

## rHuEpo before high-dose therapy allows autologous peripheral stem-cell transplantation without red blood cell transfusion: a pilot study

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### Summary:

To decrease red blood cell (RBC) transfusion requirements during high-dose therapy (HDT) for hematological malignancies, we conducted a pilot study to assess the effect of recombinant human erythropoietin (rHuEpo) given during chemotherapy before HDT and autologous peripheral stem-cell transplantation (AP SCT). The transfusion histories of 15 HDT and AP SCT for hematological disease performed in 11 consecutive patients who received rHuEpo (10 000 U subcutaneously three times/week) were compared to those of 22 HDT and ASCT performed in 17 consecutive historical controls matched for hematological parameters. rHuEpo increased the hemoglobin (Hb) level from  $10.3 \pm 2.3$  g/dl at diagnosis to  $12.9 \pm 2.2$  g/dl at the time of HDT in 11 patients; no major adverse effects occurred. Compared to historical controls (95%, 21/22), RBC transfusion requirements were significantly lower for rHuEpo recipients (26%, 4/15) ( $P = 0.00001$ ) and rHuEpo responders (15%, 2/13) ( $P = 0.00002$ ). After HDT and AP SCT, fewer RBC transfusions were needed: 3.3, 1.2 and 0.3 RBC units for controls, rHuEpo recipients and rHuEpo responders, respectively ( $P = 0.006$  and  $0.00002$ ). Therefore, rHuEpo should be administered before, and not after HDT and AP SCT, to lower RBC transfusion requirements after HDT and AP SCT.

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High-dose therapy (HDT) with autologous peripheral stem-cell transplantation (AP SCT) has improved the prognosis of aggressive lymphoproliferative malignancies. Transplantation-associated mortality is now less than 5%;

however, transplantation-associated morbidity is still high. Profound suppression of all three hematopoietic lineages is uniformly observed. Combining granulocyte colony-stimulating factors (G-CSF) and peripheral blood stem cells (PBSC) has successfully accelerated myeloid engraftment and neutrophil recovery without any effect on red blood cells (RBC). Anemia is ubiquitous in this setting and almost all patients who undergo AP SCT require RBC transfusions with potential side effects such as infectious disease transmission, transfusion reaction and iron overload. Although several controlled trials have established that high-dose recombinant human erythropoietin (rHuEpo) increases the hemoglobin (Hb) level in 60–80% of the patients with multiple myeloma (MM), non-Hodgkin's lymphoma or chronic lymphocytic leukemia,<sup>1</sup> rHuEpo administered after HDT and AP SCT has failed to lower the need for RBC transfusions.<sup>2–7</sup> Therefore, we designed a pilot study to determine whether a strategy of giving rHuEpo during chemotherapy before HDT and AP SCT could effectively reduce RBC transfusion requirement in this setting.

### Patients and methods

#### Patients and historical controls

From November 2001 to May 2002, 11 consecutive patients with lymphoproliferative disease scheduled to receive HDT and AP SCT were included in a pilot study and received rHuEpo during chemotherapy before HDT. For comparison, all 17 consecutive patients matched for hematological disease and treatment, who received matched HDT and AP SCT in our department from January 2000 to November 2001, were retrospectively analyzed and served as historical controls. Since MM patients were enrolled in a double transplant program, 15 and 22 HDT and AP SCT were analyzed for RBC transfusion requirements in 11 rHuEpo recipients and 17 historical controls (Table 1). Patients who had experienced seizures or had uncontrolled hypertension were excluded. Patients with MM at diagnosis received four to six cycles of VAD<sup>8</sup> before HDT. One MM patient given rHuEpo had a retroperitoneal plasmocytoma resistant to two cycles of VAD; three cycles of ESHAP<sup>9</sup> were necessary to induce a good partial response. For MM,

