merization and proteolysis of vWF. The method is simple, based on well-tested techniques such as agarose gel electrophoresis, SDS-PAGE, and immuno-blotting; 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). At the molecular level, it fuses genes encoding PML on chromosome 15 and the nuclear retinoic acid receptor-α (RARα) on chromosome 17. The subsequent expression of PML/RARα fusion mRNA provides a potential molecular marker that can be detected in leukemic cells taken from patients with APL. Using PML and RARα sequence specific primers, reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed for detection of PML/RARα transcript in leukemic cells obtained from patients; these RT-PCR assays are more sensitive than conventional cytogenetic analysis.

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/RARα transcript. We have now applied RT-PCR assay for PML/RARα 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. 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/RARα transcript by RT-PCR analysis.

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 donor. Fourteen patients were submitted to autologous BMT. 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. Cytogenetic studies were performed as reported. RT-PCR analysis was performed as described elsewhere. Concerning the specificity and sensitivity of our RT-PCR method, we can detect one PML/RARα-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/RARα 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.
(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). The persistence of the PML/RAR\textsubscript{x} transcript in early post-remission APL samples has been associated with early clinical relapse within a few months. Several recent studies indicate that molecular monitoring of the PML/RAR\textsubscript{x} fusion transcript in APL could allow identification of patients who need further anti-leukemic therapy. On the other hand, we and others have reported that long term survival of APL is associated with eradication of cells carrying the specific PML/RAR\textsubscript{x} rearrangement, indicating that PCR negativity should be considered the therapeutic goal in these patients.

Regarding the role of consolidation and maintenance chemotherapy, 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-PCR sensitivity levels, of

\begin{table}[h!]
\centering
\caption{Clinical and therapeutic characteristics of the APL patients.}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
UPN & Pt. & Age & Sex & Breakpoint & Year of diagnosis & Induction & Consolidation & Maintenance & BMT & 1st CR duration & Survival (months) & Status \\
\hline
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 \\
7 & P.E. & 31 & M & BCR1 & 1986 & Dauno+ARAC & AMSA+ARAC & No & Auto & 141 & 142 & CR \\
9 & C.F. & 51 & M & BCR1 & 1993 & ATRA & IDA+ARAC & No & Auto & 44 & 46 & 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 \\
18 & O.M. & 50 & F & BCR1 & 1991 & IDA & ARAC+IDA & Mitox+VP16 & No & No & 37 & 53 & Died from APL relapse \\
\hline
\end{tabular}
\end{table}
residual cells expressing the PML/RARα transcript.

However, the prognostic significance of a positive RT-PCR post-induction treatment in APL is better defined than in CML, and it is clear that persistence of the PML/RARα 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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References

Granulocyte colony-stimulating factor administered as a single intraperitoneal injection modifies the lethal dose $D_{95/30}$ in irradiated $B_{6}D_{2}F_{1}$ 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 $B_{6}D_{2}F_{1}$ mice when administered as a single intraperitoneal dose of 1 mg/kg 2 hours after a lethal dose ($LD_{95/30}$) irradiation. In our model, rhG-CSF is also able to modify the $LD_{95/30}$ in irradiated animals and 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).

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. In animal models, G-CSF is able to stimulate granulocyte recovery and to promote survival after lethal irradiation when administered as daily injections, indicating a possible influence on more primitive progenitors. In these cases, G-CSF modifies both the lethal dose $D_{95/30}$ and $D_{50/30}$ ($LD_{95/30}$ and $LD_{50/30}$) providing evidence that G-CSF protects animals from the lethal effects of irradiation. 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 $LD_{95/30}$ irradiation significantly improves survival of lethally irradiated $B_{6}D_{2}F_{1}$ mice (78% vs 7%, p<0.001). Herein, we want to report the effect of rhG-CSF on survival after different doses of total body irradiation (TBI) and the $LD_{95/30}$ variation in our model.

Eight week $B_{6}D_{2}F_{1}$ 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 $^{60}$Co source (Alcyon II, Compagnie General de Radiologie, General Electric) was used to deliver total-body $^{60}$Co gamma irradiation (1.25 MeV). Mice were initially irradiated up to a total dose of 1000 cGy at a dose rate of 50 cGys/min, previously established as the $LD_{95/30}$. Irradiation was progressively increased to a total dose of 1100 cGy at the same dose rate in order to find the $LD_{95/30}$ 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 µg) and diluted in saline to a final volume of 250 µL, 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 cGy 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](https://example.com/figure1.png)

**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 µL of physiological saline 2 hours after the irradiation procedure.