duration of IFN-α treatment prior to study entry was 30 months (range 12-54), and the daily dose administered varied from 3 to 9 MIU (Table 1). Interferon-α was discontinued 15 days before. Filgrastim was administered (s.c) at a dose of 15 mg/kg/day for 5 days. Leukaphereses were then started and performed daily, using a Cobe Spectra blood separator, until the target cell yield was obtained (> 1×10^6 $CD34^+$ cells/kg b.w.). Apheresed cells were then cryopreserved. The Ph+ cell contamination both in the patients' bone marrow and in the leukapheresis products was evaluated with a quantitative competitive PCR technique (QC-PCR).[8] The priming treatment with filgrastim was well tolerated. Bone pain (WHO grade I-II) occurred in three cases. Seven out of 9 patients (78%) yielded more than 1×10^6 $CD34^+$ cells/kg in one (4 cases) or 2 to 4 (3 cases) collections (Table 2). In 5 out of the 9 mobilized cases the levels of BCR/ABL transcript in the first apheresis product were 10 to 700 fold higher than the levels of BCR/ABL transcript measured in the pre-apheresis bone marrow samples (Table 2). In 3 of these 5 patients the amount of BCR/ABL transcript decreased significantly in the subsequent aphereses reaching pre-G-CSF mobilization values (Table 2). Using the QC-PCR to assess the Ph^+ minimal residual disease in the leukapheresis products we found that priming treatment with filgrastim could induce an earlier mobilization of Ph^+ cells. This could be due to functional impairment of adhesion molecules necessary to retain progenitors in the bone marrow microenvironment,[9] or alternatively, to the induction of differentiation of CML cells[10] and their partial elimination by earlier aphereses. No significant correlation (r = 0.0069; p = 0.78) was found between the level of BCR/ABL transcript and the number of $CD34^+$ cells collected. No patient has been autografted as yet, because all patients remain in complete or major cytogenetic remission after collection. We therefore cannot provide data concerning the repopulating ability of the collected $CD34^+$ cells. We did, however, show that Ph-neg $CD34^+$ cells could be collected from patients who were treated with IFNα for a long time, were in cytogenetic remission and had a hypocellular marrow.

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Key words

CML, IFNα, G-CSF, autografting

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All-trans retinoic acid potentiates the *in vitro* inhibitory effects of IFN-α in parental and p210-bcr/abl transfected murine myeloid cell lines

Sir,

Recently, a great deal of interest has been focused on the use of ATRA in the treatment of Ph+ CML.[1-3] Preclinical observations showed that all-trans retinoic acid (ATRA) synergizes with IFNα to induce suppressive effects on Ph+ CML progenitor cells.[4,5] As

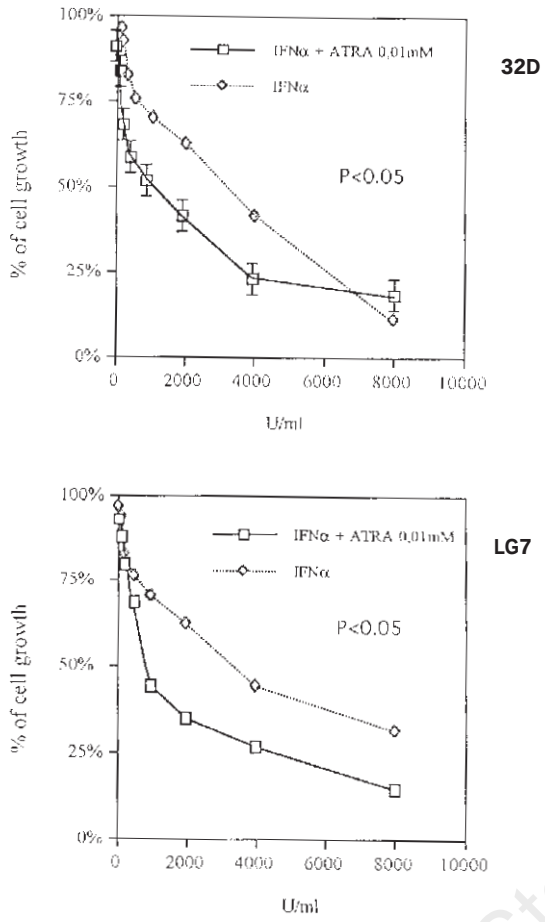

Figure 1. Suboptimal concentrations of ATRA (0.01 mM) added (2 hours before) to scalar concentrations of IFNα induce a significant increase ($p < 0.05$) of growth inhibition in comparison to IFNα alone, both in 32D and LG7 cell lines

