Treatment of anaemia in myelodysplastic syndromes with prolonged administration of recombinant human granulocyte colony-stimulating factor and erythropoietin

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Summary. Treatment with recombinant human erythropoietin (rhEPO) improves anaemia in ≈ 20% of the patients with myelodysplastic syndromes (MDS). Recent reports suggest that a combination treatment with rhEPO plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) given for up to 18 weeks may result in a higher erythroid response rate than with rhEPO alone. We investigated the potential advantage of an even more prolonged schedule of combined rhG-CSF and rhEPO treatment to obtain and maintain stable responses. In a phase II study, 33 patients with MDS [17 with refractory anaemia (RA), eight with RA with ringed sideroblasts (RARS), eight with RA with excess blasts (RAEB) with bone marrow blast counts less than 20%] were scheduled to receive at least 36 weeks of combined therapy with rhG-CSF and rhEPO. Seventeen of 28 evaluable patients demonstrated an erythroid response [61%; 95% confidence interval (CI) 41–78] after 12 weeks of treatment. The erythroid response rate was 80% (20 of 25 evaluable patients; 95% CI 59–93) after 36 weeks. Seven of these responses developed between week 12 and week 36, whereas two initially responding patients became refractory. The cytokine therapy was generally well tolerated. Nineteen of the 20 patients responding after 36 weeks continued to be treated with both cytokines. After 1 year and 2 years of continuous combined treatment, 50% of the initially included patients showed a continuing response. Our results suggest that a prolonged combination treatment with rhG-CSF and rhEPO is highly effective in achieving a stable and long-lasting erythroid response in many patients with MDS and low blast count.

Keywords: myelodysplastic syndromes, granulocyte colony-stimulating factor, erythropoietin, prolonged cytokine combination treatment, erythroid response.

Patients with myelodysplastic syndromes (MDS) have cytopenias often leading to an increased risk of infections and transfusion requirements of red blood cells. MDS patients also have an increased risk of progression to acute myeloid leukaemia (Sanz *et al*, 1989). In low-risk MDS, anaemia is often the major clinical problem (Dreyfus, 1976; Linman & Bagby, 1978; Ganser & Hoelzer, 1992a; Greenberg *et al*, 1997). Standard treatment options are limited because of low efficacy of standard cytoreductive chemotherapy in this disease and because of the advanced age of these patients (Armitage *et al*, 1981; Spriggs *et al*, 1986).

Cytopenias in MDS may in some cases be improved by treatment with haematopoietic growth factors. Neutrophil responses have been observed in the majority of patients treated with recombinant human granulocyte colony-stimulating factor (rhG-CSF), but have so far not been shown to have a positive impact on survival (Kobayashi *et al*, 1989; Negrin *et al*, 1989, 1990; Ohyashiki *et al*, 1989; Yoshida *et al*, 1991; Kaczmarski & Mufti, 1993). Recombinant human erythropoietin (rhEPO) has been used in several investigations to correct anaemia in MDS. The overall erythroid response rate ranged between 10% and

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28% (Geissler *et al*, 1997). Higher response rates were recently reported in two studies in which rhG-CSF and rhEPO were given in combination. Negrin *et al* (1996) treated patients for an initial 8 weeks with rhEPO alone and for the subsequent 8 weeks with daily doses of 100–300 U/kg rhEPO and 1 µg/kg rhG-CSF (range 0·1–5 µg/kg). Twenty-one of 44 (48%) evaluable patients [14 with refractory anaemia (RA), 21 with RA with ringed sideroblasts (RARS), nine with RA with excess blasts (RAEB)] showed an erythroid response. Hellstroem-Lindberg *et al* (1998) treated patients with 5000–10 000 U/d rhEPO and 30–150 µg/d rhG-CSF for 12–18 weeks. Of 47 evaluable patients, 18 (38%) showed an erythroid response. However, the response criteria used in this trial were somewhat more strict than those used by Negrin *et al* (1996). In both studies, after these treatment periods, no fixed maintenance strategies were adopted and options ranged from cessation of treatment to continuation with both cytokines or with rhEPO only. Longer follow-up in both trials showed that responses were lost in over 50% of initially responding patients. Another small phase II trial (Imamura *et al*, 1994) failed to reproduce similar results.

