



## Alglucerase enzyme replacement therapy used safely and effectively throughout the whole pregnancy of a Gaucher disease patient

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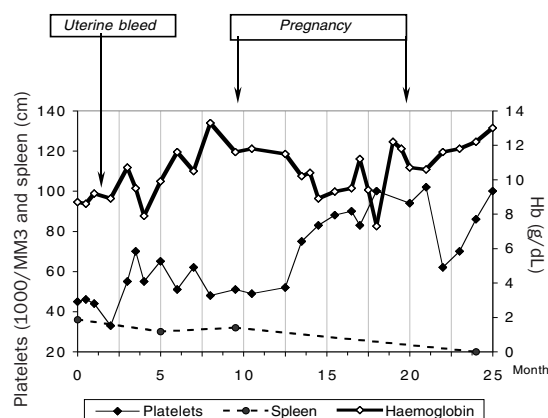
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**We present the case of a woman with Gaucher disease who was being given alglucerase as enzyme replacement therapy. She was found to be pregnant: the treatment was continued. She gave birth to a healthy son after a spontaneous vaginal delivery at term.**

Type I Gaucher's disease (GD) is an inherited metabolic disorder resulting from the deficient activity of  $\beta$ -glucocerebrosidase and the subsequent accumulation of the glycolipid glucosylceramide in macrophages.

A 30-year-old female Type I GD patient, had received enzyme replacement therapy (ERT) for 13 months with alglucerase (Ceredase™, Genzyme Corp. Cambridge, MA, USA) at a dose of 30 IU/kg every two weeks, when she was found to be 15 weeks pregnant during a routine ultrasound to monitor the size of her spleen. This patient was first seen at our hospital at the age of 24 with extreme pallor, cachexia, hepatosplenomegaly and a history of anemia and thrombocytopenia. In the following years she developed some typical GD symptoms such as bleeding, anemia, massive hepatosplenomegaly and slight skeletal Erlenmeyer flask deformity.

Treatment with alglucerase was started on February 18<sup>th</sup>, 1994. In May the patient experienced an episode of severe uterine bleeding, with hemoglobin dropping (8.6 g/dL) even below baseline values, after an initial adequate hematologic response (hemoglobin from 8.6 to 10.5 g/dL and platelets from 48,000 to 56,000/mm<sup>3</sup>) (Figure 1). An ultrasound showed a right sided cystic tumor and signs of endometriosis. To control the hormonal dysfunction, Gynacrin-therapy (Leuproveline; Gn-RH/LH-RH) was started while maintaining ERT. As expected, the patient became amenorrheic, and no further bleeding episodes occurred. In the following months her clinical condition improved again. On April 7, 1995 the pregnancy was detected. Conception had presumably occurred at the end of December 1994 (10 months after the start of ERT). As, by then, the first trimester of pregnancy was already over and uterine ultrasound showed no fetal abnormalities, it was decided to continue ERT. In the 40<sup>th</sup> week of pregnancy spontaneous contractions started, resulting in a successful vaginal delivery of a 3.5 kg boy, with no abnormalities (Apgar test: 9 at 1 and 10 min). Uterine bleeding was in the low-normal range and the placenta was



**Figure 1. Follow-up of hemoglobin, platelets and spleen during enzyme replacement therapy.**

judged normal. Given the satisfactory clinical course it was decided to maintain ERT and allow maternal breast-feeding. The baby continues to have a steady and healthy development. The patient recovered well and continued to respond to ERT (Figure 1).

ERT has shown to be safe and effective in the treatment of Type I GD.<sup>1</sup> So far, no complete clinical descriptions of pregnant GD patients treated with alglucerase throughout pregnancy have been published. There is only one abstract briefly describing 10 patients receiving alglucerase during any part of pregnancy,<sup>2</sup> revealing two adverse outcomes, and only one full term delivery while on ERT for the whole pregnancy. Obstetric aspects of GD patients not receiving ERT have already been described.<sup>3-5</sup> These reports show that GD patients are at risk of complications during pregnancy. Therefore, it might be suggested that alglucerase could contribute to increase the patient's health status during pregnancy, and delivery. As no reproductive studies have been performed on the use of alglucerase, the decision to treat GD patients during pregnancy should be made on an individual basis by the patients physician, considering the disease status of the patient and possible risks.

### Key words

*Gaucher disease, pregnancy, alglucerase*

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### Long-term follow-up of non-Hodgkin's lymphoma patients treated with ProMACE-CytaBOM: an effective regimen for the intermediate grade subtype

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**Long-lasting results achieved in 54 patients with aggressive non-Hodgkin lymphoma treated with ProMACE-CytaBOM regimen were evaluated. Twenty-four out of 54 (45%) patients achieved a complete remission and 13 of them are still in continuous remission with a median survival of 53.5 months. Interestingly, in 16 patients with intermediate grade histology we obtained an overall response rate of 100%.**

Among third-generation chemotherapy regimens, ProMACE-CytaBOM is considered a very effective protocol in patients with aggressive non-Hodgkin lymphomas (NHL), producing about a 70% rate of complete remission (CR) as first reported by Fisher and confirmed by subsequent studies.<sup>1-4</sup>

In our study 54 patients with aggressive NHL, 25 of whom at diagnosis, received ProMACE-CytaBOM chemotherapy according to the scheme proposed by Fisher *et al.* and updated by Longo *et al.*<sup>2,5</sup> The patients' clinical and histological characteristics are summarized in Table 1. All responding patients received a minimum of six cycles and almost all patients received 100% of the planned dose. Overall, 24 out of 54 (44.5%) patients achieved complete response (CR) and 20 (37%) a partial response (PR), reaching an overall response rate of 81%.

As expected, patients at diagnosis or with a more favorable histology and with a normal LDH serum level achieved better results, while differences in response rates were not observed when patients were analyzed according to age, sex, stage of disease, and mean dose intensity calculated for each single drug (Table 1). On the other hand, CR rates were significantly higher ( $p < 0.05$ ) in previously untreated patients and in those with intermediate grade histology.

