

Adding growth factors or interleukin-3 to erythropoietin has limited effects on anemia of transfusion-dependent patients with myelodysplastic syndromes unresponsive to erythropoietin alone

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Background and Objectives. Recombinant erythropoietin (r-EPO) induces erythroid responses in patients affected by myelodysplastic syndromes (MDS). However, the response rate declines to 10-15% in MDS with substantial transfusion needs. Both *in vitro* and *in vivo* studies have suggested that the addition of growth factors (G-CSF, GM-CSF) or interleukin-3 (IL-3) may potentiate the effect of r-EPO on dysplastic erythropoiesis. The aim of this study was to evaluate the effects of the combination of r-EPO with G-CSF, GM-CSF or IL-3 on the anemia of heavily transfusion-dependent MDS patients, previously unresponsive to r-EPO alone.

Patients and Methods. Sixty patients with transfusion-dependent MDS, already treated without significant erythroid response with r-EPO alone, were scheduled to receive, for at least 8 weeks, r-EPO subcutaneously at the dose of 300 U/kg t.i.w. in combination with G-CSF (300 µg s.c. t.i.w., 27 patients), or GM-CSF (300 µg s.c. t.i.w., 23 patients), or IL-3 (5 µg/kg s.c. t.i.w., 10 patients), after a two-week pre-phase during which G-CSF, GM-CSF and IL-3 were administered daily at the same dose, as single drugs.

Results. Ten patients were not evaluable for erythroid response because of relevant side effects related to GM-CSF or IL-3 administration. Overall, among 50 patients who completed the study, there were 3 erythroid responses (as determined by complete abolition of red-cell transfusions): 1 (4%) in the G-CSF + r-EPO and 2 (10.5%) in the GM-CSF + r-EPO treated groups. No patient responded to the combination of r-EPO + IL-3. All responders had inappropriate serum levels of endogenous EPO and a relatively short disease duration. Both responders to GM-CSF + r-EPO developed acute myeloid leukemia 2-9 months after the start of the combined therapy. A third elderly patient, treated with the same association, developed marrow hypoplasia. A significant increase in leukocyte count occurred in 96% of patients who received r-EPO + G-CSF, 78.9% of those treated with r-EPO + GM-CSF and 66% of subjects receiving r-EPO + IL-3. A significant increase in platelet count was observed in a single patient receiving r-EPO and GM-CSF, while a slight decrease in platelet count with respect to baseline levels occurred in about 20% of patients.

Interpretation and Conclusions. Our results suggest that the combination of r-EPO with G-CSF, GM-CSF or IL-3, at least at the doses and schedules employed in the present study, has limited efficacy on the anemia of heavily transfusion-dependent MDS patients previously unresponsive to r-EPO alone. However, in this setting of patients, the combination of G-CSF or GM-CSF + r-EPO may occasionally be effective in subjects with low circulating levels of serum EPO and short disease duration.

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Key words: erythropoietin, G-CSF, GM-CSF, interleukin-3, anemia, myelodysplastic syndromes

Many clinical studies have indicated that about 20-25% (range 0-56%) of patients with myelodysplastic syndromes (MDS) treated with recombinant erythropoietin (r-EPO) alone may have variable benefits in terms of reduction of red cell transfusions and improvement of hemoglobin levels.¹⁻⁴⁰ Although the exact mechanisms of action are still not completely elucidated, it has been suggested that pharmacologic doses of this drug could: i) stimulate residual non-clonal erythropoiesis; ii) promote erythroid differentiation of clonal progenitors with reduced but still not abolished responsiveness; iii) neutralize the activity of cytokines with inhibitory effects on erythropoiesis (i.e. tumor necrosis factor (TNF) and interleukin (IL-1β)); iv) reduce the apoptotic processes of the neoplastic clone.⁴¹

The most useful parameters for predicting response to r-EPO in MDS (low baseline levels of serum EPO and no transfusion requirements)⁴² identify only a minority of patients, since endogenous production of EPO in MDS is usually adequate for the degree of anemia^{43,44} and a large proportion of patients with MDS are transfusion-dependent. This is in substantial agreement with the unsatisfactory results obtained using r-EPO alone *in vivo* in these patients.⁴⁵ Indeed, when more stringent criteria of response are adopted (complete and stable interruption of transfusions) or selected groups of

Table 1. Clinical trials with combined cytokine therapies in myelodysplastic syndromes.