It was the objective of our phase II study to examine the potential benefit of a prolonged combination treatment with rhEPO and rhG-CSF in MDS patients. We enrolled patients with RA and RARS exhibiting a bilineage or trilineage cytopenia and patients with RAEB with less than 20% bone marrow blast cells. We decided to treat these patients for at least 36 weeks with both cytokines and to continue treatment thereafter in all responders. The major objective was to monitor onset and duration of the erythroid response, the effects on the other haematopoietic cell lineages, safety and transition to acute myeloid leukaemia (AML).

MATERIALS AND METHODS

Patients. From January 1994 to December 1997, 33 patients were included in the study. All patients signed informed consent forms and the protocol was approved by the local ethics committees. Diagnosis of MDS was established by blood and bone marrow findings (histopathological or cytomorphological) and classified according to the criteria of the French–American–British (FAB) Cooperative Group (Bennett *et al*, 1982).

Entry criteria for the study included a clinical diagnosis of primary and secondary MDS subtype RA/RARS with either bicytopenia or a history of severe infections within the last 6 months. Furthermore, MDS subtype RAEB was included. Cytopenias were defined as haemoglobin < 9 g/dl or red blood cell (RBC) transfusion requirements, neutrophil counts $< 3·0 \times 10^9$/l, platelet counts $< 1·0 \cdot 10^9$/l. Further inclusion criteria were age ≥ 18 and Karnofsky performance status $> 60\%$. Haematopoietic growth factor use 8 weeks before study entry was not permitted.

Treatment protocol. All patients received standard supportive care. Patients were treated as needed with antibiotics for infection, RBC transfusions for anaemia and platelet transfusions to treat thrombocytopenic bleeding. RhG-CSF (Neupogen) was administered as a daily subcutaneous injection at a starting dose of 1·5 µg/kg/d. Doses were adjusted every 2 weeks to maintain a normal absolute neutrophil count (ANC) ($3·0 \times 10^9$/l $\leq$ ANC $\leq 10·0 \times 10^9$/l). Simultaneously, rhEPO (Erypo) was given subcutaneously at a starting dose of 200 U/kg three times weekly. After 6 weeks of treatment, the dose of rhEPO was escalated to 400 U/kg if no erythroid response had occurred. Treatment was scheduled to be given for a minimum of 36 weeks. After completion of the 36-week treatment period, continuation was left to the decision of the treating physician, but all responding patients were recommended to stay on treatment. Cytokine treatment was discontinued because of side-effects, patient withdrawal or if an increase of bone marrow blasts $> 30\%$ occurred.

Full blood counts and reticulocyte counts were obtained weekly. Blood chemistry, coagulation profiles, iron, transferrin, ferritin, serum EPO levels, bone marrow aspirates, bone marrow biopsy and cytogenetic analyses were performed before treatment and at each scheduled treatment evaluation.

Response evaluation. Patients were evaluated for haematological response after 12 weeks of treatment (first evaluation) and after 36 weeks of treatment (second evaluation). Good erythroid response (GER) was defined according to Negrin *et al* (1996) as complete loss of transfusion requirement or as an increase of the haemoglobin level by more than 2 g/dl if transfusion was not required. A partial erythroid response (PER) was defined as a reduction of the transfusion frequency by at least 50% averaged over the preceding 2 months or an increase of the haemoglobin by 1–2 g/dl compared with initial values if transfusion was not required.

The disease status was classified as stable if no change in the MDS subtype occurred. Disease progression was defined as any change of the FAB subtype or an increase of the bone marrow blasts beyond 30%.