The estimated 5-year overall survival was 53%. When the analysis was done according to histologi-

**Table 1. Patients' characteristics and results achieved in 54 patients treated with ProMACE-CytaBOM.**

Characteristics	CR	PR	CCR
Age			
≤ 60 years	12/24 (50%)	8/24 (33%)	7/12 (58%)
> 60 years	12/30 (40%)	12/30 (40%)	6/12 (50%)
Sex			
male	13/32 (40%)	13/32 (40%)	7/13 (54%)
female	11/22 (50%)	7/22 (32%)	6/11 (54%)
Stage			
I-II	9/18 (50%)	7/18 (39%)	6/9 (67%)
III-IV	15/36 (42%)	13/36 (36%)	7/15 (47%)
Histology			
intermediate grade	16/22 (73%)	6/22 (27%)	8/16 (50%)
high grade	8/32 (25%)	14/32 (44%)	5/8 (62%)
Therapy			
untreated	16/29 (55%)	9/29 (31%)	11/16 (69%)
1 regimen	8/21 (40%)	9/21 (43%)	2/8 (25%)
> 1 regimen	0/4	2/4 (50%)	0/4
LDH			
≤ 450	16/29 (55%)	11/29 (38%)	8/16 (50%)
> 450	8/25 (32%)	9/25 (36%)	5/8 (62%)
IPI			
low/low-intermediate	16/27 (59%)	10/27 (37%)	11/16 (69%)
intermediate-high/high	8/27 (29%)	10/27 (37%)	2/8 (25%)
Total	24/54 (44%)	20/54 (37%)	13/54 (24%)

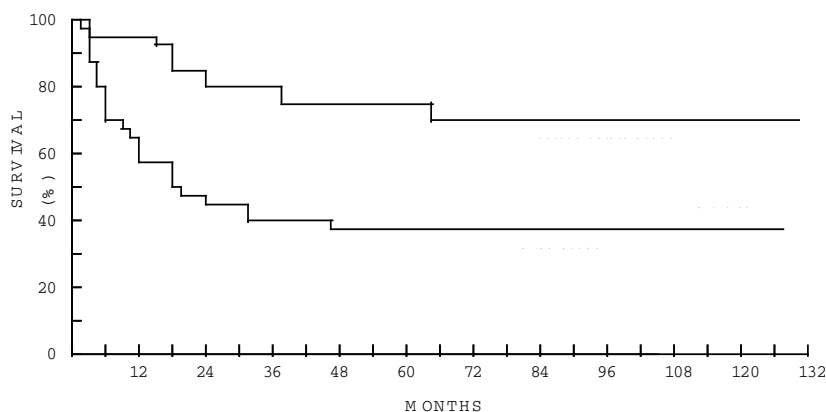
Abbreviations: CR: complete response; PR; partial response; CCR: continuous CR.

cal subtypes and previous therapy, untreated patients and those with intermediate histology showed statistically significant ( $p < 0.05$ ) better survival curves (Figures 1 and 2).

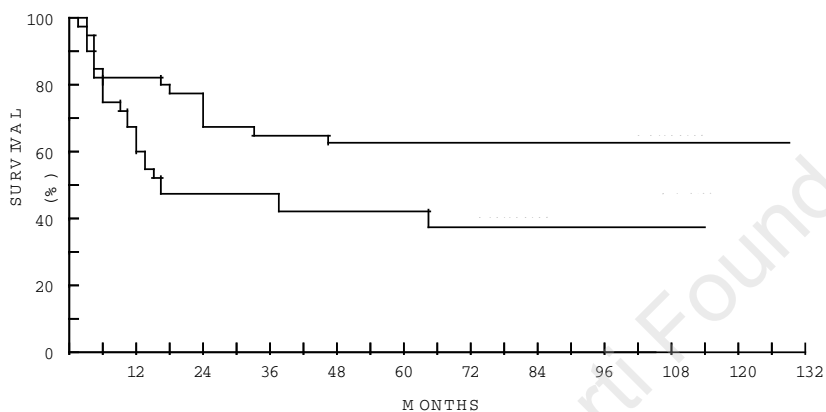
Grade 3-4 neutropenia (WHO classification) recorded in 15 patients, and nausea and vomiting were the most important and frequent hematologic and non-hematologic side effects.

Our results, in agreement with previous reports,<sup>4,6-10</sup> document an overall response rate of 81% with an apparent reduced number of CRs probably due to the higher median age of our patients, to a not negligible number of pre-treated cases and probably also to the very strict criteria adopted in defining CR. However, despite the reduced CR rate, 54% of complete responders in our study are still in CR after a considerable period. Interestingly, patients with intermediate grade of malignancy showed a response rate of 100% regardless of the previous therapy. The small number of this very lucky series of patients does not allow further analysis.

In conclusion our results, although less impressive in terms of CR rate than other previous reports<sup>3-6</sup> document that 54% of complete responder patients are still in CR after a median of 53 months. Hematologic toxicity and side effects were acceptable and never



**Figure 1.** Survival curves of 54 NHL patients according to histological subtype (high grade vs intermediate grade).



**Figure 2.** Survival curves of 54 NHL patients according to treatment (previously untreated vs pretreated).

lifethreatening, suggesting that the ProMACE-CytaBOM regimen is an effective and safe chemotherapeutic scheme.

#### Key words

*Non Hodgkin lymphoma, ProMACE-CytaBOM regimen, long-term follow-up*

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## Philadelphia positive acute lymphoblastic leukemia 16 years after the apparent cure of acute lymphoblastic leukemia. New leukemia or late relapse?

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**A Philadelphia-positive ALL in an adult occurring 21 years after the initial diagnosis is reported here. This case raises the question as to whether or not this event is a relapse or a new leukemia. A possible role of interferon- $\alpha$  previously administered to the patient for a chronic viral hepatitis is discussed too.**

Acute lymphoblastic leukemia (ALL) relapses occurring more than five years after achieving complete remission (CR) are unusual<sup>1</sup> and raise the possibility of a new neoplasm.<sup>2</sup>

We report a second leukemia 16 years after the cure of a childhood ALL. This second leukemia was Philadelphia chromosome (Ph1) positive, a poor prognostic factor. In addition, we discuss herein the possible role played by interferon- $\alpha$  (IF- $\alpha$ ) in the etiopathogenesis of this new leukemia.

### Case Report

A three-year-old child affected by ALL in 1974 received induction treatment, holocranial radiotherapy and intrathecal methotrexate and he achieved CR. Maintenance treatment was given until June 1977. After therapy for testicular relapse treatment was stopped in 1979.