Reference	No. pts	G-CSF	EPO	Duration (weeks)	Erythroid response (complete+partial)
Negrin <i>et al.</i>	24	0.2-5 µg/kg/d	100-300 U/kg/d	8-16	10/24 (42%)
Hellstrom-Lindberg <i>et al.</i>	22	0.3-3 µg/kg/d	60-120 U/kg/d	12	8/21 (38%)
Negrin <i>et al.</i>	55	0.2-5 µg/kg/d	100-300 U/kg/d	8-16	21/44 (48%)
Hellstrom-Lindberg <i>et al.</i>	50	30-150 µg/d	5000-10000 U/d	10-12	18/47 (38%)
Remacha <i>et al.</i>	14	1 µg/kg/t.i.w.	300 U/kg t.i.w.	8	5/11 (45%)
Himamura <i>et al.</i>	10	400-1200 µg/sqm/d i.v.	100-400 U/kg t.i.w.	10	0/10 (0%)
Musto <i>et al.</i>	12	300 µg/t.i.w.	300 U/kg/t.i.w.	8	0/12 (0%)
Mantovani <i>et al.</i>	33	1.5 µg/kg/d (adjusted)	200-400 U/kg t.i.w.	12-36	20/25 (80%)

Reference	No. pts	GM-CSF	EPO	Duration (weeks)	Erythroid response (complete+partial)
Hansen <i>et al.</i>	13	1.5-3 µg/kg/d	60-120 U/kg/d	12	5/11 (45%)
Runde <i>et al.</i>	10	150 µg/sqm for 10 days	100/200 U/kg/d from d 11	8-33	2/8 (25%)
Bernell <i>et al.</i>	13	200 µg/d weeks 1-6	10.000 U t.i.w. weeks 5-14	14	3/13 (23%)
Economopoulos <i>et al.</i>	19	3 µg/kg days 1-2	60-120 U/kg days 3-5	12	10/19 (52.6%)
Stasi <i>et al.</i>	31	1 µg/kg/d adjusted	150-300 U/kg alternate days	12	9/26 (34.6%)
Thompson <i>et al.</i>	66	0.3-5 µg/kg/d	150 U/kg t.i.w.	12	4/45 (9%)

Reference	No. pts	IL-3	EPO	Duration (weeks)	Erythroid response (complete+partial)
Miller <i>et al.</i>	22	0.75-3 µg/kg/d	150-300 U/kg t.i.w	12	2/21 (9.5%)
Verhoef <i>et al.</i>	8	5-10 µg/kg/d	500 U/kg/t.i.w.	6	1/8 (16.6%)

patients are evaluated (only severely anemic transfusion-dependent patients), the response rate to r-EPO in MDS declines to 10-15% or even less.^{36,42,45} Further criteria for a good response, such as absence of ring sideroblasts, low marrow blast count, a recent diagnosis and low circulating serum levels of TNF and IL-1, have also been proposed, but still not extensively confirmed.^{36,42,46}

The combination of r-EPO with additional growth factors in MDS could be justified by the frequent association of anemia with other cytopenias which can respond to different cytokines. In particular, pooled data from clinical trials have shown a significant increase in leukocyte count in 90%, 76% and 36% of MDS patients treated with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3, respectively, as single drugs.⁴⁷⁻⁵⁰ Occasionally, improvement of platelet count has also been reported. In addition, some *in vitro* studies have suggested a possible synergistic effect between these cytokines and r-EPO on the regulation of erythropoiesis.⁵¹⁻⁵⁴ Thereby, several phase I/II clinical trials involving combinations of these growth factors, administered either simultaneously or sequentially, have been performed in order to target proliferation and differentiation of both early and late stages of hematopoiesis (Table 1). In this setting, the combination of G-CSF with r-EPO has yielded impressive results in some studies, with erythroid responses

observed in up to 80% of cases.⁵⁵⁻⁶¹ However, the clinical and laboratory characteristics of treated patients (in particular the levels of hemoglobin and those of serum EPO) seem to affect the possibility of an erythroid response significantly.⁶² Indeed, other studies, including our own initial experience, did not show any significant improvement using this combined therapy with respect to erythropoietin alone.^{63,64} The results of the association of r-EPO with GM-CSF are even more complex to evaluate, since they are often obtained in small and heterogeneous groups of patients with variable response rates in different studies.⁶⁵⁻⁷⁰ Finally, the data regarding the combination of IL-3 and r-EPO in MDS are quite scanty and disappointing.^{71,72}

