medication. No increased incidence of adverse events of special interest (hypertension, angina pectoris, cerebral ischemia, or malignancy progression) was found in the rHuEpo groups with respect to the control group.

**DISCUSSION**

rHuEpo corrects anemia, eliminates transfusion requirement, and improves quality of life in anemic patients with renal failure. This remarkable efficacy is explained by the major role that defective endogenous Epo production plays in the pathogenesis of renal anemia. Blunted Epo production has been shown to contribute to the anemia associated with other disorders, including MM and NHLs. Beguin et al. investigated the pathophysiology of erythropoiesis in 62 patients with MM. Overall, about a quarter of those studied had inadequate Epo production, and this proportion increased to about 50% in the ones with advanced disease. Although defective Epo production was mainly observed in patients with concomitant renal failure, a number of MM patients with normal renal function also showed inadequate Epo levels. In the present study, 70% of the examined MM patients presented serum Epo levels inadequate for the degree of anemia (O/P ratio ≤0.9).

The mechanisms of anemia associated with malignant lymphomas are largely similar to those of MM, except for renal failure, which is quite uncommon in lymphoproliferative disorders. Neoplastic BM infiltration and chemotherapy seem to play a major role in the pathogenesis of anemia associated with malignant lymphomas. In a previous study, a blunted endogenous Epo production was found in all the patients with malignant lymphoma undergoing chemotherapy. This was confirmed by the results of the present clinical trial, which show that 86% of NHL patients had serum Epo levels inadequate for the degree of anemia.

Table 5. Results of the CART Analysis of the Prognostic Importance of Various Covariates on Time to Response

<table>
<thead>
<tr>
<th>rHuEpo Dose</th>
<th>Cutoff Level or Subgroups</th>
<th>Response (%)</th>
<th>P Value</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,000/10,000</td>
<td>Serum Epo</td>
<td>50 mU/mL</td>
<td>78</td>
<td>25</td>
<td>.0004</td>
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<tr>
<td></td>
<td>Serum Epo</td>
<td>70 mU/mL</td>
<td>73</td>
<td>18</td>
<td>.0028</td>
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<tr>
<td></td>
<td>O/P ratio</td>
<td>0.8</td>
<td>75</td>
<td>31</td>
<td>.0015</td>
</tr>
<tr>
<td></td>
<td>O/P ratio</td>
<td>0.9</td>
<td>70</td>
<td>27</td>
<td>.0127</td>
</tr>
<tr>
<td></td>
<td>Baseline neutrophils</td>
<td>1.6 × 10⁹/L</td>
<td>44</td>
<td>71</td>
<td>.0328</td>
</tr>
<tr>
<td></td>
<td>Baseline PLT count</td>
<td>100 × 10⁹/L</td>
<td>33</td>
<td>69</td>
<td>.0423</td>
</tr>
<tr>
<td></td>
<td>Baseline Hb</td>
<td>10 g/dL</td>
<td>53</td>
<td>79</td>
<td>.0446</td>
</tr>
<tr>
<td>2,000</td>
<td>Average PLT count</td>
<td>150 × 10⁹/L</td>
<td>0</td>
<td>50</td>
<td>.0016</td>
</tr>
<tr>
<td></td>
<td>Average PLT count</td>
<td>250 × 10⁹/L</td>
<td>2</td>
<td>23</td>
<td>.0105</td>
</tr>
</tbody>
</table>

Abbreviation: PLT, platelet.

* For quantitative variables, two subgroups were generated, ≤cutoff and >cutoff.
Inadequate endogenous Epo production was also found to be a predictor of response to rHuEpo in nonrenal anemia, which is in keeping with the biological action of the erythroid hormone. In fact, Epo is both a survival factor and a mitogenic. Epo prevents apoptosis or programmed cell death of CFU-Es and proerythroblasts. When it is present in adequate amounts, large numbers of CFU-Es can proliferate and differentiate, generating erythroid precursors that mature to red blood cells. On the other hand, inadequate levels of Epo result in premature death of most erythroid progenitors and in insufficient production of red blood cells. Though the algorithm of Ludwig et al showed a predictive change in Hb concentration (<0.5 g/dL) and serum Epo level, nonresponders showed serum Epo levels >100 mU/mL, and no change in Hb level. It has already been pointed out that the accumulation of exogenous Epo in the plasma of nonresponders is difficult to explain. Although the algorithm of Ludwig et al showed a predictive power of about 95% in their study, it remains to be seen whether this will be of value in other clinical settings.

Results of this study show that prediction of response to rHuEpo can be reasonably based on an evaluation of endogenous Epo production at baseline, and that this might be improved by using indicators of residual BM function and indicators of early response.