In December 1990 a chronic viral C hepatitis was diagnosed and IF- $\alpha$  was given for three years, but was subsequently discontinued as it was ineffective.

In 1995, 21 years after the first diagnosis the patient presented with clinical and analytical signs of an apparent ALL relapse. Immunophenotyping revealed a B lineage common ALL. Reverse-transcription polymerase chain reaction (RT-PCR) with nested primers specific for *minor breakpoint* rearrangements (primers supplied by Oncogen RP, Cambridge, MA, USA) was positive giving rise to the expected bands in *bcr/abl* e1a2 rearrangements. No amplification was noted when transcripts from the patient were assayed with primers specific for *mayor breakpoint* rearrangements.

After receiving a single chemotherapy course, he died of septic abdominal infection.

In patients achieving long-lasting CR, late relapses are unusual and raise a controversial issue: relapse versus new leukemia.

Pagano *et al.*<sup>3</sup> revealed that the actuarial estimated cumulative proportion of ALL patients with a secondary haematologic neoplasm at 5 and 10 years

were 0.59% and 3.63%, respectively.

In our case, the lack of immunologic and molecular data from the first leukemia hampers understanding of whether we are facing a genuine relapse or a distinct ALL. The hypothesis of a different neoplasm is supported by two facts. Firstly, the strikingly long duration of our patient's relapse-free survival (therefore, a drug-induced leukemia cannot fully discarded). Secondly, although in pediatric ALL, Ph1 can be absent at diagnosis, subsequently emerging as a consequence of clonal evolution,<sup>4</sup> and a subset of good prognosis Ph1 ALL could exist,<sup>5</sup> Ph1 is usually associated with aggressive disease, poor prognosis and no short term remissions.<sup>6</sup>

Nevertheless, a relapse cannot be completely ruled out. In this case IF- $\alpha$ , as an immune modulator, administered to the patient in previous years may have prompted the leukemia relapse.

IF- $\alpha$  can activate B-cells in malignant and non-malignant lymph nodes to proliferation and blast transformation.<sup>7</sup> IF- $\alpha$  has been shown to regulate B-cell differentiation and to act as a natural regulator of B-cell functions. In some neoplasms of B-cell origin, especially myeloma, IF- $\alpha$  is able to stimulate the proliferation of Interleukin-6 dependent cells of clonogenic tumor cells *in vitro*<sup>8</sup> as well as *in vivo*.<sup>9</sup>

### Key words

Acute lymphoblastic leukemia, late relapse, Philadelphia chromosome, interferon- $\alpha$ .

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### ***bcr-abl* rearrangement in adult T-lineage acute lymphoblastic leukemia**

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**The *bcr-abl* rearrangement in T-lineage ALL has been rarely described. In the last three years we studied all new patients with ALL at diagnosis by cytogenetic and molecular analysis. Three out of eleven T-lineage ALL patients presented the rearrangement and only one was Philadelphia positive.**

The Philadelphia (Ph) chromosome, t(9;22)(q34;q11), is present in more than 95% of patients with chronic myelogenous leukemia (CML) and in 15-25% of adults with acute B-lineage lymphoblastic leukemia (ALL).<sup>1,2</sup> In T-lineage ALL it has rarely been reported<sup>3,4</sup> and singular cases of T-lineage adult ALL carrying the *bcr-abl* rearrangement have been recently described.<sup>5-8</sup> *bcr-abl* rearrangement in T lineage ALL is thus a rare event and the clinical relevance of this translocation is currently unknown.

In the last three years we studied 25 new consecutive cases of ALL (14 B-lineage and 11 T-lineage). We present here the clinical, immunologic, cytogenetic and molecular features of three out of the eleven T-lineage ALL patients presenting *bcr-abl* rearrangement at diagnosis.

**Patient #1.** A 15-year-old male was referred with a recent history of cough and fatigue. He was treated with daunomicin, vincristine, asparaginase, and prednisone and obtained a complete remission. He relapsed 7 months later, did not obtain a second remission and died 4 months later.

**Patient #2.** A 32-year-old male was admitted with acute leukemia. He was treated with idarubicin, cytarabine, vincristine and prednisone and obtained complete remission. He was later submitted to allogeneic peripheral blood transplantation from his HLA-identical sister while in first CR. The patient developed acute but not chronic GVHD. He relapsed 18 months later. He is actually in second CR after reinduction treatment.

**Table 1. Clinical and biological characteristics of the patients.**

Patients	#1	#2	#3
Age/Sex	15/M	32/M	47/M
Hb g/dL	13.7	6.9	12.9
WBC x 10 <sup>9</sup> /L	59.4	19.6	9.6
Plt x 10 <sup>9</sup> /L	329.	39.	436.
LDH U/L	1575	2290	711
Splenomegaly	-	+	-
Mediastinal enlargement	++	-	+++
Lymph-node enlargement	+	+	-
Phenotype	TdT,CD34,cCD3,CD4,CD5,CD7,CD13	TdT,cCD3,CD1,CD2,CD4,CD5,CD7,CD8	TdT,CD1,CD2,CD4,CD5,CD7,CD8
Karyotype	46,XY,t(9;22)(q34;q11)	46,XY	46,XY,6q-,7-,9,-,+2mar
BCR type	M	M	M
Response to induction	CR	CR	CR
Survival (months)	11	24+	5

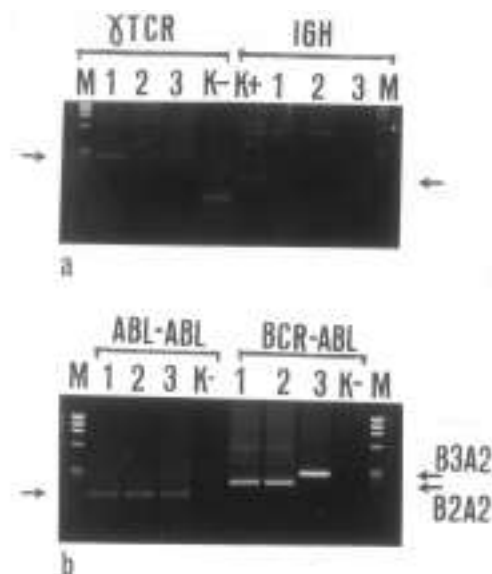
**Patient #3.** A 47 year-old male was admitted with acute pericarditis. The chest X-ray and chest CT scan showed massive mediastinal enlargement and pleural and pericardial effusion. Blood counts were normal, but differential counts showed 20% blast cells. Bone marrow aspiration revealed 50% blast cells with cerebroid nucleus. Pleural fluid contained 117.0×10<sup>9</sup>/L blast cells. The patient was treated with daunoblastin, vincristine, asparaginase and prednisone and obtained complete remission. He was submitted to autologous peripheral stem cell transplantation, but died of adult respiratory distress syndrome two weeks later.

