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Effects of recombinant human granulocyte-macrophage colony-stimulating factor in an intensive treatment program for children with Ewing's sarcoma

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Background and Objectives. A treatment program including polychemotherapy at progressively escalating doses and sequential hemi-body irradiation (HBI) was adopted between 1987-1994 at our Pediatric Unit for high risk Ewing's sarcoma. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was added to the treatment program in a phase II study fashion to evaluate, in a pediatric setting, its tolerability, as well as its impact on drug dose escalation and on the need for supportive care.

Design and Methods. The study was open-label and sequential; GM-CSF administration (5 μg/Kg s.c./d ×10) was planned after each chemotherapy cycle and after each HBI session in 18 consecutive patients (group A). Thirty-eight additional patients (group B) were treated by the same therapeutic program, without GM-CSF. In 12 patients (6 in each group) long-term bone marrow cultures (LTBMC) were performed to evaluate the myeloproliferative potential throughout the chemotherapeutic program.

Results. Seven of 18 (39%) patients experienced side effects from GM-CSF; 3/7 discontinued GM-CSF due to anaphylactic symptoms. The degree of neutropenia, as well as the frequency of infectious episodes and the need for supportive care were significantly lower in group A than in group B. Iatrogenic thrombocytopenia, and the possibility of performing drug-dose escalation were similar in the two groups. The 5-year event-free survival probabilities for group A and B were similar. LTBMC showed that the chemotherapy-related depletion of myeloid precursors could be more pronounced in patients receiving GM-CSF cyclically.

Interpretation and Conclusions. In this series, GM-CSF was shown to be effective on iatrogenic neutropenia and related complications, with no impact on thrombopoiesis, drug dose escalation and outcome.

Key words: GM-CSF, Ewing's sarcoma, chemotherapy, radiotherapy

Granulocyte-macrophage colony-stimulating factor (GM-CSF) was one of the first cytokines available for clinical use and its effects have been investigated in a variety of cytopenic conditions, both in adults and in children.[1,2] In the present study, we investigated the effects of GM-CSF in children with newly diagnosed high-risk Ewing's sarcoma (ES) of the bone admitted to our Pediatric Unit and managed according to an intensive treatment program. The treatment program was adopted in the period 1987-1994 and consisted of 8 cycles of chemotherapy with progressively escalating intrapatient drug doses (depending on the degree of myelotoxicity from the previous cycle) followed by sequential hemi-body irradiation (HBI) as consolidation (Table 1). The results obtained in the first 22 consecutive patients treated between 1987 and 1989 showed that iatrogenic leuko-thrombocytopenia was the factor limiting the feasibility of drug dose escalation. In fact, in 50% of the administered cycles drug doses could not be escalated, and only in 6% of the cycles was it possible to deliver a dose 1.6 times as high as the initial one. The problem of HBI-related myelotoxicity was even more important; after 68% of the administered HBI sessions concomitant leukopenia

and febrile episodes were observed. In 1990, human recombinant GM-CSF became available for a phase II study in pediatric patients at our Institution. We, therefore, decided to test this cytokine in a subsequent group of patients recruited in the same treatment program. The aims of the study were to evaluate, in a pediatric setting: a) the tolerability of GM-CSF with the schedule we adopted, b) the hematologic effects of GM-CSF, c) the impact of GM-CSF on drug dose escalation as well on the compliance to chemotherapy and HBI schedules; d) the impact of GM-CSF on the need for myelotoxicity-related supportive care; e) the effect of the repeated GM-CSF administration on the myeloproliferative potential, tested by a long-term bone marrow culture (LTBMC); f) the role of GM-CSF on patients' outcome.