Statistics. Statistical analyses were performed using Student's *t*-test, Mann–Whitney's *U*-test and the Wilcoxon's matched pair rank test. The χ^2 test and the McNemar test were used to test statistical significance in contingency tables. Fisher's exact test was applied when appropriate for small samples. To evaluate the agreement between two measurements, the kappa-coefficient was calculated.

Survival time, time to AML evolution, time to treatment failure and duration of erythropoietic response were analysed using Kaplan–Meier curves and the log-rank test. *P*-values less than 0·05 were considered statistically significant.

All survival time estimates started at the time of start of cytokine treatment. Survival included deaths as events for whatever reason. Time to AML considered time at first detection of more than 30% bone marrow blasts or onset of overt AML as event. Time to treatment failure considered failure of erythroid response at 36 weeks or later as events as well as transformation to AML or death, whichever came first. The erythroid response duration was estimated by censoring deaths and onset of AML and considering failures of erythropoietic response at week 36 or any time point thereafter as an event.

RESULTS

Patient characteristics

Thirty-three patients were enrolled. A summary of the patients characteristics is shown in Table I. Seventeen patients had RA, eight had RARS and eight had RAEB. Twenty-seven patients belonged to the low-risk group (SANZ score 0–3) and six to the high-risk group (SANZ score ≥ 4) (Sanz *et al*, 1989). However, the low-risk profile was accentuated by the occurrence of bicytopenias (19 cases) and tricytopenias (13 cases). Only one case had monocytopenia. Twenty-seven patients were dependent on RBC transfusions in the prestudy period (median two RBC units/month). Cytokine treatment was discontinued before the first evaluation for erythroid response in five patients (one because of patient's request at week 4, one because of bone pain at week 3, one because of unrelated disease at week 4 and two because of transition to AML, one at week 6 and one at 11). Thus, 28 patients completed the 12 weeks combined administration of rhG-CSF and rhEPO therapy.

Erythroid response: evaluation after 12 weeks of treatment

Of 28 patients evaluable after 12 weeks of treatment, 12 patients achieved a good erythroid response (GER 43%), five patients a partial erythroid response (PER 18%) and 11 patients showed no improvement (Table II). Hence, the erythroid response rate was 61% [95% confidence interval (CI) 41–78]. The number of responders in the different MDS subtypes were 10/15 (67%), 2/6 (33%) and 5/7 (71%) for RA, RARS and RAEB respectively. The difference in response rate in the different subgroups was not statistically significant. The haemoglobin increase is shown in Fig 1. Ten patients (36%) became transfusion independent.

Erythroid response: evaluation after 36 weeks of treatment

Twenty-five patients were evaluable for erythroid response after 36 weeks of cytokine treatment (Table II). The overall erythroid response rate was 80% (95% CI 59–93). Fourteen patients achieved a GER and six a PER. Five patients who were non-responders after 12 weeks of treatment developed a late response (one GER and four PER) during further cytokine treatment. Two patients with a PER after 12 weeks converted into good responders after 36 weeks. In two patients who had responded after 12 weeks, haematological parameters worsened and they were classified as non-responders after 36 weeks of combined cytokine therapy. The change in response rate after weeks 12 and 36 of these 25 patients was not statistically significant ($P = 0.453$).

Figure 1A and B shows the increase of the haemoglobin levels between the first and second evaluation in both groups of patients whether they had achieved an initial erythroid response after 12 weeks or not. For responders and also for patients who were classified as non-responders after an initial 12 weeks of treatment, the difference in haemoglobin levels between the first and second evaluations is significant ($P = 0.033$ and $P = 0.031$ respectively), suggesting that there may be delayed stimulatory effects in both groups.

Table I. Baseline patients' characteristics.