Clinical and biological data are shown in Table 1. *bcr-abl* transcript was detected by RT-PCR and the rearrangements occur in all patients within the 5.8-kb M-*bcr* region associated with P210 *bcr-abl* expression; monoclonal rearrangement of the TCR $\gamma$  gene, but not of the IGH locus was also detected by PCR (Figure 1).

Ph+ CML is known to arise in a multipotent hematopoietic stem cell.<sup>9</sup> This is also shown by the fact that Ph translocation and/or *bcr-abl* expression can be simultaneously found in cells of myeloid and lymphoid lineage. Occasional reports of Ph+ T-cell blast crisis of CML provide evidence that T-cell precursors can be involved in Ph+ leukemic transformation.<sup>10</sup> Single cases of T-lineage ALL with Ph translocation or *bcr-abl* rearrangement have also been reported.<sup>3-8</sup>

The characteristics of our three *bcr-abl* T-ALL cases are indistinguishable from other T-lineage ALL. Two presented with mediastinal enlargement and one patient had intermediate characteristics between T-ALL and T mediastinal lymphoblastic lymphoma.

We do not know whether the presence of *bcr-abl* gene rearrangement makes the prognosis of T-lineage ALL worse, but two out of three patients had bad prognosis characteristics such as early T phenotype,



**Figure 1. Molecular characteristics of our cases. A) PCR amplification of  $\gamma$ TCR and IGH locus. B) RT-PCR detection of BCR-ABL mRNA. Lane numbers refer to patients listed in table 1. Arrows indicate the products of amplification. M = size markers, K- = negative control, K+ = positive control. The samples were electrophoresed through agarose and DNA visualized by staining in ethidium bromide.**

high WBC count and extranodal involvement. The presence of *bcr-abl* rearrangement in T-ALL is a rare event. Our cases are probably an occasional series. Nevertheless the real incidence and the significance of *bcr-abl* rearrangement in T-lineage ALL is not known. We think that further studies on the molecular biology of T-ALL could be useful.

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### AIDS-related non-Hodgkin's lymphomas from an Italian area

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**In a retrospective study, 42 (7.7%) of 545 patients with AIDS from a single area of Italy had non-Hodgkin's lymphoma (28 systemic and 14 primary central nervous system lymphomas). The improved outcome and survival of treated patients outlines the clinical benefit of antineoplastic treatment in selected cases.**

It has been widely recognized that patients with HIV-related immunosuppression are at increased risk of developing non-Hodgkin's lymphomas (NHL) that include systemic, primary central nervous system (P-CNS-L) and body cavity-based lymphomas.<sup>1</sup> At present in Italy the incidence of NHL is 3.5% according to the Centro Operativo AIDS.<sup>2</sup> However, this figure represents only the first AIDS-defining condition, potentially missing lymphomas occurring at a later stage.

We have retrospectively evaluated the epidemiological and clinical characteristics of 42 cases (7.7%) of NHL among 545 patients with AIDS admitted to the Infectious Diseases Departments of the Verona area up to June 1997. Baseline characteristics of patients are shown in Table 1. Systemic-NHL were of B-cell type and classified as follows: 18 large cell, 4

**Table 1. Baseline characteristics of patients.**

	Systemic NHL (28 patients)	P-CNS-L (14 patients)
Sex		
male	20	10
female	8	4
Risk group		
IVDA	13	10
homosexuals	10	1
heterosexuals	2	3
transfusion recipients	2	
unknown	1	
Median age, years (range)	32 (25-53)	31 (28-42)
Previous AIDS diagnosis	4 (15%)	9 (75%)
Median Karnofsky score (range)	70 (60-90)	70 (50-80)
Median CD4 cell/ $\mu$ L (range)	122 (2-668)	32 (2-114)

NHL= non-Hodgkin's lymphoma; P-CNS-L= primary central nervous system lymphoma; IVDA= intravenous drug abusers.

**Table 2. Treatment, outcome and survival of patients.**

	# of pts.	Treatment				Outcome			Median surv. months (range)
		CT	CT+RT	RT	S	CR	PR	NR	
NHL	17	10	3	2	2	8	4	5	11 (1-66)
P-CNS-L	11	-	-	11	-	3	6	2	9 (3-12)

NHL= non-Hodgkin's lymphoma; P-CNS-L= primary central nervous system-lymphoma; CR= complete remission (response); PR= partial remission (response); NR= no response; CT= chemotherapy; RT= radiotherapy; S=surgery.

Burkitt's-type, 4 large cell immunoblastic, 2 low-grade B lymphomas. Three patients (11%) had exclusive nodal involvement and 25 one or more extranodal sites: 6 stomach, 4 each in liver, lung, bone marrow, 2 in the CNS, 1 each in testicle, spleen, gut, tongue, bladder and heart. Twenty patients (71%) presented with B symptoms. At diagnosis, 2 patients were in Ann Arbor stage I, 4 in stage II, 8 in stage III and 14 in stage IV. The diagnosis of P-CNS-L (B large cell lymphoma) was obtained by stereotactic brain biopsy in 12 cases and by post-mortem examination in 2 cases. The outcome and survival of treated patients are shown in Table 2. Chemotherapeutic and dose-intensity (range) regimens for patients with systemic-NHL were as follows: 6 patients CHOP (0.93-0.98), 2 ACVP (0.83-0.85), 3 ACVBP (0.61-0.69), 2 VACOP-B (0.82-0.87), 1 ProMACE-MOPP (0.69). Seven patients received in addition intrathecal methotrexate (3-4 cycles). Five patients underwent radiation therapy (involved fields, total dose 30.5-40 Gy delivered over 3-4 weeks). All patients

received trimethoprim/sulfamethoxazole prophylaxis and were not given anti-retroviral therapy up to completion of chemotherapy. A total of 7 patients had neutropenia and received G-CSF. The 8 patients (47%) who achieved complete remission did not have a previous AIDS diagnosis or a baseline CD4<sup>+</sup> count < 100/mL. In contrast, 4 of the 9 remaining patients who showed partial remission or no response had a previous AIDS diagnosis and CD4<sup>+</sup> count < 100/mL. The overall survival of 10 untreated patients (median 1 month, range 1-14) was significantly ( $p < 0.01$ ) shorter than that of treated patients. Eleven patients with P-CNS-NHL underwent radiation therapy: 7 whole brain radiation (total dose 32-50 Gy delivered over 3-5 weeks) and 4 gamma-knife treatment (radiosurgical dose 46-70 Gy delivered to the tumor center in a single session).