**Table 1** Characteristics of patients treated with rHuEpo and historical controls

|  | Patients |               | Controls |               | P-value |
|--|----------|---------------|----------|---------------|---------|
|  | Mean     | (s.d.)        | Mean     | (s.d.)        |         |
| MM (number of tandem transplant)           | 7 (4)    |               | 10 (5)   |               |         |
| MCL  | 1        |               | 3        |               |         |
| Lymphoma in relapse                        | 3        |               | 4        |               |         |
| Age (range)                                | 57       | (9.5) (33–66) | 53       | (7.7) (34–69) | 0.25    |
| Number of CD34 ( $\times 10^6$ /kg) (s.d.) | 4        | (1.8)         | 4.8      | (3.6)         | 0.4     |
| Day of hospitalization                     | 22.6     | (3.3)         | 25.6     | (4.1)         | 0.02    |
| Number of platelet transfusion             | 2.2      | (2.4)         | 2.9      | (2.4)         | 0.38    |
| Hb level at diagnosis (g/dl)               | 10.3     | (2.3)         | 11.6     | (1.5)         | 0.17    |

the AP SCT conditioning regimen consisted of melphalan 200 mg/m<sup>2</sup> (three rHuEpo recipients and five controls) or melphalan 140 mg/m<sup>2</sup> followed 2 months later by melphalan 200 mg/m<sup>2</sup> for tandem transplant (four rHuEpo recipients and five controls). Stage IV mantle cell lymphoma (MCL) (one rHuEpo recipient and three controls) received six cycles of VAD and chlorambucil (12 mg/day on days 20–29) prior to the conditioning regimen combining melphalan (140 mg/m<sup>2</sup>) and total body irradiation (8 G in four fractions). The conditioning regimen for diffuse large-cell lymphoma (DLCL) was BEAM,<sup>10</sup> given in second complete remission (CR) that was obtained with three or four ESHAP cycles as second-line chemotherapy for first relapses. All patients had previously received four to eight CHOP regimen with or without rituximab (four to eight infusions of 375 mg/m<sup>2</sup>/day). One rHuEpo recipient suffering from bulky mediastinal Hodgkin's disease was treated with three VABEMP cycles<sup>11</sup> and local radiotherapy; an early mediastinal relapse was treated with six cycles of GNC (gemcitabine 1 g/m<sup>2</sup>, vinorelbine 30 mg/m<sup>2</sup> and cisplatin 40 mg/m<sup>2</sup> every other week) before HDT with BEAM.

The local Research Ethics Board approved the study.

#### rHuEpo treatment

Enrolled patients received rHuEpo (Eprex<sup>®</sup>, Ortho Biotech Europe/Jansen-Cilag) as soon as their Hb level fell <11 g/dl during induction chemotherapy, whereas none of the historical controls received rHuEpo during their treatment. Prior to rHuEpo administration, no patient had major bleeding, hemolysis, or iron, folates or vitamin B<sub>12</sub> deficiency. rHuEpo (10 000 IU) was injected subcutaneously three times/week for 4 weeks. If the Hb level at that time had increased by >1 g/dl, rHuEpo was continued at the same dose. Otherwise, it was doubled to 20 000 IU three times/week. If the Hb level exceeded 15 g/dl at any time, rHuEpo was withheld until the concentration decreased to <12 g/dl, at which time it was restarted. rHuEpo was administered for a maximum of 12 weeks of chemotherapy, which included the period after the last chemotherapy dose before HDT. rHuEpo was withheld during PBSC collection and during hospitalization for HDT. For patients who received tandem transplants at a 2-month interval, rHuEpo was reintroduced at the time of

discharge after the first transplant up to the admission for the second one. No oral iron supplementation was added.

#### HDT procedure

PBSC were collected upon recovery from short-term chemotherapy-induced cytopenia. CD34<sup>+</sup> cells were selected for patients with MCL. HDT was performed in the intensive care unit. Supportive care followed our hematology department's standard procedures and was the same for rHuEpo recipients and historical controls: the transfusion thresholds were 8 g/dl and  $20 \times 10^9$ /l for RBC and platelets, respectively. G-CSF was administered from day 7 after AP SCT until neutrophils reached  $1.5 \times 10^9$ /l for 2 consecutive days. Only RBC transfusions given during the hospitalization for HDT and ASCT were considered.

#### Statistical analysis

Continuous variables and percentages were compared using Student's *t*-test and Fisher's exact test, respectively.

## Results

#### Pre-HDT Hb levels

rHuEpo, administered for a mean of 9 weeks before AP SCT (range 4–12, s.d. 2.7), increased the Hb levels of nine (82%) patients (rHuEpo responders), but two patients failed to respond (rHuEpo nonresponders) (Table 2). One nonresponder had MM with severe diffuse osteosclerosis and cytopenia. Despite increasing the rHuEpo dose to 20 000 IU three times/week, this patient's Hb level did not rise. The second nonresponder underwent major abdominal surgery at diagnosis for a bulky mesenteric DLCL and developed a chronic inflammatory syndrome without bone marrow involvement. Owing to poor local tolerance, his rHuEpo dose could not be increased. No other adverse effect of rHuEpo was observed. Side effects were recorded at the time of every cycle of conventional chemotherapy before HDT.