In order to define the effect on anemia of these combined therapies in a well defined subgroup of patients, the present study was undertaken to assess the therapeutic role of the combination of r-EPO plus G-CSF, r-EPO plus GM-CSF or r-EPO plus IL-3 in heavily transfusion-dependent MDS patients, selected on the basis of previous unresponsiveness to r-EPO alone.

Design and Methods

Sixty transfusion-dependent (Hb levels < 8 g/dL) MDS patients, seen at our Institution between 1994 and 2000, were evaluated. According to FAB and WHO classifications,^{73,74} there were 30 cases of refractory anemia

(RA, 2 of which were secondary to previous chemoradiotherapy), 20 RA with ring sideroblasts (RARS), and 10 RA with excess of blasts (RAEB), 5 of which had a percentage of marrow blasts between 11 and 20%. Thirty-four, 20 and 6 patients had low, intermediate or high risk MDS, respectively, according to the International Prognostic Scoring System (IPSS).⁷⁵ In addition to supportive transfusional therapy, previous treatments mainly included steroids, folates and other vitamins, danazole and desferrioxamine, for iron overload. Furthermore, all patients had previously received r-EPO alone (up to 300 U/kg s.c., t.i.w., for at least two months), without significant improvement of Hb levels or transfusion requirement. r-EPO treatment had been performed 6 to 24 months (mean 11 months) before combined therapy was started.

Twenty-seven patients were selected to receive a combination of r-EPO + G-CSF, 23 patients to receive r-EPO + GM-CSF, and 10 patients r-EPO + IL-3. Table 2 reports the patients' main clinical and laboratory characteristics. The treatment was generally administered on an outpatient basis, after a wash-out period of three months during which patients underwent only transfusional and iron-chelating treatments.

The following schedule was employed in each group of patients: a) pre-phase (14 days) with G-CSF (Granulokine, Roche; Granocyte-34, Rhône-Poulenc-Rorer; Neupogen, Dompé-Biotec) 300 µg/d s.c., or GM-CSF (Mielogen, Schering-Plough; Leucomax, Novartis) 300 µg/d s.c., or IL-3 (SDZ-ILE 964, Sandoz/Novartis) 5 µg/kg/d s.c. The duration of the pre-phase was shortened if the WBC count exceeded 10,000/µL or if relevant side effects occurred; b) at the end of the pre-phase, all patients continued to receive the same dose of G-CSF or GM-CSF or IL-3 plus r-EPO (300 U/kg s.c.), each t.i.w., for 2 months. Overall, 10 weeks of treatment were considered enough and needed for evaluation. Patients or relatives were instructed to draw up the appropriate

amount of drugs into a syringe and inject the drugs subcutaneously.

Clinical examination, hematologic parameters and other routine laboratory tests of liver, renal and clotting function were monitored at least every two weeks. Bone marrow morphologic and karyotype analyses were performed at baseline and at the end of the study in each patient. In order to evaluate modifications of erythropoiesis under cytokine therapy, serum levels of soluble transferrin receptor (sTfR), a marker of marrow erythroid activity, and the automated count of high fluorescence reticulocytes (HFR), an index of effective erythropoiesis, were also monitored, as previously described.³⁴ The possible development of functional iron deficiency was evaluated by means of plasma ferritin levels and the automated count of hypochromic erythrocytes (HE), as previously reported.³⁴

Complete interruption of red cell transfusions, with stable Hb levels maintained above 8.5 g/dL for at least one month, was considered as the only criterion for erythroid response.

Results

Ten patients (6 while receiving GM-CSF and 4 during administration of IL-3) did not complete the pre-phase because of relevant side effects (fever, arthralgias, abdominal pain, hypotension, general malaise) and were not evaluable for final analysis. These and other adverse events occurring during or immediately after the study are reported in Table 3.