Design and Methods

Patient population and treatment program

Our study included a cohort of 56 consecutive children with biopsy-proven high-risk ES at onset, admitted to the Pediatric Unit of the Istituto Nazionale Tumori, Milan, between 1987 and 1994. The term *high-risk* identified both metastatic disease and non-metastatic disease with maximum tumor diameter > 5 cm or axial localization. These patients entered the treatment program depicted in Table 1. Local treatment was planned after the fourth cycle of chemotherapy, and consisted of conservative surgery and/or radiotherapy (RT) at the primary tumor site. In patients with metastatic disease, RT to the site of metastases was delivered according to each patient's needs. Clinical characteristics of the whole series of patients are summarized in Table 2.

Study design

The present study was carried out in a single institute, in the Pediatric Unit of the Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan. For the purposes of this study we defined as group A the 18 patients treated between 1990-1992 who received GM-CSF after each cycle of chemotherapy and session of HBI; group B was formed of 38 patients who received the same treatment program, without GM-CSF (22/38 were treated between 1987-1989 and 16/38 between 1993-1994). Since one of the main endpoints was the evaluation of the tolerability of GM-CSF in children, the study was designed as non-randomized and sequential.

GM-CSF (5 µg/kg/d) was administered subcutaneously once a day for ten consecutive days starting the day after each cycle of chemotherapy and after each HBI session. Excepting the first administration, the cytokine was given in an out-patient setting.

Table 1. Treatment program for high-risk Ewing's sarcoma.

Week	*No.*		
0	I	V vincristine 1.4 mg/m^2 + E etoposide 75 mg/m^2/d ×4 days + P cis-platinum 20 mg/m^2/d ×4 days + E epirubicin 15 mg/m^2/d × 4 days	
3	II	V E P + I ifosfamide 1g/m^2/d × 4 days	
19	7	III	VEPE
11	IV	VEPI	
		↓	
13		Local treatment	
		↓	
20	15	V	VEPE
21	19	VI	VEPI
23	VII	VEPE	
27	VIII	VEPI	
		↓	
31	I	Hemi-Body Irradiation (2 Gy/d × 5 consecutive days)	
35	II	Hemi-Body Irradiation (2 Gy/d × 5 consecutive days)	

GM-CSF 5 µg/kg/d subcutaneously × 10 days starting the day after the end of each chemotherapy cycle and each HBI session in 18/56 patients. In each patient, doses of drugs to be escalated by 20% at every following cycle (first cycle = 100%), with the exception of vincristine, in case of nadir after the previous cycle with WBC >1,000/mm^3 and/or platelets >70,000/mm^3. Doses of drugs to be reduced by 20% in case of nadir of WBC <1,000 and/or platelets <70,000/mm^3 (minimal doses given =100%).

At the time of diagnosis, complete blood cell counts, bone marrow aspirate and biopsy, and blood chemistry were routinely performed in all patients. Complete blood cell count was repeated every 2 days after the end of each chemotherapy cycle and each HBI session up to the recovery from myelotoxicity; bone marrow aspiration was repeated after the 4th and 8th chemotherapy cycles. In order to evaluate the effectiveness of GM-CSF, the following parameters were compared in groups A and B: total leukocyte and thrombocyte nadirs, and duration of grade IV neutropenia (defined as an absolute neutrophil count of less than 1×10^9/L) after chemotherapy and after HBI; duration of febrile neutropenia episodes; number of transfu-

sions; duration of hospital stay for supportive care; compliance to the scheduled treatment; number of chemotherapy cycles at escalated doses; event-free survival (EFS) probability at 5 years. Patients with febrile neutropenia – defined as grade IV neutropenia along with body temperature 38°C or higher - were hospitalized and treated with i.v. antibiotics, after appropriate cultures had been obtained. The policy for the use of supportive care did not change during the study period.

Safety of GM-CSF

All GM-CSF side effects were recorded according to the Common Toxicity Criteria of the National Cancer Institute. Patients who experienced grade 1 or 2 adverse reactions continued at the same dose. Patients who experienced grade 3 or 4 toxicity discontinued GM-CSF.