	All patients* ($n = 33$)	Patients evaluable for response at 12th week ($n = 28$)
Male/female	18/15	15/13
Median age (range)	62 (51–86)	62 (51–86)
MDS subtype: RA/RARS/RAEB	17/8/8	15/6/7
SANZ score		
0–3	27	23
4–5	6	5
Disease duration		
< 6 months	25	21
$\geq$ 6 months	8	7
RBC transfusion dependent, yes/no	27/6	23/5
Median RBC transfusion, units/month (range)	2 (1–8)	2 (1–8)
Anaemia, Hb < 9·0 g/dl	27	23
Neutropenia, ANC < 3×10^9/l	30	26
Thrombocytopenia, platelets < 100×10^9/l	21	18
Monocytopenia	1	0
Bicytopenia	19	17
Tricytopenia	13	11
Median bone marrow blasts, % (range)	2 (0–18)	2 (0–18)
Median serum erythropoietin, U/l (range)	62·5 (12·7–326) ($n = 24$)	68 (12·7–326) ($n = 23$)

* One patient with preinclusion haemoglobin levels of 11·4 g/dl was included in the study because of clear cytomorphological and histological established diagnosis of MDS, subtype RA, with leucopenia (WBC $2\cdot 2 \times 10^9$/l), thrombocytopenia (platelets 66×10^9/l) and pretreatment haemoglobin levels fluctuating between 10·0 and 11·4 g/dl without substitution treatment.

Table II. Erythroid response to rhG-CSF plus rhEPO treatment.

MDS subtype	After 12 weeks of cytokine treatment				After 36 weeks of cytokine treatment			
	No. of patients evaluable	GER	PER	NER	No. of patients evaluable	GER	PER	NER
RA	15	6	4	5	14	9	2	3
RARS	6	1	1	4	6	1	4	1
RAEB	7	5	–	2	5	4	–	1
Total	28	12	5	11	25	14	6	5
		17/28 (61%)				20/25 (80%)		
		(95% CI 41−78%)				(95% CI 59−93%)		

GER, good erythroid response (loss of transfusion requirement or haemoglobin increase > 2 g/dl).
PER, partial response (> 50% reduction in transfusion frequency or haemoglobin increase >1−2 g/dl).
NER, no response.

Long-term erythroid response
Nineteen of 20 responding patients continued to receive the combined cytokine therapy after 36 weeks. Median duration of continued treatment for these patients reached 106 weeks (range 44−214). Figure 2 describes the duration of the erythroid response in the entire cohort of all 28 evaluable patients counting failure to achieve GER or PER at 36 weeks or later as event, while censoring for onset of AML or death. This figure indicates that long-lasting erythroid responses could be obtained in about 45% of the patients with stable MDS disease.

Factors influencing erythroid response
To search for factors influencing response to cytokines, we contrasted erythroid responders with non-responders (Table III). Responders and non-responders did not differ significantly with respect to age, sex or diagnosis. The haemoglobin pretreatment level was the only significant univariate predictor for response ($P = 0.002$). Responding patients had somewhat lower pretreatment serum EPO levels (median 52·5 U/l, range 12·7−241) than non-responders (median 104 U/l, range 25·8−326). Although the difference in pretreatment levels was not significant ($P = 0.249$), we noted no responses in patients with EPO levels > 250 U/l. Seven patients had pretreatment transfusion requirement ≤ 2 RBC units/month and EPO levels < 100 U/l. Six of the patients showed an erythroid response (five with RA and one with RARS). All cases with transfusion requirements < 2 RBC units per month responded (three RA).

Furthermore, we applied the scoring system of Hellstroem-Lindberg *et al* (1997) for predicting response to rhG-CSF and rhEPO treatment in the patient cohort. This score incorporates the initial serum EPO levels and the RBC transfusion needs (Hellstroem-Lindberg *et al*, 1997).

The response rate at the first and second evaluation was 71·4% and 85·7%, respectively, in the group with score > +1 and 68·8% and 81·3%, respectively, in the group with scores between −1 and +1. No patient was classified in the group with score lower than −1.

We found no agreement between this score and the erythroid response in our data at the first evaluation

(kappa = 0·02, $P = 0.898$) as well as at the second evaluation (kappa = 0·03, $P = 0.789$).