Fifty patients completed the programmed treatment and were evaluable for hematologic response (Table 4). An erythroid response occurred in 3 patients (one receiving r-EPO + G-CSF and two receiving r-EPO + GM-CSF). Table 5 summarizes the characteristics of erythroid responders. All of them had relatively low serum levels of endogenous EPO and a short history of disease. No improvement of anemia was observed in any patient

Table 2. Demographic, clinical and laboratory characteristics.*

	Total	G-CSF+EPO	GM-CSF+EPO	IL-3+EPO
Male/Female	30/30	12/15	13/10	5/5
Age	65.3 (19-85)	66.4 (35-81)	64.5 (39-85)	65.1 (58-79)
FAB/WHO				
RARS	20	7	9	4
RA	30	14	11	5
RAEB	10	6	3	1
IPSS (L/I/H) ^o	34/20/6	15/9/3	13/8/2	6/3/1
Hb (g/dL)	7.1 (4.8-7.9)	7.3 (5-7.9)	7 (6.2-7.9)	7.2 (4.8-7.8)
Neutrophils (x10 ⁹ /L)	3.2 (0.3-8.2)	3.1 (0.3-7.3)	3.2 (0.9-5.2)	3.4 (1-8.2)
Platelets (x10 ⁹ /L)	165 (6-628)	188 (6-511)	135 (18-628)	149 (40-260)
Transfusions (unit/month)	3.6 (1-8)	3.8 (2-6)	3.6 (2-6)	3.3 (1-8)
Serum EPO (miu/µL)	570 (39-3670)	551 (39-3670)	459 (45-2880)	690 (110-3060)
Time from diagnosis (months)	34 (6-73)	32 (6-73)	37 (8-60)	30 (12-58)

*Age, hemoglobin (Hb), neutrophils, platelets, transfusions, serum EPO and time from diagnosis are expressed as mean values (ranges in brackets); ^oInternational Prognostic Scoring System (low, intermediate, high risk).

Table 3. Adverse events and side effects occurred during or after combined therapy.

	G-CSF + EPO	GM-CSF + EPO	IL-3 + EPO
Skin rash/erythema	0	2	2
Myalgia/arthralgia	1	10	5
Fever	0	6	8
Bone pain	2	4	2
Abdominal pain	0	3	0
Hypotension	0	0	2
Headache	1	1	3
Edema	0	2	2
Splenomegaly	0	2	0
Thrombocytopenia	4	3	2
Asthenia/fatigue	1	6	6
Marrow hypoplasia	0	1	0
Leukemic evolution	0	2	0

Table 4. Hematologic responses to combined therapy in evaluable patients.

	G-CSF + EPO	GM-CSF + EPO	IL-3 + EPO
Hb	1/25 (4%)	2/19 (10.5%)	0/6 (0%)
WBC	24/25 (96%)	15/19 (78.9%)	3/6 (50%)
PLT	0/25 (0%)	1/19 (5.2%)	0/6 (0%)

Table 5. Characteristics of erythroid responders.

Sex	Age (years)	FAB/WHO classification	Hb (g/dL) pre/post	Transf./month pre/post	IPSS	Serum EPO (miu/mL)	Time from dx (months)	Combined therapy
M	58	RA	6.4/8.6	4/0	Low	65	8	G-CSF+EPO
F	56	RA*	6.3/9.5	4/0	Low	108	10	GM-CSF+EPO
F	53	RAEB	7/10.8	2/0	High	98	11	GM-CSF+EPO

*Secondary. dx: diagnosis.

receiving r-EPO + IL-3. Serum levels of sTfR and HFR rose significantly in all responders and preceded the increase of hemoglobin by about 10 days. No case of sTfR increase without concomitant improvement of HFR count, as expression of stimulation of ineffective erythropoiesis, was observed (data not shown). No patient developed functional iron deficiency, as evaluated by HE count, which never exceeded 7%, and plasma ferritin values, which also remained substantially unchanged even in responders.

