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Hematologic and Cytogenetic Findings in Myelodysplastic Syndromes Treated with Recombinant Human Granulocyte Colony-stimulating Factor

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We administered recombinant human granulocyte colony-stimulating factor (rhG-CSF) to four patients with myelodysplastic syndrome (MDS) and three patients with non-MDS (two malignant lymphoma and one lung cancer) as a part of a phase II trial and analyzed the effects of rhG-CSF on the neoplastic cells of MDS by performing sequential chromosome analyses on the bone marrow cells. A greater than 3-fold increase in neutrophil count was observed in the MDS patients after rhG-CSF infusions, whereas the number of blasts in the bone marrow did not increase and none of them progressed into the leukemic phase. After rhG-CSF treatment, the bone marrow cells obtained from patients without MDS did not show any particular chromosome abnormalities such as chromosomal breakage. On the contrary, two of the four MDS patients with acquired chromosome abnormalities showed a change in the frequency of marrow cells with clonal abnormalities after rhG-CSF treatment; the proportion of metaphase cells with additional numerical chromosome abnormalities decreased in these two MDS patients. After discontinuation of the treatment, the constitution of marrow cells with chromosome changes reverted to that before treatment. The remaining two MDS patients did not show any particular chromosome changes after the rhG-CSF treatment, indicating that rhG-CSF may not promote the characteristics of dyshematopoiesis in MDS, and act on cells derived from an MDS clone.

Key words: Granulocyte colony-stimulating factor — Myelodysplastic syndrome — Chromosome aberration

Recent molecular technology has made it possible to isolate and clone the hematopoietic growth factors which regulate hematopoiesis.[1-5] Recent articles have described attempts to treat myelodysplastic syndrome (MDS) patients with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF).[6-9] Vadhan-Raj *et al.* reported the *in vivo* effect of rhGM-CSF in MDS patients from the viewpoint of the enrichment of peripheral blood leukocytes, including granulocytes.[6] Although they did not observe patients in which the treatment caused leukemic progression, they found an increase in the number of peripheral blood blasts and a response in multiple hematopoietic components in some of the MDS patients[7]; Antin *et al.* have also reported an increase in myeloblast numbers in two of the seven MDS patients treated with rhGM-CSF.[8] In contrast, Ganser *et al.* recently reported that some MDS patients treated with rhGM-CSF evolved into the leukemic phase and they speculated that a difference in response to rhGM-CSF among MDS patients may exist.[9] Thus, the main issue we have to address is how such kinds of hematopoietic growth factors act on cells derived from a neoplastic clone, when they are given to patients with hematological neoplasia, including MDS.

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is also available and its effect on restricted granulopoiesis has been documented.[4,10-14] Although *in vivo* effects of rhG-CSF on hematopoietic progenitors were demonstrated, the effect on patients with MDS is unclear.[15] In this study, we performed clinical trials of rhG-CSF on patients with MDS and with malignancies other than MDS, as a part of a phase II trial. To ascertain the effects of the treatment on the proliferative kinetics of bone marrow cells in MDS patients, we performed sequential cytogenetic investigations.

MATERIALS AND METHODS

Patients We administered rhG-CSF to seven patients, four newly diagnosed MDS patients who had not received any previous chemotherapy, one lung cancer patient and two non-Hodgkin's lymphoma patients (stage IV), after obtaining their written informed consent. Among the four MDS patients, three were diagnosed as having refractory anemia (RA) and the remaining one as having refractory anemia with excess blasts (RAEB) based on the French-American-British (FAB) criteria (Table I).[16] Routine bone marrow examination was performed at least twice before rhG-CSF infusion and

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Table I. Hematologic Findings at the Diagnosis of MDS Patients Treated with Recombinant Human Granulocyte Colony-stimulating Factor