AIDS-related NHL is quite frequent in our area and similar to that recently reported in USA by others.<sup>3</sup> Our results confirm that the outcome and survival after therapy of systemic NHL substantially depend on both the underlying immune status and the stage of HIV disease.<sup>4,5</sup> The prognosis of P-CNS-L is generally poor despite therapy since this tumor occurs in patients who are much more severely immunosuppressed.<sup>6</sup> The surprisingly long survival<sup>7,8</sup> of our patients treated with cerebral irradiation is due to exclusion from brain biopsy of patients with a life-expectancy of less than 6 months.<sup>9</sup>

### Key words

AIDS-related NHL

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## Two-dimensional analysis of the structure of human von Willebrand factor

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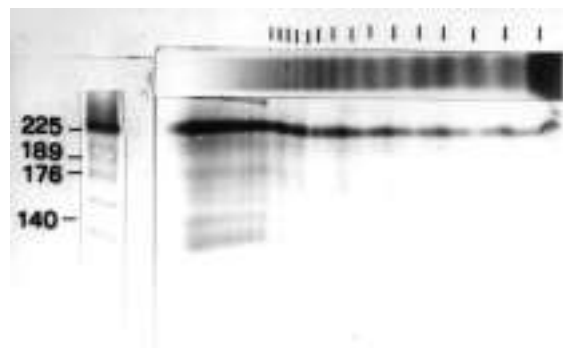
**Human von Willebrand factor (vWF) is synthesized as an extra large polymer; then, it is converted to lower molecular weight plasma multimers, originally composed of intact 225-kDa subunits, by a metalloproteinase.<sup>1,2</sup> Proteolysis generates two fragments of 140- and 176-kDa, which originate from cleavage of peptide bond Tyr842-Met843 and which represent vWF residues 1-842 and 843-2050, respectively; a very small amount of 189-kDa fragment can also be found in normal plasma.<sup>3</sup> We describe here a two-dimensional (2-D) method to analyze plasma vWF structure.**

Agarose gel electrophoresis was used as the first dimension to resolve the multimeric structure of vWF; the second dimension, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), allowed us to obtain information about proteolysis *in vivo* of this molecule. One-dimensional electrophoresis was performed on a vertical mini-gel apparatus (Mini-Protean II, Bio-Rad, Hercules, CA, USA). Plasma from normal subjects and type 2 von Willebrand disease (vWD) patients was diluted 1:5 in sample buffer; 30  $\mu$ L were applied on 1.7% low gelling temperature agarose and run at 14 V for 20 h.<sup>4</sup>

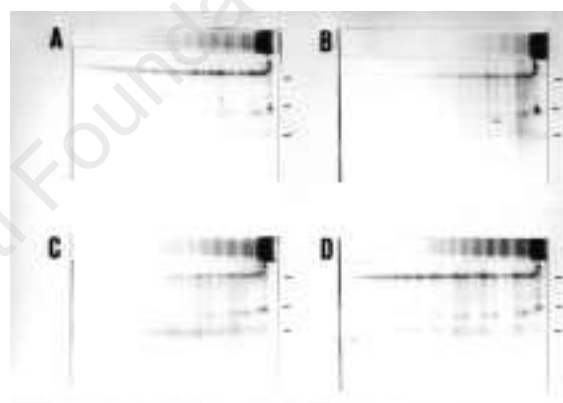
vWF lanes were excised from the agarose gel, washed in distilled water for 30' and soaked in reducing buffer (1% dithiothreitol and 1% SDS in stacking gel buffer, pH 6.8) for 30' at room temperature under gentle agitation.

SDS-PAGE (5% polyacrylamide)<sup>5</sup> was performed on mini-slab gel. The reduced lanes were applied on the top of the gel and electrophoresis was run at 200 V until the dye front reached the bottom of the gel.

Immunodetection was performed as described elsewhere;<sup>4</sup> briefly, after electroblotting of 1-D gel, nitrocellulose membranes were incubated with rabbit anti-human vWF antiserum followed by incubation with alkaline phosphatase-labeled anti-rabbit antibody.



**Figure 1. 2-D analysis of plasma normal vWF. Multimeric and subunit composition of vWF are shown, as references, on the top and on the left of the figure, respectively.**



**Figure 2. 2-D analysis of type 2A (A,B,C) and type 2B (D) vWF. Dashes indicate 225-, 176-, and 140-kDa subunits, respectively.**

BCIP/NBT was used as the chromogenic substrate.

Normal vWF shows a predominance of the intact 225-kDa subunit; the proteolytic fragments are present in low amounts (Figure 1). The 189-kDa fragment is poorly recognizable due to its low concentration in plasma. All subunits are resolved as broad bands having a whiter central area; the high glycosylation degree of vWF<sup>6</sup> may be responsible for this pattern. Moreover, better detection of vWF subunits may be achieved using a pool of monoclonal antibodies rather than a polyclonal antibody.

Type 2A and 2B vWF lack the higher molecular weight multimers;<sup>7</sup> 2-D analysis shows that the lower molecular weight multimers are composed of higher amounts of proteolytic fragment than normal plasma (Figure 2).

The proposed method allows 2-D analysis of vWF; in this way, we could achieve information on multi-



merization and proteolysis of vWF. The method is simple, based on well-tested techniques such as agarose gel electrophoresis, SDS-PAGE, and immunoblotting; it is performed by mini-gel equipment, thus minimizing reagent consumption and analysis time. Moreover, vWF subunits are immunoenzymatically detected, without need of radiolabeled reagents.