Erythroid response disappeared after suspension of the combined treatment in the G-CSF + r-EPO treated patient due to patient's choice after 4 months of effective, adjusted therapy. Both responders to r-EPO + GM-CSF (1 RA secondary to breast cancer previously treated with chemo-radiotherapy and 1 RAEB) transformed into acute leukemia (AML), the former after 2 months

of continued combined therapy, the latter 4 months after the interruption of the treatment. In this case, r-EPO plus GM-CSF therapy had been prolonged for 5 months and then stopped because of the patient's refusal to continue the treatment. A third, elderly patient receiving the same combination developed bone marrow aplasia after 9 weeks of therapy.

A significant increase in leukocyte counts occurred in 24/25 (96%) of patients who received r-EPO + G-CSF, 15/19 (78.9%) of those treated with r-EPO + GM-CSF and 4/6 (66%) of subjects receiving r-EPO + IL-3. A dramatic increase in platelet count was observed in a single patient receiving r-EPO plus GM-CSF (from 19 to 126×10⁹/L), while a slight decrease of platelet count with respect to baseline levels occurred in about 20% of patients (Table 4).

Marrow morphology did not change significantly under combined treatment. Some patients with high neutrophil response showed a transient, mild increase of myeloid precursors and, in some cases, of blast percentage. No substantial quantitative modifications of erythropoiesis occurred in responders. All karyotypes remained unchanged at the end of therapy.

Discussion

The aim of this study was to evaluate the clinical effects on anemia of r-EPO in combination with G-CSF, GM-CSF or IL-3, in a selected cohort of 60 heavily transfusion-dependent patients with MDS previously unresponsive to r-EPO alone. Overall, three significant erythroid responses, accounting for 4% in the G-CSF + r-EPO and 10.5% in the GM-CSF + r-EPO groups, were observed.

Despite the fact that IL-3 is an early growth factor stimulating the *in vitro* proliferation and differentiation of erythroid precursors, which could then theoretically be induced to complete maturation by r-EPO, treatments employing these two cytokines have so far been disappointing (Table 1). In fact, only 3 out of 30 (10%) reported patients who received such a combination of drugs showed an erythroid response, a result comparable with that obtained by r-EPO alone.^{71,72} In these studies worrisome findings were the high general toxicity of IL-3 and the frequent development of thrombocytopenia in treated patients, probably related to the induction of TNF, a potent inhibitor of megakaryocytopoiesis.⁷⁶ Our results confirm that the association of r-EPO and IL-3 is quite toxic and has poor effects on erythropoiesis in MDS.

The role of the combination of GM-CSF with r-EPO is more difficult to evaluate, since heterogeneous results have so far been reported (Table 1). Hansen *et al.*⁶⁵ treated 13 MDS patients with GM-CSF and r-EPO for 12 weeks, after 6 weeks of GM-CSF alone, obtaining 3 complete responses in untransfused patients and the reduction of transfusional needs in a further 2 patients. Runde *et al.*⁶⁶ gave sequential therapy with GM-CSF and r-EPO to 10 patients with MDS. Two out of 8 evaluable patients showed a temporary loss of transfusion requirements which, in the Authors' view, was related to r-EPO

treatment. Bernell *et al.*⁶⁷ treated 13 MDS patients previously resistant to r-EPO alone with a second phase study in which, after 6 weeks of GM-CSF alone, r-EPO was administered for ten additional weeks. In this study 3 patients had an erythroid response. Economopoulos *et al.*⁶⁸ treated 19 patients with MDS using brief, multiple weekly cycles of sequential GM-CSF plus r-EPO for 12 or more weeks. Seven patients were reported to have a good erythroid response, while a further 3 subjects achieved a partial erythroid response. Prolonged responses were obtained, in particular, in 6 out of 8 cases of RARS. In a recent study by Stasi *et al.*⁶⁹ 31 patients with low or intermediate risk MDS were enrolled in a 12-week trial in which they received adjusted daily doses of GM-CSF plus r-EPO on alternate days. Nine out of 26 evaluable patients achieved a clinically significant erythroid response (2 good and 7 partial responses). However, no effect on anemia was seen among 7 patients who had not responded to prior treatment with r-EPO alone. In the largest study so far reported, Thompson *et al.*⁷⁰ found no more than a trend towards reduced transfusion requirement in patients with serum EPO < 500 miu/mL as a result of a randomized, double blinded, placebo-controlled trial including 66 MDS patients and comparing the administration of GM-CSF plus placebo versus GM-CSF plus r-EPO. In this study, 4 out of 45 patients receiving GM-CSF plus r-EPO (9%) had an hemoglobin response versus 1/21 (5%) in the GM-CSF + placebo group ($p \geq n.s.$). In our experience, here reported, 2 out of 19 patients temporarily lost their transfusion dependence while receiving r-EPO plus GM-CSF. However, both these patients (one with high risk RAEB, the other with a history of secondary MDS) developed secondary acute myeloid leukemia during the follow-up. Although a strict correlation between combined therapy and leukemic evolution could not be assessed, these events, along with the development of marrow aplasia in a further old subject treated with r-EPO and GM-CSF, suggests caution in administering such a combination to elderly patients with MDS.