Long-term bone marrow cultures (LTBMC) with clonal assays for hematopoietic progenitor cells

The myeloproliferative potential throughout the treatment program was evaluated by LTBMC in twelve consecutive patients, of whom six were consecutive patients in group A and six consecutive patients in group B. The cultures were performed according to the method described by Coutinho *et al*.[3] LTBMC were performed at diagnosis, four weeks after the 4th and the 8th chemotherapy cycles, when myelodepression from the previous cycle recovered. The total non-adherent cell production and non-adherent colony-forming unit granulocyte-macrophage (CFU-GM) in LTBMC of the patients were measured weekly.

Statistical considerations

The analysis comparing quantitative variables in groups A and B was performed using Student's t test. Mean values of these variables are given with their 95% confidence interval (CI). The Wilcoxon ranked sums test was used to compare, in groups A and B, the leukocyte and platelet nadirs and the duration of neutropenia during the entire treatment program. The EFS probabilities were calculated using the Kaplan-Meier estimation technique, and the two Kaplan-Meier curves were compared using the log-rank test.

No formal hypothesis testing was performed for the results of the LTBMCs in the twelve cases we analyzed, because of the small sample sizes.

Results

As of December 2000, the median follow-up of group A patients was 97 months. Fourteen of 18 had completed the treatment program, which was discontinued in 3/18 patients because of disease progression (after the 6th and 7th cycles of chemotherapy, and after the first session of HBI) and in 1 patient because of prolonged thrombocytopenia after the first HBI session. GM-CSF had to be discontinued in 3/18 because of its side effects, as detailed below.

The median follow-up for the 38 patients in group B as of December 2000 was 108 months; the therapeutic program was completed in 26 of 38 patients, and discontinued in 12 of 38: 8 because of disease progression (during the chemotherapeutic phase-6, following the first HBI session-2), and 4 because of prolonged leuko-thrombocytopenia induced by the first HBI session and consequent deletion of the second session of HBI. Table 2 shows the number of chemotherapy cycles and HBI sessions evaluable, as well as the number of cycles and sessions not evaluable and the reasons why.

Table 2A. Clinical characteristics of the patients.

	Given GM-CSF (group A)	*Not given GM-CSF (group B)*
Number of patients	18	38
Localized Ewing's sarcoma	14 (78%)	29 (76%)
Limbs	9	21
Axial	5	8
Metastatic Ewing's sarcoma	4 (22%)	9 (24%)
Site of primary:		
Limbs	3	6
Axial	1	3
Sites of metastasis:		
Bones	2	5
Lungs	1	3
Lungs + bones	1	1
Male/female ratio	1.1	1.2
Age range (median)	1-16 yrs (8)	1-18 yrs (9)

Table 2B. Evaluable cycles of chemotherapy and sessions of hemibody irradiation.

	Given GM-CSF (group A) (18 pts.)	*Not given GM-CSF (group B) (38 pts.)*
Total number of cycles of CT	141	284
Unevaluable	15	4
GM-CSF discontinuation	15	-
Incomplete information	0	4
Evaluable	126 (89%)	280 (98%)
Total number of HBI sessions	30	58
Unevaluable (incomplete information)	1	4
Evaluable	29 (97%)	54 (93%)

Table 3. Side-effects of GM-CSF.

Total number of patients treated with GM-CSF:	18	
Number of patients with side effects:	7 (39%)	
Symptom	*grade 1-2 # of pts.*	*grade 3 # of pts.*
Malaise	6 (33%)	0
Flu-like syndrome	5 (28%)	0
Nausea/vomiting	5 (28%)	0
Tachycardia	4 (22%)	3 (17%)
Hypotension	3 (17%)	2 (11%)
Rash at site of inoculum+edema	5 (28%)	1 (5%)