### Key words

*von Willebrand factor, two-dimensional analysis*

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### Long-term disease-free acute promyelocytic leukemia patients really can be cured at molecular level

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**The characteristic t(15;17) translocation involving chromosomes 15 and 17 is specifically associated with both the common and the variant subtypes of acute promyelocytic leukemia (APL) (M3 according to FAB classification).<sup>1</sup> At the molecular level, it fuses genes encoding PML on chromosome 15 and the nuclear retinoic acid receptor- $\alpha$  (RAR $\alpha$ ) on chromosome 17. The subsequent expression of PML/RAR $\alpha$  fusion mRNA provides a potential molecular marker**

**that can be detected in leukemic cells taken from patients with APL.<sup>1</sup> Using PML and RAR $\alpha$  sequence specific primers, reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed for detection of PML/RAR $\alpha$  transcript in leukemic cells obtained from patients; these RT-PCR assays are more sensitive than conventional cytogenetic analysis.<sup>1,2</sup>**

We and others reported previously that the majority of the acute promyelocytic leukemia (APL) patients with long-lasting disease free survival were negative for PML/RAR $\alpha$  transcript. We have now applied RT-PCR assay for PML/RAR $\alpha$  analysis on bone marrow samples from 18 APL patients (8 female, 10 male; median age 31 years; range 14-59) with long-lasting complete remission (CR), after induction chemotherapy and consolidation (median 59 months; range 38-142 months from CR) in order to verify the validity of these observations further. All patients were in clinical and cytogenetic CR at the time of molecular evaluation. Nine of these patients had already been studied.<sup>2</sup> In eleven patients karyotypic analysis on bone marrow aspirates was performed at diagnosis and confirmed the presence of the t(15;17) translocation. In the other 7 patients, using bone marrow samples frozen at the time of diagnosis we were able to detect the presence of the PML/RAR $\alpha$  transcript by RT-PCR analysis.<sup>3</sup>

Patients received different protocols of induction chemotherapy including an anthracycline (daunorubicin or idarubicin) alone or in combination with cytosine arabinoside (biological and clinical data are given in Table 1). After achievement of CR, one patient (PS in Table 1) was submitted to allogeneic bone marrow transplantation (BMT) from an HLA matched available donor. Fourteen patients were submitted to autologous BMT.<sup>4</sup> Only two patients (GL and OD) were submitted to maintenance chemotherapy, and one patient (OM) withdrew from maintenance chemotherapy owing to hepatic toxicity. Remission bone marrow aspirates were obtained after achievement of CR and used for molecular analysis.<sup>1</sup> Cytogenetic studies were performed as reported.<sup>3</sup> RT-PCR analysis was performed as described elsewhere.<sup>2</sup> Concerning the specificity and sensitivity of our RT-PCR method, we can detect one PML/RAR $\alpha$ -positive cell diluted in  $10^{-3}$ - $10^{-4}$  PML/RAR-negative cells.

The results of RT-PCR analysis in remission samples are schematically represented in Figure 1. Only the molecular results regarding the last sample for each patient are presented. In all cases but one, no PML/RAR $\alpha$  transcripts were visible either on the ethidium bromide gels or after silver staining. At present, all but one of the patients are in continuous CR with a median follow up of 59 months (range 38-142). The patient who died (OM) had been persistently PCR positive at different times of analysis (+13, +15 and +32 months). After 39 months of CR, she presented a cytogenetic and a clinical relapse. A second CR was achieved after therapy with all-trans retinoic acid

**Table 1. Clinical and therapeutic characteristics of the APL patients.**

UPN = unidentified progressive number; name = initials of name of the patients; duration of the 1st CR = time in months of observation disease free survival after achieving 1st CR; induction = drugs employed in induction therapy; consolidation = drugs employed in consolidation therapy; MTX = Methotrexate<sup>®</sup>; VP16 = Vepesid<sup>®</sup>; IDA = Zavedos<sup>®</sup>; ARAC = Aracytin<sup>®</sup>; 6TG = Thioguanine<sup>®</sup>; 6MP = Purinethol<sup>®</sup>; DNR = Daunoblastine<sup>®</sup>; AMSA = Amsacrine<sup>®</sup>; maintenance = drugs employed in maintenance therapy; BMT = bone marrow transplantation; Allo = allogeneic BMT; Auto = autologous BMT; age = age in years of the patients at the time of diagnosis; sex = sex of the patients F= female; M = male; FAB and M3 = French-American-British Cooperative Group classification.<sup>5</sup>