The combination of r-EPO with G-CSF (Table 1), after a pre-phase with G-CSF alone, gave an erythroid response in 8/21 (38%) and 10/24 (42%) of MDS treated, respectively, in two initial studies.^{55,56} In the European trial patients with RARS responded very well to the combined treatment,⁵⁶ but this was not confirmed in the study by Negrin *et al.*⁵⁵ Bessho *et al.*⁵⁷ obtained 4 erythroid responses (57%) in 7 MDS patients treated with G-CSF plus EPO. All responders had serum EPO levels inappropriate for the degree of anemia. A long-term evaluation of a second, larger American study³⁸ showed that a substantial proportion of erythroid responses obtained with the association of r-EPO and G-CSF were stable and long-lasting. In this study, the suspension of G-CSF determined a decrease of hemoglobin levels in about 50% of responders. Resumption of G-CSF was required for recurrent erythroid responses in most of these patients, thus suggesting a possible *in vivo* synergistic effect on erythropoiesis provided by the two

cytokines. The American and Scandinavian patients were then pooled to develop a predictive scoring system for erythroid response in MDS patients treated with r-EPO plus G-CSF based on pre-treatment serum EPO levels and transfusional needs.⁶² Using this score, non-transfused (or transfused with less than 2 units/month) MDS patients with low (< 100 miu/mL) EPO levels had a very high possibility (74%) of responding to the combined treatment, while a few subjects (7%) undergoing more than 2 transfusions/month and with high circulating EPO (> 500 miu/mL) were expected to have an erythroid response. A further phase II, randomized trial in 50 MDS patients evaluated the efficacy of the combination of r-EPO plus G-CSF at fixed doses, after an initial treatment with r-EPO or G-CSF alone,⁵⁹ and substantially confirmed an erythroid response rate of 38%, with the possibility of stable and long-lasting responses under adjusted doses of both drugs (again, in particular in RARS) and established that pre-treatment with G-CSF is not necessary. In a more recent report, Remacha *et al.*⁶⁰ added G-CSF to r-EPO in 14 patients with RA or RARS, achieving a total of 7 (50%) erythroid responses (2 complete responses in 3 patients with partial response after r-EPO alone and 1 complete response + 4 partial responses in 11 subjects completely resistant to r-EPO). Finally, Mantovani *et al.*⁶¹ have very recently obtained an erythroid response rate of 61% (12 good and 5 partial erythroid responses) after 12 weeks of combined G-CSF and r-EPO treatment in 28 MDS patients with short disease duration (less than 6 months). In this trial, the erythroid response rate reached 80% (14 good and 6 partial responses) in 25 patients who prolonged the combined therapy up to 36 weeks.

Thus, the most striking difference between our study and previous works lies in the very low proportion of erythroid responses we observed in MDS patients treated with r-EPO plus G-CSF. This finding, however, confirms our preliminary report, in which we did not observe any significant erythroid response in 12 transfusion-dependent MDS patients previously unresponsive to r-EPO alone and then treated with r-EPO plus G-CSF.⁶⁴ Similar results have also been reported by Himamura *et al.*⁶³ Thus, why such a discrepancy? First of all, it should be stressed that all previous studies have included variable proportions of non transfusion-dependent patients (in two reports even selected from among subjects with unusual, low baseline serum EPO),^{60,61} a population which has the best possibility of achieving an erythroid response after r-EPO and G-CSF (as well as after r-EPO alone!).^{42,62} Indeed, by reviewing the characteristics of single patients included in some studies, it emerges that the large majority of erythroid responses after r-EPO plus G-CSF were achieved in subjects with no or very low transfusion needs.^{55,56,61} These patients were not represented in our selected population of heavily transfusion-dependent MDS. The exclusion from our analysis of the so-called *partial responses*, which generally have limited clinical impact, should also be considered in the evaluation of our data. Therefore, on this basis, the pos-