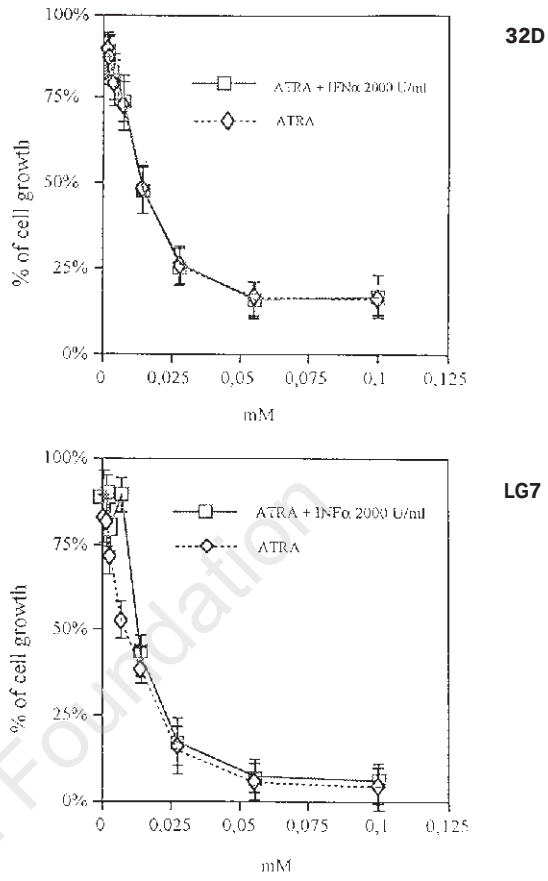

Figure 2. Suboptimal concentrations of IFNα (2,000 U/mL) combined (2 hours before) to escalating doses of ATRA do not induce any additive or synergistic effects in comparison to the growth inhibition induced by ATRA alone either in 32D or in the LG7 cell line.

the same combination did not show similar effects on progenitors from patients with Ph-negative myeloproliferative diseases it has been suggested that the suppressive effect of ATRA, either alone or in combination with IFNα, could be related to the expression of p210-bcr/abl oncoprotein.[4] To dissect the role of p210 bcr/abl expression regarding the cell sensitivity to ATRA, in this study, we evaluated the effects of ATRA with and without IFNα on parental (32D)[6] and p210 transfected (LG7)[7] murine myeloid cell lines. Proliferation of 32D and LG7 cell lines was assayed by the 3-(4,5-dimethylthiazol,2-yl)-2,5-diphenyltetrazoliumbromide MTT method.[8] The detection and quantification of p210 bcr/abl transcript in LG7 cells was made by using a quantitative, competitive polymerase chain reaction (PCR).[9] The results were expressed as mean values ± SD of the data obtained from three experiments performed in triplicate. Data were analyzed by the Student's t test and differences with $p < 0.05$ were considered statistically significant.

We observed that the incubation of 32D and LG7 cell lines with scalar concentrations of ATRA or IFNα inhibited the cell growth in a dose-dependent manner. However, no significant difference in growth inhibition was found between the parental (32D) and the p210 transfected (LG7) cell line. In both cell lines the ATRA inhibition dose 50 (ID_{50}) was 0.01 mM, while the IFNα ID_{50} was 2,000U/mL. When suboptimal concentrations of ATRA (0.01 mM) were added (2 hours before) to scalar doses of IFNα a significant increase ($p < 0.05$) of cell growth inhibition compared to that produced by IFNα alone was observed both on 32D and LG7 cell lines (Figure 1). In contrast, when suboptimal concentrations of IFNα (2,000 U/mL) were combined (2 hours before) to escalating doses of ATRA we did not observe any additive or synergistic effects in comparison to the growth inhibition induced by ATRA alone (Figure 2). To verify that the effects of ATRA, either alone or in combination with IFNα, were not due to the absence

of p210 bcr/abl fusion protein in LG7 cells, detection and quantification of p210 bcr/abl transcript in these cells was done using a quantitative, competitive PCR.[9] The presence of bcr-abl transcripts in the LG7 cell line was further demonstrated by western blot analysis (data not shown). Our data suggest that ATRA can potentiate the inhibitory effects of IFNα both on Ph-negative and Ph+ leukemic cells. The mechanism (s) of synergism is unknown. It does not seem to be related to p210 expression, but appears to be influenced by preincubation of target cells with ATRA. These findings suggest that pretreatment with ATRA could induce activation of IFNα-induced genes[10] which in turn could favor the clinical response to IFNα.

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Key words

ATRA, IFNα, Ph+ CML, p210 bcr/abl, cell lines

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Possible evolution of human parvovirus B19 infection into erythroleukemia

Sir,

Since its discovery in 1975, HPV B19 has been identified with an acute form of bone marrow failure in susceptible hosts. It is now well established that HPV B19 is cytotoxic for human erythroid precursors and causes a lytic process in infected cells.[1]

We report the case of a patient with HPV B19 bone marrow infection who developed erythroleukemia (EL).

A 69-year old man was admitted to a local hospital following several days of high fever and pancytopenia. The patient was given antibiotics and several units of red blood cells and then transferred to our institution for further evaluation.

Cytologic and histologic examination of bone marrow cellularity showed markedly reduced and dysplastic erythropoiesis. Blasts cell such as myeloblasts and monocytic blasts accounted for 20% of bone marrow cells. We were impressed by the high number of giant, frequently binucleated, erythroblasts, some of which were morphologically normal while others showed atypical irregularly-shaped nuclei, prominent nucleoli and vacuoles (Figure 1a). Cell pictures suggesting a fusion phenomenon were occasionally observed (Figure 1b). A provisional diagnosis of myelodysplastic syndrome was formulated.

One week after admission a rapid increase in WBC, platelet and reticulocyte count was observed. Bone marrow examination was repeated ten days after admission; the marrow showed marked erythropoietic hyperplasia, a few giant proerythroblasts were still present and there were numerous megakaryocytes while the number of blast cells was reduced. A clinical diagnosis of transient aplastic crisis due to HPV B19 was considered. Serologic examination revealed the presence of elevated anti-HPV B19 IgM antibodies and a low level of IgG, by ELISA immunoassay. Virus DNA, tested by PCR, was negative. In the following days a dramatic reticulocytosis followed, the blood count returned to normal values, the patient's clinical condition improved and he was discharged. He remained in good health for the next six months. He then started complaining of