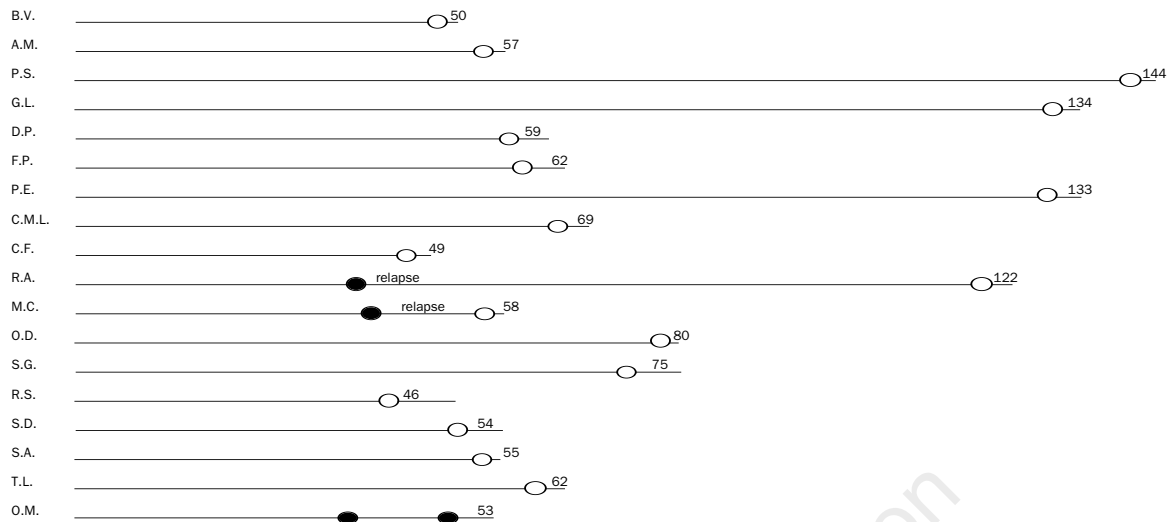
UPN	Pt.	Age (yrs)	Sex	Breakpoint	Year of diagnosis	Therapy				1 <sup>st</sup> CR duration	Survival (months)	Status
						Induction	Consolidation	Maintenance	BMT			
1	B.V.	43	M	BCR3	1993	ATRA	Dauno+ARAC	No	Auto	46	47	CR
2	A.M.	37	M	BCR1	1993	ATRA+Dauno	ATRA	DAE	Auto	52	54	CR
3	P.S.	19	F	BCR1	1985	Dauno+ARAC	Dauno+ARAC	No	Allo	138	141	CR
4	G.L.	59	F	BCR1	1986	Dauno+ARAC	Dauno+ARAC	ARAC+6TG	No	129	131	CR
5	D.P.	25	F	BCR3	1992	ATRA+Dauno	Dauno+ARAC	DAE	Auto	50	53	CR
6	F.P.	36	M	BCR1/2	1992	IDA+ARAC	IDA+ARAC Mitox+VP16 Dauno+6TG	DAE	Auto	55	56	CR
7	P.E.	31	M	BCR1	1986	Dauno+ARAC	AMSA+ARAC	No	Auto	141	142	CR
8	C.M.L.	29	F	BCR3	1992	IDA+ARAC	Dauno	ATRA	Auto	54	55	CR
9	C.F.	51	M	BCR1	1993	ATRA	IDA+ARAC	No	Auto	44	46	CR
10	R.A.	31	F		1987	Dauno	AMSA+MetilGAG AMSA+ARAC AMSA+ARAC	No	Auto	7	131	CR after 2 <sup>nd</sup> relapse
11	M.C.	37	M	BCR1	1992	IDA+ARAC	IDA+ARAC Mitox+VP16 IDA+6TG+ARAC	No	Auto	18	65	CR after 2 <sup>nd</sup> relapse
12	O.D.	17	M	BCR1	1991	IDA+ARAC	IDA+ARAC Novan+ARAC IDA+ARAC+6TG	6MP+MTX	No	75	77	CR
13	S.G.	20	M	BCR3	1991	IDA	IDA+ARAC Nov+VP16	MTX+6MP	Auto	72	73	CR
14	R.S.	16	M	BCR1	1994	ATRA	IDA+ARAC	No	Auto	42	43	CR
15	S.D.	14	F	BCR3	1993	ATRA+Dauno	Dauno+ARAC	No	Auto	51	52	CR
16	S.A.	28	M	BCR3	1993	ATRA+IDA	IDA+ARAC	No	Auto	53	54	CR
17	T.L.	42	F	BCR1	1992	IDA+ARAC	IDA+ARAC Mitox+VP16 ARAC+6TG	MTX	Auto	58	59	CR
18	O.M.	50	F	BCR1	1991	IDA	ARAC+IDA Mitox+VP16	No	No	37	53	Died from APL relapse

(ATRA). Although she remained in CR for 5 months at a molecular level she was persistently positive. After a further relapse, she died from disease progression.

Recently, *in vitro* amplification of leukemia-specific fusion transcripts by RT-PCR has been applied to the detection of minimal residual leukemia (MRL).<sup>5</sup> The persistence of the PML/RAR $\alpha$  transcript in early post-remission APL samples has been associated with early clinical relapse within a few months.<sup>1,6</sup> Several recent studies indicate that molecular monitoring of the PML/RAR $\alpha$  fusion transcript in APL could allow identification of patients who need further anti-leukemic therapy.<sup>7</sup> On the other hand, we and others<sup>8</sup> have reported that long term survival of APL is associated with eradication of cells carrying the spe-

cific PML/RAR $\alpha$  rearrangement, indicating that PCR negativity should be considered the therapeutic goal in these patients.

Regarding the role of consolidation and maintenance chemotherapy,<sup>9</sup> most of our APL patients received, as a consolidation of the cytotoxic chemotherapy induction of APL, an allogeneic (1 patient) or autologous bone marrow re-infusion after chemotherapy ablation (14 patients). The only patient in our series who had a clinical relapse did not receive any *consolidation* therapy because of intercurrent infections. These observations suggest that the cure of APL by transplantation is accompanied by elimination, at least below our RT-PC sensitivity levels, of



**Figure 1.** Display of RT-PCR results of all tested patients. The figure shows the data of our series of patients after the achievement of CR. The time of follow-up is denoted in months as the number at the end of each line, which represent the patients' follow-up. Each dot represents an RT-PCR assay performed at the indicated time after achievement of CR. Open dots (○) represent samples that were negative for the presence of a PML/RAR $\alpha$  transcript by RT-PCR analysis. Full dots (●) represent samples that were positive for the presence of a PML/RAR $\alpha$  transcript by RT-PCR analysis. Only samples with adequate RNA quality for amplification of control RNA are included.

residual cells expressing the PML/RAR $\alpha$  transcript.

However, the prognostic significance of a positive RT-PCR post-induction treatment in APL is better defined<sup>5,6,10</sup> than in CML, and it is clear that persistence of the PML/RAR $\alpha$  transcript is fatally associated with clinical relapse (as in our patient OM). This means that the RT-PCR assay is a useful prognostic tool not only in the induction and consolidation treatment phases, but also after transplantation and during long-lasting follow-up.

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We wish to thank Dr. Francesco Lo Coco and Daniela Diverio for supporting our study. We are grateful to Mr. Robin M.T. Cooke for checking the English version of the manuscript.

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### Granulocyte colony-stimulating factor administered as a single intraperitoneal injection modifies the lethal dose<sub>95/30</sub> in irradiated B<sub>6</sub>D<sub>2</sub>F<sub>1</sub> mice

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**Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic growth factor that stimulates the proliferation of progenitor myeloid cells. We have previously demonstrated that recombinant human G-CSF (rhG-CSF) significantly improves survival of lethally irradiated B<sub>6</sub>D<sub>2</sub>F<sub>1</sub> mice when administered as a single intraperitoneal dose of 1 mg/kg 2 hours after a lethal dose (LD)<sub>95/30</sub> irradiation. In our model, rhG-CSF is also able to modify the LD<sub>95/30</sub> in irradiated animals and 1.1 has been found to be the dose modification factor (the ratio of LD<sub>95/30</sub> for mice treated with rhG-CSF to that for control animals).**