Patient No.	1	2	3	4
Age (yr)/Sex	33/Male	29/Male	54/Male	30/Male
Peripheral Blood				
WBC (/μl)	1000	3300	1500	2200
Hb (g/dl)	7.5	5.4	10.3	8.1
Plt ($\times 10^4/\mu$l)	7.68	3.01	15.1	11.4
Blasts (%)	0	0	0	0
Bone marrow				
M/E ratio	3.0	2.0	5.47	1.4
Blasts (%)	3.4	4.2	5.4	1.4
Diagnosis	RA	RA	RAEB	RA

Abbreviations: RA, refractory anemia; RAEB, refractory anemia with excess of blasts; WBC, white blood cell; Hb, hemoglobin; Plt, platelet; M/E ratio, myeloid erythroid ratio.

again after treatment. Some of the marrow cells were used for the chromosome studies. The MDS patients were followed up 4 to 13 months after discontinuation of the rhG-CSF administration.

The rhG-CSF used in this study was kindly provided by Kirin Brewery Co., Ltd. (Tokyo). rhG-CSF was dissolved in 100 ml of 5% glucose solution and was administered intravenously over a period of 30 min.[17] The dose and period of treatment with rhG-CSF for the MDS patients are shown in Table II. rhG-CSF was administered to three non-MDS neoplastic patients for 14 days with the same protocol which was utilized for the MDS patients (200 μg/m^2 to 400 μg/m^2 daily); it was administered 72 h after anti-neoplastic chemotherapy had been stopped, in accordance with the proposed protocol for the phase II trials of rhG-CSF and with the approval of the Institutional Review Board. Bone marrow and cytogenetic examinations were performed on day 14 of the treatment.

Chromosome analysis Bone marrow cells were cultured for 48 h in RPMI 1640 culture medium supplemented with 15% fetal calf serum (FCS). Cells were arrested by adding colcemid for the final 1 h of culture followed by treatment with 0.075 *M* KCl hypotonic solution for 20 min and fixation with three changes of methanol:acetic acid (3:1, v/v). Chromosome preparations were stained with Hoechst 33258 and the quinacrine mustard sequential staining method.[18] Chromosome abnormalities are described according to the International System for Human Cytogenetic Nomenclature.[19]

In addition, *in vitro* chromosome analysis following the addition of the rhG-CSF (final concentration: 10 ng/ml in culture) was also carried out on bone marrow cells obtained from two MDS patients (patients no. 3 and 4) before rhG-CSF administration to the patients. The cells were harvested after 48 h in culture, and 100 metaphase cells were analyzed cytogenetically using a double-staining Q-banding procedure as mentioned above.

RESULTS

Hematological findings In all of the MDS cases, a more than 3-fold increase in neutrophil count was noted at the peak (Fig. 1).[17] Two of the three MDS patients who were given rhG-CSF for more than 2 weeks exhibited a biphasic increase in neutrophil count; rapid elevation within a week after the beginning of rhG-CSF administration followed by a further gradual elevation in neutrophil counts was noted in both MDS patients (patients no.