Other haematological responses
After 12 weeks of treatment, 26/28 patients (93%, 95% CI 76−99) showed neutrophil counts $> 3·0 \times 10^9$/l. After
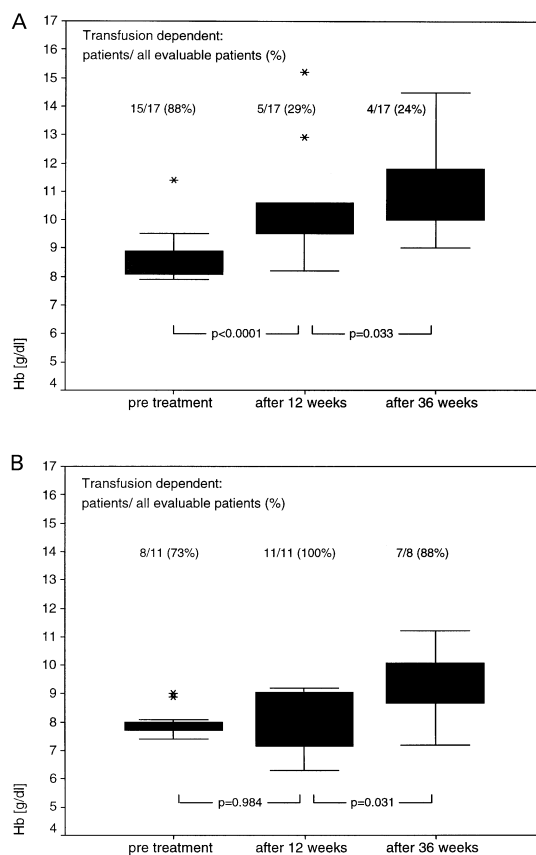

Fig 1. Comparison of haemoglobin levels (g/dl) and transfusion dependency pretreatment after 12 weeks and after 36 weeks of combined cytokine therapy in patients who achieved erythroid responses (A) or who were non-responders (B) at the first evaluation (12 weeks) (box plots showing median, 25% and 75% percentiles).
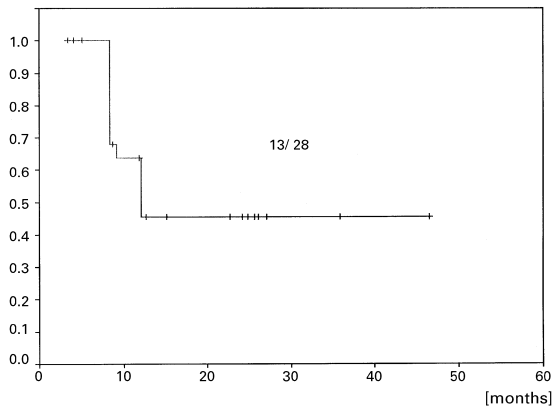
Fig 2. Duration of erythroid response (GER and PER). Death and progression to AML were censored.

36 weeks of treatment, all 25 evaluable patients had a complete neutrophil response. The combination treatment did not induce any significant changes in the platelet counts at any time point during evaluation.

Growth factor doses administered
RhG-CSF was administered at a starting dose of $1 \cdot 5$ μg/kg/d. The dose was modified based on neutrophil counts. The median dose of rhG-CSF at the time of response was $1 \cdot 5$ μg/kg/d (range $0 \cdot 7$–12) at the first as well as at the second evaluation.

After the first 6 weeks, rhEPO dose was doubled in 12 of 28 patients. Of the 17 responding patients after 12 weeks of treatment, 12 achieved an erythroid response with a rhEPO dose of 200 U/kg.

Side-effects
No infection episodes occurred during the first 12 treatment weeks. Treatment-induced thrombocytopenia was not observed. Only one patient showed a moderate decline in the platelet count after 1 year of treatment. No death due to cerebral or other haemorrhage occurred. There was no episode of thrombosis, seizures or therapy-related hypertension.