At the time of HDT, the mean Hb level was significantly higher in rHuEpo recipients than in controls ( $12.9 \pm 2.2$  vs

**Table 2** RBC transfusion analysis

|                                   | RHuEpo recipients (s.d.) | Controls (s.d.) | P-value  |
|-----------------------------------|--------------------------|-----------------|----------|
| Number of patients                | 11                       | 17              |          |
| Number of transplant analyzed     | 15                       | 22              |          |
| Transplant requiring transfusion  | 4/15 (26%)               | 21/22 (95%)     | 0.00001  |
| Hb level at transplant (g/dl)     | 12.9 (2.2)               | 11.4 (1.5)      | 0.002    |
| Hb level at discharge (g/dl)      | 9.9 (1.6)                | 9.9 (1.0)       | 0.9      |
| Number of RBC unit transfused     | 1.2 (2.5)                | 3.3 (2.1)       | 0.006    |
| <i>rHuEpo responders</i>          |                          |                 |          |
| Number of patients                | 9                        | 17              |          |
| Number of transplant analyzed     | 13                       | 22              |          |
| Transplant requiring transfusion  | 2/13 (15%)               | 21/22 (95%)     | 0.000002 |
| Hb level at transplant (g/dl)     | 13.7 (0.8)               | 11.4 (1.5)      | 0.000001 |
| Hb level at discharge             | 9.6 (1.6)                | 9.9 (1.0)       | 0.5      |
| Number of RBC unit transfused     | 0.3 (0.7)                | 3.3 (2.1)       | 0.00002  |
| <i>RBC according to pathology</i> |                          |                 |          |
| MM first transplant               | 1/7 (14%)                | 9/10 (90%)      | 0.003    |
| MM second transplant              | 1/4                      | 5/5             | 0.04     |
| MCL                               | 0/1                      | 3/3             | 0.2      |
| Lymphoma (refractory/relapse)     | 2/3                      | 4/4             | 0.4      |

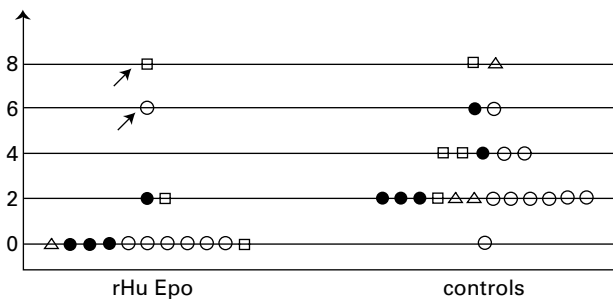
11.4 ± 1.5 g/dl, *P* = 0.002), especially when only rHuEpo responders were analyzed (13.7 ± 0.8, *P* = 0.000001).

*Comparison of the RBC transfusion requirements*

rHuEpo recipients and historical controls were similar in terms of diagnosis, initial Hb levels, conditioning regimens, numbers of CD34<sup>+</sup> cells infused and platelet transfusions, but duration of hospitalization for HDT was a few days longer for controls (Table 1). The RBC transfusion requirement fell from 95% (21/22) of historical controls to 26% (4/15) (*P* = 0.00001) of rHuEpo recipients and to 15% (2/13) of the rHuEpo responders (*P* = 0.000002) (Table 2). The mean number of RBC units transfused after HDT and AP SCT was significantly lower for patients who had received pre-HDT rHuEpo than for historical controls (1.2 ± 2.5 vs 3.3 ± 2.1 U, respectively; *P* = 0.006). As expected, the mean number of RBC units transfused was even lower when the analysis was restricted to rHuEpo responders (0.3 ± 0.7, *P* = 0.00002). Compared to controls, MM patients who received rHuEpo required fewer RBC transfusions requirement: 90% (9/10) vs 25% (1/4) after one transplant and 100% (5/5) vs 25% (1/4) after the second transplant (Figure 1). The reduced need for RBC transfusion in lymphoma patients is less clearcut (Figure 1), especially for patients in relapse, but the small number of patients precludes any definitive conclusion.