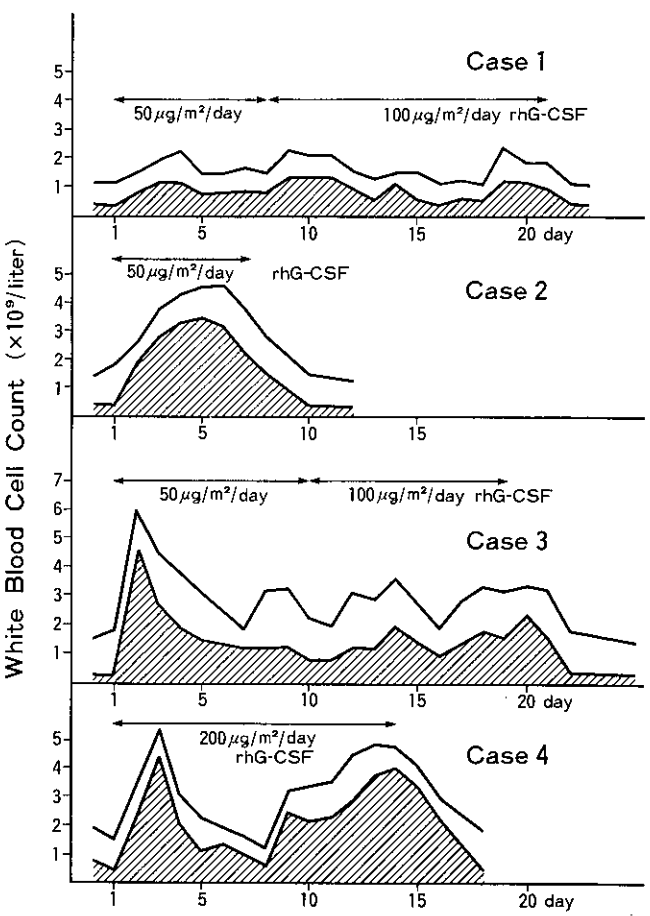

Fig. 1. Daily changes in white blood cell levels and neutophils (shaded area) in the four MDS patients treated with recombinant human granulocyte colony-stimulating factor, showing a greater than 3-fold increase in neutrophil counts after rhG-CSF infusions. Patient no. 4 showed a typical biphasic increase in neutrophil count.

3 and 4). After discontinuation of rhG-CSF infusions, the neutrophil counts declined within 3 days to reach the pretreatment levels in all patients. None of the MDS patients showed particular changes in eosinophil, basophil, or lymphocyte counts in the peripheral blood during the rhG-CSF treatment, whereas monocyte counts increased in three of the four MDS patients. Bone marrow aspirates obtained from patients no. 1, 3, and 4 showed that percentages of myeloid cells slightly increased after rhG-CSF treatment. The maturation index of the

Table II. Changes in Bone Marrow Findings during Treatment with Recombinant Human Granulocyte Colony-stimulating Factor

Patient No.	Dose ($\mu g/m^2$) and period (days)	Myeloid cells (%)		Blasts (%)		Pro+Myelo+Meta (%)		Stab+Seg (%)		Maturation index[b]	
		Before	After	Before	After	Before	After	Before	After	Before	After
1	50×7, 100×14	38.2	47.6 (10)[a]	3.4	4.0	21.2	28.0	13.6	15.8	6.4	9.0
2	50×7	60.0	55.8 (7)	4.6	3.8	28.2	25.8	27.6	34.4	4.8	7.0
3	50×10, 100×11	65.5	80.2 (14)	5.4	2.4	24.0	37.4	32.8	38.0	9.8	9.0
4	200×14	30.8	54.0 (14)	2.6	2.8	9.6	27.6	14.8	15.8	4.3	9.4

a) Parentheses indicate days after the beginning of the rhG-CSF administration.
b) Maturation index: (myelocytes+metamyelocytes+stabs+segmented neutrophils):(blasts+promyelocytes).
Abbreviations: Pro+Myelo+Meta, promyelocytes, myelocytes, and metamyelocytes; Stab+Seg, stab and segmented cells.

Table III. Chromosome Findings of Bone Marrow Cells Obtained from Patients Treated with rhG-CSF