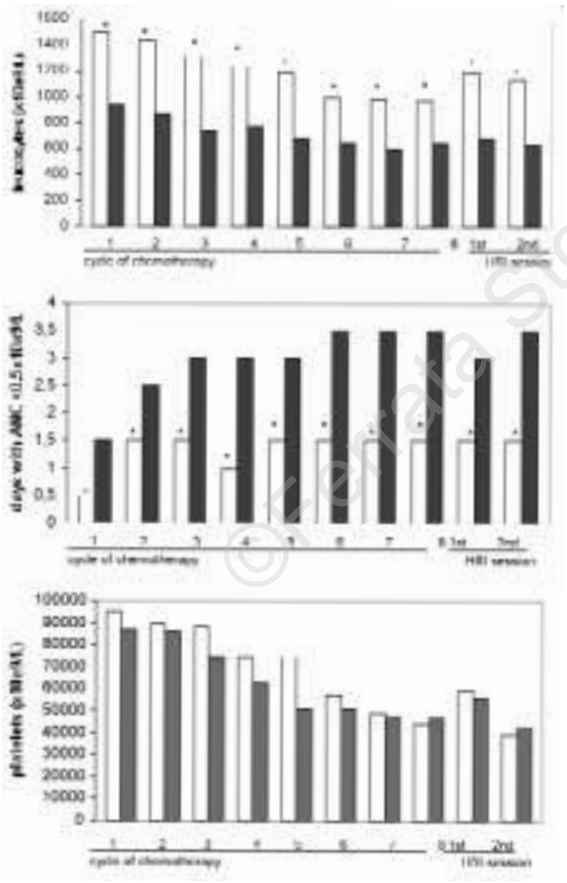

Figure 1. Mean leukocyte count nadir (A), mean duration of neutropenia (B) and mean platelet count nadir (C) after each cycle of chemotherapy and HBI session.
Given GM-CSF (group A) = open bars; not given GM-CSF (group B)= solid bars. *p<0.05 by the Wilcoxon rank-sum statistic.

Safety of GM-CSF

The side effects of GM-CSF administration are summarized in Table 3. Seven of 18 patients (39%) experienced side-effects, and in 3 of them (17%) grade 3 acute toxicity was the reason for GM-CSF discontinuation (Table 3). One of the 3 discontinued GM-CSF after the second day of the first cytokine course because of hypotension, tachycardia, diffuse rash, fatigue, malaise, nausea and vomiting, occurring 20 minutes after each inoculum of GM-CSF; the second patient experienced mild tachycardia after each administration of GM-CSF during the last three days of the first and second course, and discontinued the cytokine after the first administration of the third course due to severe hypotension, tachycardia and fever, having been pre-medicated with chlorphenamine 10mg. i.m. The third patient experienced intense skin-rash and urticaria at the site of inoculum and tachycardia, and discontinued GM-CSF after 6 courses.

The remaining 4 patients showed grade 1 or 2 side effects consisting of skin rash at the site of inoculum, a 'flu-like syndrome, nausea and/or vomiting.

GM-CSF efficacy

The entity of the nadir of WBC, the duration of severe neutropenia and the need for hospital stay and supportive care were significantly less considerable in group A than in group B (Figure 1 and Table 4). The actual incidence of febrile neutropenia in group A and group B was 22% and 39%, respectively ($p < 0.05$). The need for transfusional support (Table 4) and the severity of thrombocytopenia (Figure 1) were not significantly reduced by the use of GM-CSF. The patients given the cytokine did not benefit in terms of dose-intensity: chemotherapy dose escalation and total treatment duration were similar in the two groups. In fact, the percentages of drug doses delivered per patient in groups A and B were almost the same (103% and 114.5% of the initial planned doses/patient, respectively). Among those patients who completed the treatment program, the treatment duration expressed as mean ±SD in groups A and B was 260±20 and 264±21 days, respectively.

GM-CSF administration did not influence the outcome, and the 5-year EFS probabilities in the two groups were similar: 0.56 for patients with non-metastatic ES in group A versus 0.51 in group B. The EFS probabilities were not calculated for patients with metastatic disease because of the small number in this subgroup; of the 4 long-term survivors, 1 belonged to group A, and 3 to group B.

Table 4. Transfusion requirement, fever and hospitalization for supportive care.

