letter to the editor
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CIRCULATING HEMATOPOIETIC PROGENITOR CELLS IN LEUKAPHERESIS: FLOW CYTOMETRIC ESTIMATION

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Sir,

in a recent issue of Haematologica, Indovina *et al.* reported their experience on engraftment and long-term stability of hemopoiesis after autografting of peripheral blood circulating progenitor cells (CPC).[1] In particular, these authors found no correlation on harvested products between $CD34^+$ cells and colony-forming units granulocyte-macrophage colonies (CFU-GM). We would like to describe here our experience in this field, which is not in agreement with the above conclusion. Forty-one leukapheresis samples obtained in 20 patients suffering from hematologic malignancies or solid cancers and recovering from high-dose chemotherapy-induced pancytopenia, were simultaneously assayed for their $CD34^+$ cell and CFU-GM progenitor content. We used the fluorescein or phycoerythrin conjugated anti-CD34 monoclonal antibody HPCA-2 (Becton Dickinson) to stain CPC by flow cytometry (FACSORT, Becton Dickinson), according to the *Milan Protocol* as described elsewhere.[2,3] After incubation with HPCA-2 (without separation of mononuclear cells), red blood cell lysing and washing, the samples were stored and then analyzed using a scattergram with the horizontal axis as side (90°) light scatter and the vertical axis as fluorescence (red or green). With this technique true $CD34^+$ cells form a discrete cluster that exhibits low density CD34 expression and low-side scatter characteristics (Figure 1).

Enumeration of clonogenic cells (CFU-GM) was carried out in Iscove's methylcellulose cultures containing 30% fetal calf serum, 10% bovine serum albumin, 2 U/mL erythropoietin, 50 ng/mL GM-CSF, 50 ng/mL G-CSF and 10^{-4} mL β-mercaptoethanol. Five $\times 10^5$ cells were plated and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. CFU-GM were counted with an inverted microscope 14 days after initiation of cultures.

Figure 2 illustrates a simple regression analysis showing that a linear relationship could be established between $CD34^+$ cells and the absolute number of CFU-GM ($R=0.6$; $p < 0.0001$) in freshly collected apheresis products, before cryopreservation.

All patients achieved rapid engraftments that were comparable, in terms of time and quality, to those reported in previous studies. The number of $CD34^+$ cells transplanted ranged from 1.8×10^6/kg to 19×10^6/kg, while that of CFU-GM was in the range of $3.1\text{-}33\times10^4$/kg. Both the CFU-GM assay and flow cytometric quantita-

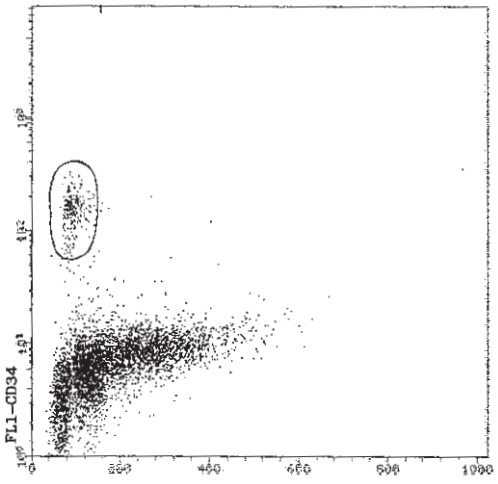

Figure 1. Dot plot generated by combining right angle side scatter on the horizontal axis and green fluorescence (FITC-conjugated CD34) on the vertical axis. The $CD34^+$ cells form a well-defined discrete cluster (gated), separated from the negative autofluorescent cells.

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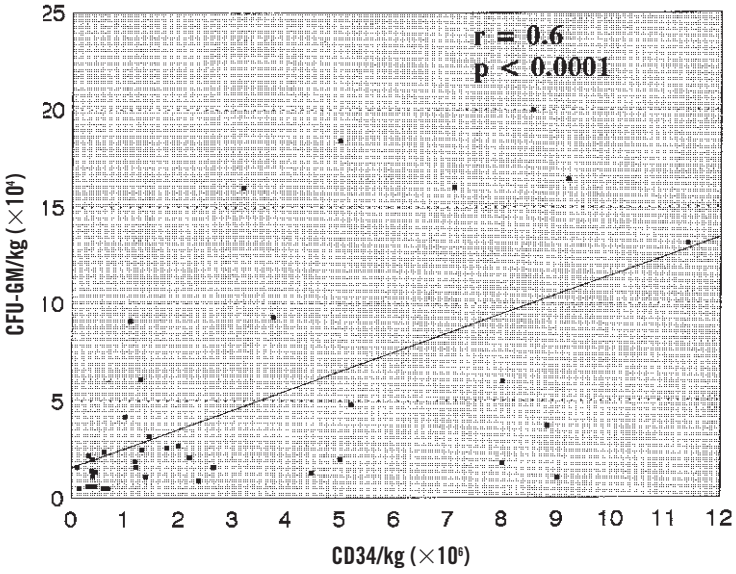

Figure 2. Correlation between CD34⁺ cells/kg and CFU-GM/kg in 41 leukapheresis products from 20 patients.

tion of cells bearing the CD34 antigen are useful tools for assessing the quality of the harvested material and estimating the number of CPC collected. Although some controversy still exists about the correlation between CFU-GM and CD34⁺ cell number,[4] several technical and biological reasons could explain the contrasting results obtained in this field.[3,5,6] The CFU-GM assay may be difficult to compare among various institutions and it also has the disadvantage of measuring only a limited number of committed progenitors. Furthermore, a period of 14 days is needed before a result is available. Phenotyping hemopoietic progenitors appears to be a more standardized technique, ready in about one hour, that allows useless leukapheresis to be avoided if sufficient CPC are collected.

Thus, in our hands, flow cytometric analysis of CD34⁺ cells by means of direct immunofluorescence, performed according to the *Milan Protocol*, permits a rapid estimation of the number of CPC required for successful engraftment and provides information comparable to that obtained with CFU-GM assay.

References

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