Case	Date of examination[a]	No. of cells analyzed	Karyotypes[b]		
			Normal (%)	Abnormal (%)	
1	Dec/9/1987 (before)	50	12	88	46,XY,1q+ (72%)/2{46,XY,1q+} (10%)/47,XY,+8,1q+ (6%)
	Dec/21/1987 (day 7)	50	6	94	46,XY,1q+(76%)/2{46,XY,1q+} (16%)/4{46,XY,1q+} (2%)
	Jan/7/1988 (day 24)	50	6	94	46,XY,1q+ (66%)/2{46,XY,1q+} (28%)
	Apr/28/1988	50	4	96	46,XY,1q+ (82%)/2{46,XY,1q+} (6%)/47,XY,+8,1q+ (8%)
	Aug/11/1988	100	5	95	46,XY,1q+ (66%)/2{46,XY,1q+} (26%)/47,XY,+8,1q+ (3%)
	Dec/3/1988	20	0	100	46,XY,1q+ (80%)/2{46,XY,1q+} (15%)/47,XY,+8,1q+ (5%)
2	Dec/12/1987 (before)	20	5	95	46,XY,5q− (15%)/44,XY,−18,−20,5q−,t(19;21) (80%)
	Jan/7/1988 (day 7)	20	5	95	44,XY,−18,−20,5q−,t(19;21)
3	Aug/17/1988 (before)	100	94	6	47,XY,+8
	Sep/1/1988 (day 14)	50	96	4	47,XY,+8
	Nov/30/1988	50	92	8	47,XY,+8
4	Oct/7/1988 (before)	100	2	98	46,XY,1q+ (14%)/49,XY,+8,+13,+16,1q+ (78%) 2{46,XY,1q+} (6%)
	Oct/28/1988 (day 14)	50	0	100	46,XY,1q+ (46%)/49,XY,+8,+13,+16,1q+ (44%) 2{46,XY,1q+} (6%)/NCA* (4%)
	Nov/15/1988	50	2	98	46,XY,1q+ (18%)/49,XY,+8,+13,+16,1q+ (72%) 2{46,XY,1q+} (8%)

a) Date of examination: parentheses indicate days after rhG-CSF injection.
b) Karyotype: findings are described in detail in the text; NCA*, non-clonal abnormalities with the 1q+ marker chromosome.

Table IV. Comparative Cytogenetic Analysis between *in vitro* Culture with rhG-CSF and Bone Marrow Cells of MDS Patients Treated with rhG-CSF

Patient No.	Karyotypes	Percentage of cells examined in culture with rhG-CSF	Percentage of marrow cells	
			Before rhG-CSF	After rhG-CSF
3	46,XY	89	94	96
	47,XY,+8	11	6	4
	No. of cells examined	100	100	50
4	46,XY	11	2	0
	46,XY,1q+	34	17	46
	49,XY,+8,+13,+16,1q+	55	78	44
	2{46,XY,1q+}	0	3	6
	NCA*			4
	No. of cells examined	100	100	50

NCA*, non-clonal abnormalities with the 1q+ marker.

marrow myeloid cells increased in three of the four MDS patients (Table II). None of the MDS patients progressed into the leukemic phase, but patient no. 2 evolved into myelofibrosis 3 months after the discontinuation of rhG-CSF treatment. Furthermore, no particular side effect, except for endurable bone pain in all the patients, was noted during the rhG-CSF infusions.

Chromosome of MDS patients treated with rhG-CSF The chromosome findings during or after rhG-CSF treatment are summarized in Table III. Before rhG-CSF treatment, all MDS patients presented here showed clonal chromosome abnormalities. The marrow cells obtained from patient no. 1 had karyotypic subclones; the prototype was 46,XY,−1,+der(1)(1pter→1q42::1q21→1q42::1q21→1qter) [46,XY,1q+]. On day 7 of rhG-CSF treatment, metaphase cells with 47,XY,+8,1q+ disappeared and those cells reappeared 3 months after discontinuation of the treatment (Table III). Approximately 6 months later, however, the patient developed RAEB.

In patient no. 2, the first chromosome analysis revealed that 95% of the bone marrow metaphase cells contained abnormal karyotypes of 46,XY,del(5)(q13q34) and 44,XY,−18,−20,del(5)(q13q34),t(19;21)(p13;q11).[17] On day 7, no additional change was found and the occupancy of cells with abnormal karyotypes in the marrow was identical to that which had existed before treatment.

The bone marrow obtained from patient no. 3 showed only an abnormality of trisomy of chromosome 8 [+8] and no particular change in the occupancy of metaphases with +8 [47,XY,+8] between before (6% of the marrow metaphase cells) and after (4%) rhG-CSF administration was found. No tetraploid cells were observed in this case.