Long-term administration of rhG-CSF and rhEPO was generally well tolerated and side-effects were usually mild. A slight elevation of serum alkaline phosphatase was found in three patients. One patient developed bone pain as a result of rhG-CSF administration, requiring treatment discontinuation.

Transition to AML and causes of death
At the time of writing, seven of the 33 enrolled patients have developed AML during the period of observation. The overall 1-year leukaemia-free survival rate was 84%. Two patients (one RARS, one RAEB) developed AML during the first 12 weeks of treatment and were not evaluable for erythroid response at that time. Two patients (two RAEB) were non-responders at the first evaluation and progressed to overt AML within a few weeks (one at 13 and one at 14 weeks after entry). Three patients (one RA, two RAEB) were good responders at the first evaluation and progressed to AML, one at 37, one at 54 and one at 61 weeks after entry into the study.

Altogether, 2/25 (10%) patients with MDS subtypes RA/RARS and 5/8 (62%) with RAEB developed AML. The Kaplan–Meier curves differed significantly for both groups ($P = 0 \cdot 0014$) (Fig 3A). The median time to leukaemia for patients with RAEB was $8 \cdot 7$ months, and for patients with RA/RARS this was not reached.

Ten of 33 patients have died. Causes of death were AML (six patients) and infections (four patients). The median time of observation is $26 \cdot 9$ months. The overall 1-year survival rate was 87% and the 2-year survival rate 66%. The survival times of RA/RARS (2-year survival rate 80%) compared with RAEB (2-year survival rate 30%) are significantly different ($P = 0 \cdot 0421$) (Fig 3B).

For the 28 evaluable patients, the overall 1-year survival rate was 89%. Four patients (one RA, three RAEB) of the 17 responders and four (two RA, two RAEB) of the non-responders have died. The Kaplan–Meier survival curves for

Table III. Comparison of responders with non-responders (pretreatment values).

|	Erythroid responders ($n = 17$)	Erythroid non-responders ($n = 11$)	P-value
Median age (range)	62 (51–86)	61 (51–74)	0·817
Median disease duration, months (range)	2·6 (0–22)	1·5 (0–6·1)	0·325
Male/female	9/8	6/5	0·773
Median SANZ score (range)	2 (0–5)	2 (0–5)	0·963
Median Hb, g/dl (range)	8·8 (7·9–11·4)	7·9 (7·4–9)	0·002
Median platelets, $\times 10^9$/l (range)	66 (8–475)	30 (15–244)	0·981
Median ANC, $\times 10^9$/l (range)	1·2 (0·4–4·9)	0·9 (0·1–2·2)	0·208
Transfusion requirement, yes/no	15/2	8/3	0·353
Median RBC transfusion, units/month (range)	2 (0–8)	2 (0–16)	0·514
Median Epo, U/L (range)	52·5 (12·7–241)	104 (25·8–326)	0·249
Ferritin, pathological, yes/no	13/4	9/2	0·734
Median bone marrow blast, % (range)	3 (0–18)	2 (0–16)	0·517
Median reticulocytes, $\times 10^9$/l (range)	28·8 (11·5–76·8)	25·2 (2·8–81) ($n = 9$)	0·597