A low serum Epo level or O/P ratio should be used to identify patients very likely to respond to, and thus good candidates for, treatment with rHuEpo (Fig 3). The cutoff points reported in our algorithm might be slightly modified in other settings (eg, 0.8 could be used for the O/P ratio), but the essential message is that the decision to use rHuEpo in an individual anemic patient with MM or NHL should be based on indicators of endogenous Epo production. For practicing physicians to gainfully use the O/P ratio for identification of inadequate Epo response to anemia, every laboratory should have a homemade reference population of anemic subjects and should routinely provide this ratio together with the serum Epo concentration. The patients gathered to calculate a reference regression equation between serum Epo and Hct (or Hb) levels should have an anemia with a single simple mechanism. Patients with iron deficiency anemia not due to neoplastic disease have the advantages of being easily found, unequivocally defined, and homogenous in nature. These patients could become the universal reference population.

Platelet and neutrophil counts could be used to decide the initial dosage, because we observed that subjects with normal response was observed in the control group or the 1,000 U group; by contrast, administration of doses of rHuEpo from 2,000 to 10,000 U led to a clear dose-dependent increase in Hb level (see Tables 2 and 3). Therefore, the minimal effective dose was 2,000 U daily SC, which means about 200 U/kg/wk. Furthermore, there was no substantial difference between 5,000 and 10,000 U, suggesting that the former amount should be used in the large majority of patients.

The baseline serum Epo level and the respective O/P ratio were found to be the best prognostic factors for response in patients treated with 5,000 or 10,000 U daily. Three of four patients with a serum Epo level ≤50 mU/mL or an O/P ratio ≤0.9 can expect an Hb increase of 2 g/dL within 8 weeks. By contrast, the probability of response is only about 25% in patients with an O/P ratio greater than 0.9. This reinforces the opinion that rHuEpo is effective in those anemic patients in whom the endogenous hormone production is inadequate. A lower rHuEpo dose of 2,000 U daily was also found to be effective in patients having a low O/P ratio, provided the platelet count was normal and, thus, BM function was not suppressed.

In a recent study Ludwig et al investigated the power of hematologic parameters to predict response to rHuEpo in chronic anemia of cancer. At baseline, only the serum level of endogenous Epo (<100 mU/mL or ≥100 mU/mL) proved to be significantly, but rather weakly, correlated with response. However, after 2 weeks of treatment, several parameters showed significant correlations, the strongest being serum Epo level and serum Epo level. Nonresponders showed serum Epo levels ≥100 mU/mL, and no change in Hb level. It has already been pointed out that the accumulation of exogenous Epo in the plasma of nonresponders is difficult to explain. Although the algorithm of Ludwig et al showed a predictive power of about 95% in their study, it remains to be seen whether this will be of value in other clinical settings.

<table>
<thead>
<tr>
<th>Step 1:</th>
<th>eEpo ≤ 50 mU/mL or O/P ratio ≤ 0.9? (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>start treatment</td>
</tr>
<tr>
<td></td>
<td>accuracy 75% (30/40)</td>
</tr>
<tr>
<td>No</td>
<td>continue treatment</td>
</tr>
<tr>
<td></td>
<td>accuracy 88% (39/40)</td>
</tr>
</tbody>
</table>

**Fig 3. Algorithm to predict response to rHuEpo treatment in patients with MM and NHL receiving 5,000 or 10,000 U/d.** Accuracy of prediction is shown for each decision, each step, and the overall procedure. Specificity and sensitivity are shown for the first step and the overall procedure.

<table>
<thead>
<tr>
<th>Step 1:</th>
<th>accuracy 77%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specificity 88%</td>
</tr>
<tr>
<td></td>
<td>sensitivity 75%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2:</th>
<th>ΔHb ≥ 0.3 g/dL at 2 weeks? (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>stop treatment</td>
</tr>
<tr>
<td></td>
<td>accuracy 100% (6/6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 1 + 2:</th>
<th>accuracy 90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specificity 93%</td>
</tr>
<tr>
<td></td>
<td>sensitivity 88%</td>
</tr>
</tbody>
</table>
residual BM function require lower doses of rHuEpo to achieve response. In these latter patients, the initial dose should be 200 to 225 U/kg/wk; patients with low neutrophil and platelet counts should be treated with higher doses, i.e., 400 to 500 U/kg/wk.

A controversial issue remains that of indicators of early response that may allow clinicians to decide whether to continue or terminate rHuEpo therapy. Variations in Hb level after 2 weeks proved to be a useful indicator in both the study of Ludwig et al11 and in the present trial.

REFERENCES


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Recombinant human erythropoietin in the anemia associated with multiple myeloma or non-Hodgkin's lymphoma: dose finding and identification of predictors of response

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