In patient no. 4, the prototypic change was 46,XY,−1,+der(1)(1pter→1qter::1q21→1qter) [46,XY,1q+]. The proportion of cell with 49,XY,+8,+13,+16,1q+ decreased on day 14, whereas cells with 46,XY,1q+ increased (Table III). At that time, metaphases with nonclonal chromosome abnormalities with the 1q+ marker were noted in the marrow after rhG-CSF treatment. However, approximately 1 month later, the constitution of chromosomes in the bone marrow reverted to that which had existed before the administration. Among these four MDS patients, no chromosome or chromatid gaps or breaks were detected in the marrow cells before or after rhG-CSF treatment.

In the patient with lung cancer, a routine chromosome study on 20 bone marrow metaphase cells after rhG-CSF infusions revealed only a normal karyotype. They had neither detectable hyperploidy nor chromosomal breakages. Patients with non-Hodgkin's lymphoma also showed no chromosome abnormalities in the marrow cells either before or after the rhG-CSF treatment. No chromosomal breakage or hyperploid metaphases were noted.

Chromosomes of an *in vitro* culture with rhG-CSF Chromosome changes in the bone marrow cells obtained from patients no. 3 and 4 following the addition of the rhG-CSF in culture are summarized in Table IV. In patient no. 3, the occupancy of cells with 47,XY,+8 slightly increased in the culture with rhG-CSF (11%), when compared to that of marrow cells obtained from the patient after rhG-CSF infusions. In patient no. 4, the percentage of metaphase cells exhibiting a karyotype of 49,XY,+8,+13,+16,1q+ decreased following the addition of rhG-CSF, whereas the occupancy of cells with a normal karyotype or with 46,XY,1q+ increased. This tendency of a decreasing number of cells with 49,XY,

+8,+13,+16,1q+ in the culture with rhG-CSF seemed to be similar to that obtained from cytogenetic studies of bone marrow cells after administration of rhG-CSF *in vivo* (Table IV).

DISCUSSION

The *in vitro* effects of rhG-CSF on granulopoiesis and *in vivo* effects of the enrichment of peripheral neutrophils in cyclophosphamide-treated primates have previously been documented.[13] The *in vitro* effects of rhG-CSF on cells from MDS patients were demonstrated by Yuo *et al.*, and the functional activation of neutrophils by rhG-CSF treatment has also been shown.[20] We reported that rhG-CSF has a capability for increasing the number of peripheral neutrophils in MDS patients, providing that the rhG-CSF acts on cells derived from an MDS clone to mobilize neutrophils from the marginal pool and to cause differentiation into neutrophils with abnormal nuclear configurations,[17] which is in keeping with the description by Kobayashi *et al.*[15] The initial rapid elevation of neutrophil count in the MDS patients could be demargination of mature neutrophils and the further gradual increase observed after 1 week is probably due to the effects of rhG-CSF on immature cells in the bone marrow to cause differentiation into neutrophils, which is in accordance with the concept that cells derived from an MDS clone sustain a capability to differentiate into mature cells on treatment with differentiation-inducing agents. In addition, Mayani *et al.* recently found that rhGM-CSF enhances colony growth in MDS patients in a dose-dependent manner,[21] indicating that some hematopoietic growth factors are able to affect progenitor cells derived from an MDS clone. In the present study, however, a colony formation assay using rhG-CSF did not show any particular relationship between the results of the assay before the treatment and the response to rhG-CSF *in vivo* (data not shown).