A

B
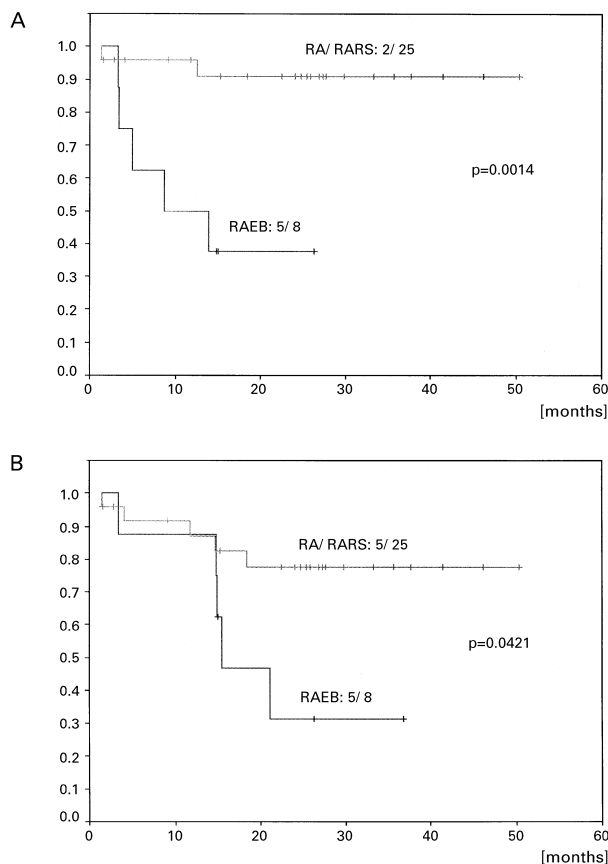
Fig 3. Time to leukaemia (A) and overall survival (B) according to MDS subtype.

responders (2-year survival rate 61%) did not differ significantly ($P = 0.308$) (survival curves not showed).

Time to treatment failure

Figure 4 shows time to treatment failure when all failures to maintain an erythroid response are considered as events, including transition to AML and death. Nineteen failures have been observed to date (three AML, three death not preceded by other failures, six non-erythroid responses after 36 weeks of treatment and seven later failures of erythroid response). The results in Fig 4 suggest the possibility that 30% of the patients were long-term survivors with good erythroid response. In fact, the longest follow-up with GER in a patient receiving the cytokine combination is now 4 years.

DISCUSSION

Our study confirms the reports by other authors that a combination therapy with rhG-CSF and rhEPO is an effective treatment of anaemia associated with myelodysplastic syndromes. Although Negrin *et al* (1996) achieved a response rate of 48%, Hellstroem-Lindberg *et al* (1998) observed similar erythroid responses (38%) with slightly stricter response criteria.
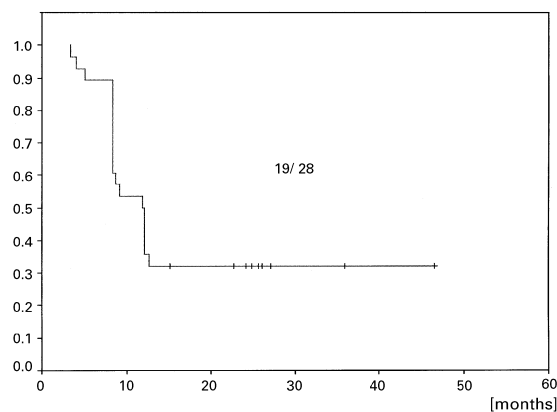

Fig 4. Time to treatment failure with failure to achieve erythroid response, death and progression to AML counted as events.

The response rates observed by us are relatively high. Using the same response criteria of Negrin *et al* (1996), we obtained erythroid response rates of 61% (95% CI 41–78) after 12 weeks of cytokine treatment and of 80% (95% CI 59–93) after 36 weeks. If we apply the somewhat stricter criteria of Hellstroem-Lindberg *et al* (1998) to our data, we find a response rate of 50% (95% CI 31–69) after 12 weeks and 56% (95% CI 35–75) after 36 weeks.

Our treatment schedule differed from that used by Negrin *et al* (1996) and Hellstroem-Lindberg *et al* (1998) in an important aspect. We decided to proceed with the combination treatment for a much longer treatment interval than in any of the other studies. The scheduled treatment duration was 36 weeks and discontinuation was not encouraged if a response was observed after this period.

A novel finding in our series was that a prolonged combination treatment beyond 12 weeks enabled conversion of non- or partial responders into partial and good responders respectively. Further, we obtained a significant increase in haemoglobin levels between 12 and 36 weeks of treatment in responding patients ($P = 0.033$) as well as in the non-responding group ($P = 0.031$). Only 24% of the responders were transfusion dependent after 36 weeks of rhG-CSF + rhEPO therapy compared with 88% before treatment was started. Another difference between our study and the other two large studies with rhG-CSF and rhEPO was the dosing frequency of rhEPO.