sible synergistic effect of r-EPO and G-CSF could be confined to less anemic, untransfused (or rarely transfused) MDS patients. However, in our opinion, it is highly questionable whether such patients really need to be treated using these very expensive drugs in view of their usually acceptable quality of life.

The good results obtained with combined G-CSF + r-EPO treatment in patients unresponsive to r-EPO alone are, again, not always convincing. In the first series by Negrin *et al.*,⁵⁵ only one patient, who had received a non-optimal r-EPO schedule, responded to G-CSF plus r-EPO out of five subjects who had been previously unsuccessfully treated with r-EPO alone. Remacha *et al.*⁶⁰ obtained only one complete erythroid response among 11 patients without any response to r-EPO alone, even within a selected cohort of MDS patients with low baseline serum levels. The possibility of late responses due to previously received r-EPO in patients undergoing combined treatment after short or no wash-out period,^{59,60} also cannot be completely ruled out. We have observed at least 3 MDS patients with delayed (up to six months after suspension!), prolonged and significant erythroid responses to r-EPO alone.

Schedules and doses could also play a significant role in this setting. It is of note that in the only randomized, placebo-controlled study so far conducted, an overall erythroid response rate of 36% was achieved using daily, relatively high dose r-EPO alone (300 U/kg/d).⁴⁰ Such a response rate is not substantially different from that reported in the largest trials employing r-EPO plus G-CSF, so that the comparison between results obtained in single and combined studies should be performed on the basis of these optimal results. Interestingly, the response rate in the Italian study dropped to 16% when r-EPO was administered at the same dose, but on an alternate day schedule, in an open-phase following the randomization.⁴⁰ Of note, in the Scandinavian experience on combined therapy,^{56,59} r-EPO was administered at daily doses corresponding to about half or even less than those used in the randomized Italian trial.⁴⁰ On the other hand, high dose G-CSF, as employed by Himamura *et al.*,⁶³ could have had negative effects by stimulating the production of cytokines with inhibitory activity on erythropoiesis.

In conclusion, our results and a critical review of the literature suggest that the combination of r-EPO with G-CSF, GM-CSF or IL-3, at least at the doses and schedules employed in the present study, has limited efficacy on the anemia of heavily transfusion-dependent patients with MDS, previously unresponsive to r-EPO alone. Thus, we do not recommend the widespread use of such combinations in this setting of patients. A possible exception, however, could be the combination of G-CSF or GM-CSF + r-EPO in MDS in younger subjects with low circulating levels of serum EPO, short disease duration and low-risk disease. For non-transfusion dependent MDS, combined therapy should be considered only within clinical trials based on well-conducted cost-effectiveness, safety and quality-of-life analyses.

Contributions and Acknowledgments

PM designed the study, was responsible for the patients' and data management and wrote the manuscript. GS, PRS, AF, CB and MC participated in the patients' care. GD, RM and GP were involved in laboratory studies. All authors contributed critically to the drafting of the article. The order of the Authors reflects their real contribution to the paper, with the exception of MC, who was the Head of our Department at the time of submission and gave his final approval to the work. He died on July 21st, 2000. This paper is dedicated to him.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript has been peer-reviewed by two external referees and by Professor Mario Cazzola, Executive Editor. The final decision to accept this paper for publication was taken jointly by the Editors. Manuscript received July 17, 2000; accepted December 11, 2000.

Potential implications for clinical practice

The addition of G-CSF or GM-CSF to erythropoietin only occasionally has significant positive effects on anemia of severely transfusion-dependent MDS patients previously unresponsive to erythropoietin alone. In this setting, a therapeutic attempt should be limited, outside of clinical trials, to patients with inappropriate endogenous erythropoietin levels and a short history of disease. In these patients, the combination of IL-3 and erythropoietin appears to be toxic and not effective.

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