treatment.[8-10] Moreover, concurrent exposure to other factors such as cadmium or methionine may modify the distribution (and therefore the levels) of selenium in peripheral tissues.[11] An influence of cadmium on selenium metabolism might explain at least in part why lower selenium levels have generally been observed in smokers as opposed to non-smokers,[8,9,12] in spite of the fact that tobacco smoke represents a source of selenium exposure.[13] The limitations of biomarkers in exposure assessment have suggested the usefulness of evaluating average selenium exposure through estimation of usual dietary intake, but unfortunately this methodology is also subject to limitations due to the high variability in the selenium content of foodstuffs in several geographical areas.[12,14] The degree of correlation between selenium intake, as estimated through food diaries or other techniques, and biomarkers as surrogate measures of exposure has been found to be satisfactory in some studies but not in all.[8,10]

An important advantage to estimating dietary intake would be the possibility of determining the specific chemical forms of selenium to which the human body has been exposed before any in vivo metabolic conversion occurs. Whichever approach is adopted in the assessment of selenium exposure – dietary intake or biomarkers – other factors such as exposure to heavy metals, which can markedly influence the biological activity of selenium, should also be considered.[5]

Despite the complexity of epidemiologic studies on the relationship between selenium and cancer, we agree with Piccinini *et al*. on the need to investigate this issue further, especially in the light of the results of two recent studies that have analyzed the effect of selective long-term selenium exposure (self-administered or accidental) on cancer risk.[7,12]

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When to perform peripheral blood progenitor cell collection in hematological patients?

Sir,

we read with interest the paper by Torretta *et al*.[1] on the experience of Pavia University regarding circulating progenitor cell collection in cancer patients. The authors stated that, using daily flow cytometric monitorization of $CD34^+$ cells in the peripheral blood (PB), collections were started when these cells reached a value of 20 µL. However, the possibility of harvesting even though circulating $CD34^+$ cells were below 20 µL (between 10 and 20 µL) was always considered in relation to the particular clinical history, state of disease and therapeutic strategy adopted for each patient.

In agreement with this latter statement, we would like to describe our experience on this topic. As Torretta *et al*. reported, we usually start leukaphereses when white cells in the PB, evaluated in the morning just before collection, are greater than 1000 µL and $CD34^+$ cells greater than 20 µL. However, when making clinical decisions, we have to consider that some patients, due to their clinical situation such as secondary myelodysplasia, exhausted marrow, heavy previous chemo- or radiotherapy may have serious problems in mobilizing $CD34^+$ progenitor cells in the blood.[2,3] This leads to the need for a larger number of procedures to obtain the optimum yield of progenitor cells for a safe engraftment. In this setting, some authors suggest a threshold dose of 2×10^6/kg $CD34^+$ cells, while others refer that less than 5×10^6/kg, although able to restore hemopoiesis in most cases, can be responsible for delayed engraftment or defective platelet reconstitution and recommend collecting not less than 8×10^6/kg $CD34^+$ cells to ensure a rapid, complete and sustained hematopoietic recovery.[4,5] We retrospectively analyzed our data on 54 patients suffering from hematological malignancies (21 multiple myelomas, 14 acute myeloid leukemias, 10 non-Hodgkin's lymphomas, 9 Hodgkin's disease) who received high-dose mobilizing chemotherapy plus growth factor administration (G-CSF and in some cases GM-CSF) at our Institution between April 1993 and July 1996. The total number of leukaphereses performed was 159 (mean number per patient 2.9, range 1-4). About 9 liters of blood were processed for each patient.

The amount of $CD34^+$ cells collected at each leukapheresis was analyzed in relation to the number of $CD34^+$ cells in the PB, in order to evaluate the predictive yield capacity of the latter. A

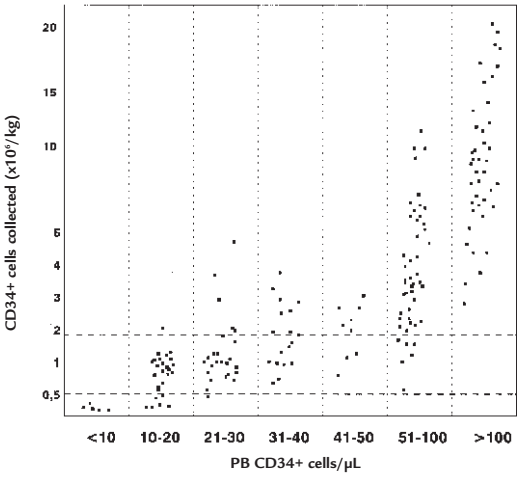

Figure 1. $CD34^+$ progenitor cell content (x 10^6/kg) in leukaphereses in relation to the PB $CD34^+$ cell concentration (/mL).

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 $0.05\text{-}0.4\times10^6$/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,[6,7] 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 coworkers[1] and Zinzani and coworkers[2] on the induction of apoptosis by the nucleoside analogs fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CdA) and 2-deoxycoformicin (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[1] and 2-CdA,[2] either alone or in combination with α-IFN, whereas DCF,[2] 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.[3] 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[4] and chronic lymphoproliferative disorders,[5] 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*[1] 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.