In this trial with four MDS patients, no consistent changes were seen in hemoglobin levels and platelet counts, indicating that rhG-CSF probably affects restricted granulopoiesis, even in MDS patients. Although it remains an open question, the finding of an elevated monocyte count in some MDS patients after rhG-CSF infusions raises the possibility that rhG-CSF has some effects on monocytes; Wong *et al.* reported that rhG-CSF has a chemotactic activity upon monocytes as well.[22]

Since MDS is a stem-cell disorder with ineffective hematopoiesis, and an autocrine action of hematopoietic growth factors in MDS patients has been suggested,[23,24] leukemic progression is one of the main problems encountered in treating this disease.[25] rhG-CSF has the potential to stimulate leukemic cells obtained from acute myeloblastic leukemia patients with or without the presence of rhGM-CSF.[26-28] Furthermore, macrophages which could produce various cytokines are also involved in MDS. Chromosomal analysis in the past demonstrated that some MDS patients contained cytogenetically identifiable subclones which may emerge from cells with a prototypic change. Although the biological implications of such additional chromosome abnormalities, especially numerical changes, in MDS patients are not thoroughly understood, some MDS patients eventually show additional chromosome changes which are associated with the progression of the disease.[29-32] Another cytogenetic feature in the progression towards the leukemic phase in MDS patients is the appearance of karyotypically unrelated clones,[31,32] which are usually found in patients with sudden leukemic transformation.[33] During rhG-CSF treatment, the proportion of the cells with additional numerical chromosome abnormalities (49,XY,+8,+13,+16,1q+) decreased in patient no. 4 and such cells (47,XY,+8,1q+) disappeared in patient no. 1, although the occupancy of cells with a normal karyotype decreased in both patients. One plausible explanation for the phenomena seen in these two MDS patients is that rhG-CSF affected the marrow immature cells to enhance the occupancy of cells with less complex cytogenetic abnormalities. Another possibility is that rhG-CSF selectively acts on the cells with additional chromosome aberrations to cause differentiation into more mature cells. Thus, it was suggested that rhG-CSF may act differently among subclones with multiple chromosomal abnormalities in MDS patients and such a subclone may not respond to the same degree as a clone with less complex abnormalities; these findings do not simply indicate that rhG-CSF has an ability to improve dyshematopoiesis. However, the constitution of chromosomes in marrow cells obtained from these two MDS patients reverted to those which had existed before rhG-CSF treatment and a sequential cytogenetic investigation revealed no further karyotypic abnormalities, indicating that rhG-CSF acts temporarily on an MDS clone without karyotypic progression. Thus, rhG-CSF probably increased the number of cells which were already present in the marrow, and which did not promote the characteristics of dyshematopoiesis in MDS. In the present trial, we could not find any marrow cells with a karyotypically unrelated clone, which is believed to be another branch towards leukemic transformation,[31-33] suggesting that rhG-CSF does not facilitate karyotypic instability in MDS patients.

The chromosomal results obtained by an *in vitro* culture with rhG-CSF seemed to correlate partially with those of marrow cells obtained from MDS patients treated with rhG-CSF. This is noteworthy, since if *in vitro* chromosome findings following rhG-CSF treatment reflected, even though partially, the *in vivo* chromosomal

constitution of MDS patients treated with rhG-CSF, chromosomal results obtained by *in vitro* co-culture with rhG-CSF might predict chromosome changes in MDS patients treated with rhG-CSF. Nevertheless, hematopoietic cells in MDS patients showed inappropriate cell division and differentiation as a result of dyshematopoiesis,[16] and hematopoietic growth factors probably act on an MDS clone.[24] Thus, an *in vitro* culture procedure might be attempted for MDS patients to select patients who are able to receive appropriate and safe administration of hematopoietic growth factors.