	Given GM-CSF group	*Not given GM-CSF group*	*p**
	No. per patient and per course of CT or HBI (mean±95% CI)		
No. of platelet transfusions			
After chemotherapy	0.4±0.3	0.5±0.3	NS
After hemibody irradiation	0.7±0.5	0.9±0.8	NS
No. of RBC transfusions			
After chemotherapy	0.8±0.3	0.9±0.8	NS
After hemibody irradiation	1.2±0.4	1.5±0.9	NS
Days with febrile neutropenia			
After chemotherapy	1.2±0.5	2.2±1.2	<.05
After hemibody irradiation	1.5±1.2	2.3±1.7	<.05
Days of hospitalization			
After chemotherapy	3.1±1.2	5.2±6.0	<.05
After hemibody irradiation	3.9±2.6	6.1±8.4	<.05

**Student's t test; NS = not significant.*

Long-term bone marrow cultures

A decline of the culture growth was observed after 4 cycles of chemotherapy, and this phenomenon was more evident after 8 cycles. The pattern of growth of the LTBMC in the 12 patients we tested was similar, but the number of CFU-GM and non-adherent cell production in the cultures of the patients in group A were 2- to 3-fold lower than those in the cultures of patients in group B (data not shown).

Discussion

The present study was focused on the use of GM-CSF in children. Only a few studies have been published on this issue;[4-17] furthermore, to our knowledge, this is the first report describing the use of GM-CSF after radiotherapy in children. In 1998, a European Panel gave some recommendations for the use of GM-CSF in children. The Panel concluded that the role of GM-CSF in lessening drug-induced neutropenia after chemotherapy was still not clear.[2] This lack of certainty about the utility of the cytokine is reflected in the heterogeneous pattern of its use among pediatric oncologists.[16]

GM-CSF was available for a phase II study in children in 1990 and was employed in an intensive program for children with high-risk ES, consisting of chemotherapy at progressively escalating drug doses and HBI. The study was not double-blind and randomized but open-label and sequential; nevertheless, we believe that its validity is based on the fact that neither the treatment policy nor the management of supportive care (support with blood products, treatment of febrile neutropenia, hospitalization criteria) changed during the entire study period. Moreover, the infectious morbidity in the group of patients who received GM-CSF was similar to that in the group not receiving GM-CSF.

The efficacy of GM-CSF on leukocytopoiesis was clearly evident and led to a lower incidence of infections and of days of hospitalization for supportive care. This result is in agreement with that observed in other studies employing GM-CSF in intensive therapeutic programs for children with solid tumors or acute lymphoblastic leukemia.[5,7,9,12,15,17] At variance, in the case series of two other studies in pediatric patients,[10,11] GM-CSF did not offset myelosuppression from chemotherapy and had no impact on supportive care. However, the schedules adopted in these two studies – in which GM-CSF was given concomitantly with chemotherapy[10] or after combined chemo- and radio-therapy[11] - were different from the other reported studies and therefore the results are not easily comparable.

In our study, GM-CSF did not have a notable effect on megakaryocytes, and the lack of effect on thrombocytopoiesis was the limiting factor for dose escalation and for compliance to the scheduled treatment plan. The effect of GM-CSF on thrombocytopoiesis in children, as in adults,[18-20] is still an open issue and will likely never be completely clarified. The results of the majority of the studies with GM-CSF showed that the cytokine had no impact on platelet recovery or on platelet transfusional support,[7,8,13,16] or else it was itself a cause of mild thrombocytopenia.[9,11] Conversely, in other studies with GM-CSF an impact on platelet recovery acceleration was observed.[5,6] These discordant data are probably related to the doses of GM-CSF administered. *In vitro*, thrombocytopoiesis is sustained at concentrations higher than those active on myelopoiesis[21] and a relationship between dosage and thrombopoietic response is suggested by the results obtained in the studies in which GM-CSF was given at higher concentrations.[5,6]

