strong correlation was found between the two variables ($r = 0.86; p<0.0001$), as suggested by linear regression analysis. The best results were obtained when the number of CD34+ cells in PB was greater than 50 µL, while the worst results were related to a level of less than 10 µL (Figure 1). Only in 5 cases, because of the particular clinical history of the patients, did we perform leukapheresis when circulating CD34+ cells were < 10 µL. None of these reached 0.5 × 10^6/kg CD34+ cells (mean value collected was 0.23, range 0.05-0.4 × 10^6/kg). With more than 10/mL CD34+ cells in PB we collected no less than 0.5 × 10^6/kg, with rare exceptions. When CD34+ cells exceeded 20/mL we usually reached more than 1 × 10^6/kg. No statistical difference was found in patients with CD34+ cell values ranging from 21 to 50 µL. Finally, in our hands, more than 50 µL circulating CD34+ cells ensured a collection greater than 2 × 10^6/kg in most patients.

Though the quality of a leukapheresis does not depend only upon the absolute number of CD34+ progenitors present,² our data confirm that daily estimation of the circulating CD34+ cell number by flow cytometry may guide our clinical decisions and may offer a useful tool for predicting the number of procedures to perform.

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All-trans retinoic acid might also induce apoptosis in freshly isolated chronic myeloid leukemia cells

Sir, we read with interest the recent letters by Martinelli and coworkers¹ and Zinzini and coworkers² on the induction of apoptosis by the nucleoside analogs fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CdA) and 2-deoxycoformycin (DCF), whether used alone or in combination with α-interferon (α-IFN), in freshly isolated leukemic cells from chronic myeloid leukemia (CML). Apoptotic cell death, as demonstrated by electrophoretic gel DNA fragmentation pattern, was induced by both FAMP and 2-CdA,³ either alone or in combination with α-IFN, whereas DCF,⁴ with or without α-IFN, failed to do so. The authors focused on the opportuneness of promoting further in vitro and in vivo studies with these two promising adenosine analogs, possibly employing assays able to measure programmed cell death. We agree with the authors about the timeliness of exploring new effective drugs capable of driving the CML clone into apoptosis, and on this point we would like to provide further evidence in support of their in vitro findings.

We recently tested the in vitro capability of FAMP and all-trans retinoic acid (ATRA) to drive peripheral myeloid cells from untreated Ph+ CML patients in chronic phase into apoptosis. Apoptosis was measured by using simple and reliable flow cytometric methods based on decreased forward light, increased right angle scatter and reduced propidium iodide fluorescence stainability.⁵ These methods, as compared to electrophoretic gel DNA fragmentation assays, which allow only bulk apoptosis measurement, are able to detect programmed cell death on a single cell basis. In our model, CML cells cultured alone in standard complete medium (RPMI 1640 plus 10% FCS) showed a low apoptotic cell rate (6.8% at 96 hours of culture) at all the different time points tested (24, 48, 72, 96 hours). By contrast, when cultures were performed in the presence of FAMP (5 µM) apoptosis reached 26.3% and 31.7% at 72 and 96 hours, respectively. Similar results were obtained when FAMP was substituted with ATRA (3 µM). This agent, which was previously shown to induce apoptosis in both acute promyelocytic leukemia and chronic lymphoproliferative disorders,⁶ also drove CML cells into apoptotic cell death (28.6% at 72 and 40.5% at 96 hours of culture). Apoptosis occurred mainly via terminal myeloid differentiation of the leukemic clone, as demonstrated by cytospin morphological and cytochemical examinations.

Taken together, these findings further support the importance of focusing on inducers of programmed cell death as promising new agents in the management of chronic phase CML, and provide a rationale for the employment of either purine analogs or ATRA in pilot clinical trials.

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Cryosupernatant in thrombotic thrombocytopenic purpura (TTP): is it really useful?

Sir, Perotti et al. in their paper entitled Cryoprecipitate-poor plasma fraction (cryosupernatant-CPP) in the treatment of thrombotic thrombocytopenic purpura at onset. A report of four cases demonstrated that CPP can induce a more rapid improvement in the clinical manifestations of TTP than fresh frozen plasma, while laboratory parameters show slow normalization. We treated six patients with plasma exchange (PE) and CPP (two relapses at four and six years, respectively; four cases at first diagnosis). The clinical manifestations of these patients at onset are reported in Table 1.

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