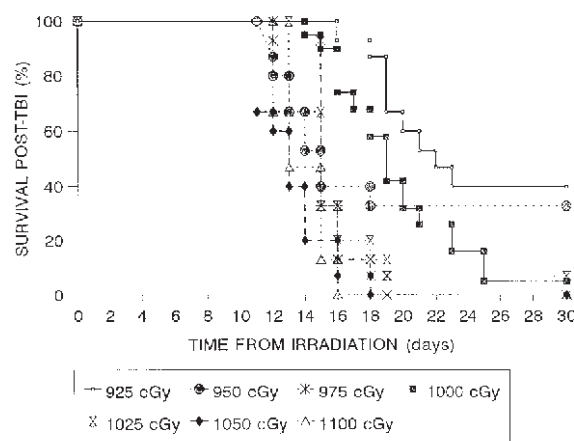
Granulocyte colony stimulating factor (G-CSF) is a hematopoietic growth factor that stimulates the *in vitro* proliferation of progenitor cells committed to the myeloid lineage.<sup>1</sup> In animal models, G-CSF is able to stimulate granulocyte recovery and to promote survival after lethal irradiation when administered as daily injections,<sup>2,3</sup> indicating a possible influence on more primitive progenitors. In these cases, G-CSF modifies both the lethal dose<sub>95/30</sub> and <sub>50/30</sub> (LD<sub>95/30</sub> and <sub>50/30</sub>) providing evidence that G-CSF protects animals from the lethal effects of irradiation.<sup>4-6</sup> We have previously demonstrated that recombinant human G-CSF (rhG-CSF) administered as a single intraperitoneal dose of 1 mg/kg 2 hours after a LD<sub>95/30</sub> irradiation significantly improves survival of lethally irradiated B<sub>6</sub>D<sub>2</sub>F<sub>1</sub> mice (78% vs 7%, p<0.001).<sup>7</sup> Herein, we want to report the effect of rhG-CSF on survival after different doses of total body irradiation (TBI) and the LD<sub>95/30</sub> variation in our model.

Eight week B<sub>6</sub>D<sub>2</sub>F<sub>1</sub> female mice were maintained in

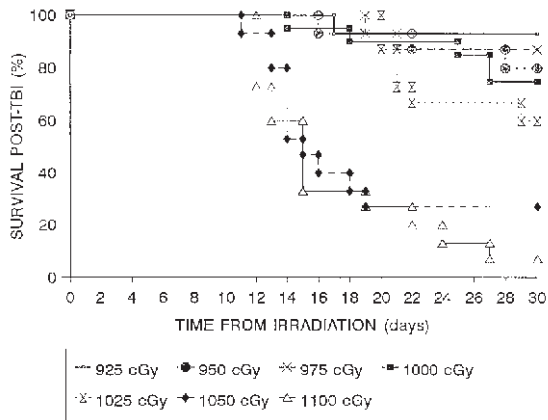
a sterile unit with filtered air on hardwood chip contact bedding (Panlab, SL) from irradiation to day +30 and provided with commercial sterile rodent chow and sterile water supplemented with neomycin sulfate (Gibco Lab, 40 mg/L) and cotrimoxazol (Soltrim®, Almirall Lab, 1.6 g/L). A <sup>60</sup>Co source (Alycon II, Compagnie General de Radiologie, General Electric) was used to deliver total-body <sup>60</sup>Co gamma irradiation (1.25 MeV). Mice were initially irradiated up to a total dose of 1000 cGys at a dose rate of 50 cGys/min, previously established as the LD<sub>95/30</sub>.<sup>8</sup> Irradiation was progressively increased to a total dose of 1100 cGy at the same dose rate in order to find the LD<sub>95/30</sub> for rhG-CSF-treated animals and subsequently decreased to 925 cGy. rhG-CSF (provided by Amgen, Thousand Oaks, CA, USA) was administered as a single dose of 1 mg/kg (20  $\mu$ g) and diluted in saline to a final volume of 250  $\mu$ L, 2 hours after the irradiation. Control mice were injected with 250 mL of physiological saline. A minimum of 30 animals from both groups was used to analyze overall survival for each one of the total doses analyzed. Surviving animals were recorded daily for 30 days. Differences in survival of irradiated rhG-CSF-treated and controls were determined using the Mantel-Peto-Cox test.

Results are shown in Figures 1 and 2. Survival post-TBI significantly increases in the control group when reducing the total dose (40% at 925 cGy vs 7% at 1000 cGy, p<0.001) (Figure 1). Nevertheless, differences in survival between both groups of animals are still significant at the 925 cGys point (40% vs 95%, p<0.005).

In the rhG-CSF group, there is a progressive decrease in survival after TBI when total dose pro-



**Figure 1. Survival of control irradiated mice receiving a total dose of 925 cGy, 950 cGy, 975 cGy, 1000 cGy, 1025 cGy, 1050 cGy and 1100 cGy on day 0. Control mice received 250  $\mu$ L of physiological saline 2 hours after the irradiation procedure.**



**Figure 2.** Effect of a single intraperitoneal injection of rhG-CSF (1 mg/kg, 2 hours after irradiation) on survival of irradiated mice with a total dose of 925 cGy, 950 cGy, 975 cGy, 1000 cGy, 1025 cGy, 1050 cGy and 1100 cGy.

gressively increases up to 1100 cGys (Figure 2); there are significant differences between survivals of rhG-CSF-treated and control animals at total doses of 1025 (60% vs 7%,  $p < 0.001$ ) and 1050 cGy (27% vs 0%,  $p < 0.025$ ). However, no significant differences can be observed at 1100 cGy (5% vs 0%, NS), as has been previously reported in a murine model with daily injections of G-CSF.<sup>5</sup> A dose of 1100 cGy can thus be considered the  $LD_{95/30}$  in our model. Consequently, 1.1 has been found to be the dose modification factor (the ratio of  $LD_{95/30}$  for mice treated with rhG-CSF to that for control animals).

In our model, rhG-CSF administered as a single intraperitoneal dose is also able to modify the  $LD_{95/30}$  in irradiated animals, as demonstrated by others when rhG-CSF is administered in daily doses.<sup>4-6</sup> Nevertheless, rhG-CSF was not effective in enhancing survival when total dose was higher than 1050 cGys, suggesting that the radioprotective effect of G-CSF requires a certain number of residual surviving stem cells.

### Key words

Granulocyte colony-stimulating factor, total body irradiation, hematopoietic injury

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