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REFERENCES

1) Metcalf, D. The granulo-macrophage colony-stimulating factors. *Science,* **229,** 16–22 (1985).
2) Metcalf, D. The molecular biology and functions of the granulocyte-macrophage colony-stimulating factors. *Blood,* **67,** 257–267 (1986).
3) Wong, G. G., Witek, J. A., Temple, P. A., Wilkens, K. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Wang, E. A. and Clark, S. C. Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. *Science,* **228,** 810–815 (1985).
4) Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, T., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, M., Nomura, H. and Ono, M. Molecular cloning and expression of cDNA for human granulocyte colony stimulating factor. *Nature,* **319,** 415–418 (1986).
5) Jacobs, K., Shoemaker, C., Rudersdorf, R., Neill, S. D., Kaufman, R. J., Mufson, A., Seehra, J., Jones, S. S., Hewick, R., Fritsch, E. F., Kawakita, M., Shimizu, T. and Miyake, T. Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature,* **313,** 806–810 (1985).
6) Vadhan-Raj, S., Keating, M., LeMaistre, A., Hittelman, W. N., McCredie, K., Trujillo, J. M., Broxmeyer, H. E., Henney, C. and Gutterman, J. U. Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic sydromes. *N. Engl. J. Med.,* **317,** 1545–1552 (1987).
7) Vadhan-Raj, S., Hittelman, W. N., Ventura, C., Buescher, D., Keating, M. J. and Gutterman, J. U. Granulocyte-macrophage colony-stimulating factor and mylodysplastic syndromes. *N. Engl. J. Med.,* **319,** 51–53 (1988).
8) Antin, J. H., Smith, B. R., Holmes, W. and Rosenthal, D. S. Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. *Blood,* **72,** 705–713 (1988).
9) Ganser, A., Volkers, B., Greher, J., Ottmann, O. G., Walther, F., Becher, R., Bergmann, L., Schulz, G. and Hoelzer, D. Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes — a phase I/II trial. *Blood,* **73,** 31–37 (1989).
10) Souza, L. M., Boone, T. C., Gabrilove, J., Lai, P. H., Zsebo, K. M., Murdock, D. C., Chazin, V. R., Bruszewski, J., Lu, H., Chen, K. K., Barendt, J., Platzer, E., Moore, M. A. S., Mertelsman, R. and Welte, K. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science,* **232,** 61–65 (1986).
11) Welte, K., Bonilla, M. A., Gillio, A. P., Boone, T. C., Potter, G. C., Gabrilove, J. L., Moore, M. A. S., O'Reilly, R. J. and Souza, L. M. Recombinant human granulocyte colony-stimulating factor. Effects on hematopoisis in normal and cyclophosphamide-treated primates. *J. Exp. Med.,* **165,** 941–948 (1987).
12) Welte, K., Bonilla, M. A., Gabrilove, J. L., Gillio, A. P., Potter, G. K., Moore, M. A. S., O'Reilly, R. J., Boone, T. C. and Souza, L. M. Recombinant human granulocyte colony-stimulating factor: *in vitro* and *in vivo* effects on myelopoiesis. *Blood Cells,* **13,** 17–30 (1987).
13) Cohen, A. M., Zsebo, K. M., Inoue, H., Hines, D., Boone, T. C., Chazin, V. R., Tsai, L., Ritch, T. and Souza, L. M. *In vitro* stimulation of granulocyte colony-stimulation factor. *Proc. Natl. Acad. Sci. USA,* **84,** 2482–2488 (1987).
14) Asano, S. and Ono, M. Human granulocyte colony-stimulating factor: its biological action and clinical implication. *Acta Heamatol. Jpn.,* **50,** 1550–1556 (1987).
15) Kobayashi, Y., Okabe, T., Ozawa, K., Chiba, S., Hino, M., Miyazono, K., Urabe, A. and Takaku, F. Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor: a preliminary report. *Am. J. Med.,* **86,** 178–182 (1989).
16) Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. and Sultan, C. Proposals for the classification of the myelodysplastic syndromes. *Br. J. Haematol.,* **51,** 189–199 (1982).
17) Toyama, K., Ohyashiki, K., Ohyashiki, J. H. and Takaku, F. Morphologic changes of neutrophils in myelodysplastic syndrome treated with recombinant human granulocyte