**Discussion**

The results obtained in this pilot study indicate that rHuEpo given during chemotherapy for hematological malignancies before HDT can drastically increase the Hb level and thereby avoid RBC transfusion during AP SCT. Historical controls received a mean number of 3.27 RBC units vs 1.2 U for rHuEpo recipients. The percentage of HDT and AP SCT procedures requiring a RBC transfusion



**Figure 1** Number of RBC transfusion during HDT (*P* = 0.006) MM (circle), tandem transplant for MM (black circle) MCL (triangle), lymphoma (square), rHuEpo nonresponding patient (arrow).

during HDT decreased from 95% of controls to 26% (4/15) of rHuEpo recipients.

rHuEpo administered intravenously during the immediate allogeneic post transplant period shortened the time needed to generate RBC, as indicated by reticulocyte counts and the number of RBC units transfused, but spared no one from transfusion.<sup>2,3,12-16</sup> However, rHuEpo given early after autologous transplant (day 1 and up to days 30-50) did not affect RBC production or transfusions,<sup>2,3,5-7</sup> even when administered simultaneously with G-CSF,<sup>4</sup> despite an *in vitro* synergistic effect. Coadministration of rHuEpo and G-CSF during PBSC mobilization before collection also did not improve erythropoiesis after transplantation.<sup>17,18</sup> In addition, rHuEpo injected at a high dose (600 IU/kg three times/week) for only 3 weeks before HDT and in combination with G-CSF failed to increase the Hb level and therefore to lower the transfusion requirements of 18 patients with relapsed lymphoma, as compared to the placebo group.<sup>5</sup>

In contrast, a positive effect of pre-HDT rHuEpo on RBC requirements after HDT was inferred from breast cancer patients, although previous chemotherapy, bone marrow involvement, durations of neutropenia and hospita-

lization are difficult to compare to those of hematological malignancies.<sup>19</sup> In that pilot study, among 10 patients given rHuEpo for 7–9 weeks from PBSC collection to HDT, only one modest responder required RBC transfusion.<sup>19</sup> Recently, Baron *et al*<sup>20</sup> reported that administering rHuEpo during the 8 weeks between first-line tandem transplants to treat MM was able to lower the RBC requirement from 10/11 during the first transplant to 1/11 during the second. That observation is in agreement with our results, but we also demonstrated that RBC transfusion requirement could be totally abrogated when rHuEpo was administered during chemotherapy, at least during first-line therapy for MM and lymphoma.

Several reasons might explain the discrepancies among our findings, the previously observed negative effect in the autologous setting and the positive effect reported after allogeneic bone marrow transplantation despite lower endogenous erythropoietin concentrations in both allogeneic and autologous transplant recipients compared to iron-deficient controls.<sup>21</sup> The suppressive effects of cyclosporin A<sup>22</sup> and allogeneic donor cell-produced cytokines<sup>23</sup> on endogenous erythropoietin production might be more easily reversed by exogenous rHuEpo.<sup>24</sup> For autologous transplants, marrow purging procedures, if any, and cryopreservation might damage and lower the number of late BFU-E and CFU-E potentially responsive to Epo-induced differentiation. In addition, our subcutaneous administration might have been more effective than the intravenous injection used in the previous studies, as has been demonstrated in renal patients and autologous blood donation programs.<sup>25,26</sup> However, the explanation may be even simpler. If one considers that one RBC transfusion unit induces a Hb increase of 1 g/dl, the loss of Hb can be calculated as follows: Hb before HDT–Hb at discharge + number of RBC units during HDT. According to this formula, the median Hb loss during HDT was similar in controls and rHuEpo recipients (4.6 *vs* 3.9 g/dl, respectively). This finding indicates that the substantially lowered RBC transfusion requirement observed after rHuEpo is secondary only to a higher Hb level at the beginning of HDT and not to an inhibition of erythroid progenitor apoptosis.<sup>27,28</sup> Indeed, Baron *et al*<sup>20</sup> observed no increased erythropoiesis after the second ASCT.

Further investigations on rHuEpo before HDT with AP SCT are needed to evaluate the frequency of side effects, such as hypertension or thromboembolic events, to identify factors predictive of the Hb response<sup>29</sup> and to determine whether parenteral iron supplementation would optimize rHuEpo efficacy because of functional iron deficiency. Whether the rHuEpo-induced Hb rise during the post-AP SCT period will be associated with improved quality of life during HDT<sup>1,30</sup> remains to be established. Finally, the cost-effectiveness of administering rHuEpo before HDT has to be evaluated by taking into account the expenditures generated by not only the RBC transfusions performed during HDT but also those given during induction chemotherapy and before PBSC collection.

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