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). 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. 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 × 10⁵ cells were plated and incubated at 37°C in a humidified atmosphere of 5% CO₂ 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 × 10⁶/kg to 19 × 10⁶/kg, while that of CFU-GM was in the range of 3.1–33 × 10⁴/kg. Both the CFU-GM assay and flow cytometric quantita-
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, several technical and biological reasons could explain the contrasting results obtained in this field. 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 leukaphereses 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

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

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Hematologica looks great on line. It would be great if you could include the address for reprint requests. Keep up the good work. I am very delighted.

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