colony-stimulating factor. *Jpn. J. Cancer Res.*, **79**, 813–816 (1988).
18) Yoshida, M. C., Ikeuchi, T. and Sasaki, M. Differential staining of parental chromosomes in interspecific cell hybrids with a combined quinacrine and Hoechst 33258 technique. *Proc. Jpn. Acad.*, **51**, 184–187 (1975).
19) Report of the Standing Committee on Human Cytogenetic Nomenclature: ISCN 1985. "An International System for Human Cytogenetic Nomenclature," ed. D. G. Harnden and H. P. Klinger, *Cytogenet. Cell Genet.* (1985). Karger, Basel.
20) Yuo, A., Kitagawa, S., Okabe, T., Urabe, A., Komatsu, Y., Itoh, S. and Takaku, F. Recombinant human granulocyte colony-stimulating factor repairs the abnormalities of neutrophils in patients with myelodysplastic syndromes and chronic myelogenous leukemia. *Blood*, **70**, 404–411 (1987).
21) Mayani, H., Baines, P., Bowen, D. T. and Jacobs, A. *In vitro* growth of myeloid and erythroid progenitor cells from myelodysplastic patients in response to recombinant human granulocyte-macrophage colony-stimulating factor. *Leukemia*, **3**, 29–32 (1989).
22) Wong, J. M., Chen, Z. G., Colella, S., Bonilla, M. A., Welte, K., Bordignon, C. and Mantovani, A. Chemotactic activity of recombinant human granulocyte colony-stimulating factor. *Blood*, **72**, 1456–1460 (1989).
23) Raskind, W. H., Tirumali, N., Jacobson, R., Singer, J. and Fialkow, P. Evidence for a multistep pathogenesis of a myelodysplastic syndrome. *Blood*, **63**, 1318–1323 (1984).
24) Russell, N. H. and Reilly, A. G. Role of autocrine growth factors in the leukemic transformation of the myelodysplastic syndromes. *Leukemia*, **3**, 83–84 (1989).
25) Mufti, G. J. and Galton, D. A. G. Myelodysplastic syndromes: natural history and features of prognostic importance. *Clin. Haematol.*, **15**, 953–973 (1986).
26) Kelleher, C., Miyauchi, J., Wong, G., Clark, S., Minden, M. D. and MuCulloch, E. A. Synergism between recombinant growth factors, GM-CSF and G-CSF, acting on the blast cells of acute myeloblastic leukemia. *Blood*, **69**, 1498–1503 (1987).
27) Nara, N., Murohashi, I., Suzuki, T., Yamashita, Y., Maruyama, Y., Aoki, N., Tanikawa, S. and Onozawa, Y. Effects of recombinant human granulocyte colony-stimulating factor (G-CSF) on blasts progenitors from acute myeloblastic leukaemia patients. *Br. J. Cancer*, **56**, 49–51 (1987).
28) Vellenga, E., Young, D. C., Wagner, K., Wiper, D., Ostapovicz, D. and Griffin, J. D. The effects of GM-CSF and G-CSF in promoting growth of clonogenic cells in acute myeloblastic leukemia. *Blood*, **69**, 1771–1776 (1987).
29) Pierre, R. V. Cytogenetic studies in preleukemia: studies before and after transition to acute leukemia in 17 subjects. *Blood Cells*, **1**, 163–170 (1976).
30) Benitez, J., Carbonell, F., Sanchez Fayos, J. and Heimpel, H. Karyotypic evolution in patients with myelodysplastic syndromes. *Cancer Genet. Cytogenet.*, **16**, 157–167 (1985).
31) Tricot, G., Mecucci, C. and Van den Berghe, H. Evolution of the myelodysplastic syndromes. *Br. J. Haematol.*, **63**, 609–614 (1986).
32) Mecucci, C., Rege-Cambrin, G., Michaux, J. L., Tricot, G. and Van den Berghe, H. Multiple chromosomally distinct cell populations in myelodysplastic syndromes and their possible significance in the evolution of the disease. *Br. J. Haematol.*, **64**, 699–706 (1986).
33) Tricot, G., Boogaerts, M. A., Wolf-Peeters, C., Van den Berghe, H. and Verwilghen, R. L. The myelodysplastic syndromes: different evolution patterns based on sequential morphological and cytogenetic investigations. *Br. J. Haematol.*, **59**, 659–670 (1985).