The same favorable effects of GM-CSF in limiting the leukopenic period after chemotherapy were observed in different studies also after wide field radiotherapy in adult patients. In fact, when GM-CSF was given after double HBI,[22,23] or was given topically on oral mucosae[24,25] or subcutaneously[26] after radiotherapy to the head and neck, a significant reduction of radiotherapy-related myelodepression and infectious episodes was observed. In the present study we obtained similar results in children. This suggests that in children the use of GM-CSF after extended-field radiation could yield clinical benefits. We suggest that the cytokine

should be administered only after the end of the radiotherapeutic program, since the concomitant administration of radiotherapy and hematopoietic growth factors has shown to increase hematological toxicity in adults.[27,28]

The benefit of GM-CSF on myelopoiesis was in part counterbalanced by its side-effects: in 3/18 patients (17%), treatment with the cytokine had to be discontinued because of signs and symptoms of anaphylaxis. Two other studies using GM-CSF reported severe side-effects in 2/29 and 2/34 children,[6,12] but other studies in children describe a good tolerability.[7,8,13,15] The results of our study suggest caution in the use of GM-CSF in children, expecially in those treated at home. When a cytokine to sustain myelopoiesis after chemotherapy is required in an out-patient setting, then G-CSF, another cytokine with good tolerability, should be used.[2] The similar EFS probabilities in groups A and B led to the speculation that GM-CSF did not elicit any of the immune accessory antitumoral activities observed with *in vitro* models;[29,30] on the other hand, this result is reassuring indirect evidence that GM-CSF does not stimulate the growth of ES cells *in vivo.*

The results of the LTBMCs in all the cases we tested show that the repeated cycles of chemotherapy induced a progressive reduction in CFU-GM production, and this was more evident in those patients treated with repeated courses of GM-CSF. The damage induced by chemotherapy on the myeloproliferative compartment has already been described by other authors using the LTBMC system.[31] The use of GM-CSF *in vivo* has been shown to increase the number of CFU-GM in rapid proliferation in the middle and at the end of the cycle of GM-CSF administration,[32] but there is also evidence from one study that a reduced number of $CD34^+$ hematopoietic stem cells is present after 2 weeks in patients treated with GM-CSF.[33] We, therefore, suggest caution in prolonged and repeated use of GM-CSF after cyclic chemotherapy, because of a potential risk of progressive depletion of the myeloproliferative compartment.

In conclusion, the results of the present study show that GM-CSF in our patient setting had a favorable impact on leukocytopoiesis and on the need for supportive care related to iatrogenic neutropenia after both chemotherapy and HBI. This favorable effect of GM-CSF was counterbalanced by quite frequent side-effects, albeit not severe in most cases. The present study joins the small number of studies on the use of GM-CSF in the setting of pediatric oncology; the experience reported in the present study and in the few others in the literature could be taken into account to design double-blind randomized studies to make a definitive assessment of the impact of GM-CSF after high-dose chemotherapy or extended-field radiotherapy.

Contributions and Acknowledgments

RL: conception, design of the study, clinical assessment of the patients and in vitro studies with bone marrow cultures, interpretation of the data, drafting the article; MM, GC, FL: design of the study, clinical assessment of the patients, important intellectual suggestions and critical revision; AF, MC: analysis, interpretation of the data and critical revision; LG: clinical assessment of the patients, important intellectual suggestions and critical revision; MT, FS: critical revision, important intellectual suggestions and help to RL in drafting the article; FFB: conception, design of the study, critical revision and final approval of the version to be submitted.

The order of the authorship was a joint decision of the co-authors. The co-authors, all together, stated the criteria for the order of appearance of their names. These criteria, in order of importance, were: concept, design of the study, the clinical assessment of the patients, the analysis and interpretation of the data, the important intellectual suggestions and the critical revision, the help in drafting the article. All the co-authors agreed with the order of authorship in the final version to be submitted.

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Disclosures

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Potential implications for clinical practice

GM-CSF can be a useful supportive care in children undergoing intensive myelotoxic treatments.

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