Our patients received rhEPO three times per week rather than daily. This schedule was sufficient to induce an erythroid response in our patients.

Furthermore, we wish to highlight that the majority of our patients (25 cases) had a short disease duration (≤ 6 months). It is supposed that patients treated shortly after diagnosis may respond better than those who have had a long history of blood transfusions.

In our data set, we have a slightly higher proportion of patients with the RA subtype compared with the other two reports. However, our patients were a negative selection of RA patients as they had multiple cytopenias. A relevant observation was that about 50% of all patients surviving and not converting to AML exhibited a long-lasting

erythroid response with an indication of a stable plateau. In terms of all patients entering the study, 30% survived 1 year and longer with a good erythroid response.

Our series was too small to detect reliably variables associated with response to treatment. However, the haemoglobin pretreatment levels showed a significance as univariate predictor in our patient series. A prognostic scoring system was recently proposed by Hellstroem-Lindberg *et al* (1997) based on the two pretreatment variables serum EPO levels and RBC transfusion need. When we applied this score to our series, we could not reproduce the prognostic value of this score. This may be due to the small numbers of patient in our study group and therefore the reduced power to detect prognostic differences. The prolonged administration of rhG-CSF and rhEPO was well tolerated without clinically relevant side-effects. Particularly, we observed no treatment-induced reduction of platelet counts or bleeding manifestations. Furthermore, among RA/RARS patients, the rate of progression to AML was small, with 90% being free of leukaemia after 3 years despite continued treatment with cytokines.

Hence, despite some difficulties in comparing the results of our study with those of Hellstroem-Lindberg *et al* (1998) and Negrin *et al* (1996), our data lend further support to the concept of a growth factor combination treatment for anaemia in MDS patients.

Pooling available literature data shows that 125 out of 515 patients who received EPO alone achieved an erythroid response (≈24%) (Bessho *et al*, 1990; Oster *et al*, 1990; Stebler *et al*, 1990; Bowen *et al*, 1991; Hellstroem *et al*, 1991; van Kamp *et al*, 1991; Laporte *et al*, 1991; Schouten *et al*, 1991; Stein *et al*, 1991; Kurzrock *et al*, 1991; Cazzola *et al*, 1992; Ganser & Hoelzer, 1992b; Rafanelli *et al*, 1992; Razzano *et al*, 1992; Shepherd *et al*, 1992; Verhoef *et al*, 1992; Aloe Spiriti *et al*, 1993; Ghio *et al*, 1993; Goy *et al*, 1993; Depaoli *et al*, 1993; Ludwig *et al*, 1993; Mittelman, 1993; Mohr *et al*, 1993; Shapiro *et al*, 1993; Stenke *et al*, 1993; Urabe *et al*, 1993; Yoshida *et al*, 1993; Zeigler *et al*, 1993; Isnard *et al*, 1994; Stone *et al*, 1994; Rose *et al*, 1995).

In comparison, although no direct randomization has been undertaken, the results of the three studies now available suggest that the erythroid response rates may be higher for combination treatment with rhG-CSF and rhEPO (range 40–60%) than for rhEPO treatment alone. This provides some evidence for *in vivo* synergy between the two cytokines on erythropoiesis in patients with MDS and stable disease.

Furthermore, our data suggested that a prolonged treatment beyond 12 weeks with rhG-CSF and rhEPO could achieve high and stable response rates in a large proportion of patients with very moderate side-effects. The response could be maintained for many years in at least 50% of the patients surviving free of AML. Our data are not sufficient to design a treatment decision strategy. However, further investigations are needed to understand better the biological basis of erythroid response to combined cytokine treatment, to determine the impact on survival of these treatments and to develop a cost-effective strategy to assign the treatment to patients for whom this treatment can provide long-lasting relief from the disease.

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