Correspondence

***In vivo* expansion of reinfused autologous peripheral blood stem cells after a myeloablative regimen, as an alternative to *ex vivo* expansion pretransplantation: an intriguing observation in a patient autografted twice**

Hemopoietic growth factors have been widely used in the context of autografting for the past 10 years. Granulocyte colony-stimulating factor (G-CSF),¹ granulocyte-macrophage colony-stimulating factor (GM-CSF)² and more recently stem cell factor (SCF)³ are used to mobilize stem cells in to the peripheral blood. G-CSF and GM-CSF have been shown to accelerate engraftment in autografted patients. A large number of teams have developed cytokine cocktails and specific culture conditions to amplify stem cells, progenitor cells, and nucleated cells *in vitro*. In our own team, the combination of SCF, interleukin-3, interleukin-6, FLT-3 ligand, G-CSF and megakaryocyte growth and development factor (MGDF), has been particularly effective on CD34⁺ selected cells, leading to a median 33-fold amplification of long-term culture-initiating cells (LTCIC).⁴ A few teams have reported on the absence of toxicity of the *in vivo* infusion of CD34⁺ stem cells expanded *in vitro*.⁵ Curiously, however, there has been, so far, no report on the administration after infusion of autologous stem cells of combinations of cytokines, that might achieve *in vivo* a similar stem cell expansion as the ones obtained *in vitro*.

In 1990,⁶ we reported in the journal, on a patient with a progressing diffuse large cell non-Hodgkin lymphoma (NHL) with marrow involvement, which we treated with the BEAM regimen (BCNU 300 mg/m², VP-16 and aracytine 800 mg/m² each, melphalan 140 mg/m²) followed by GM-CSF alone, with no reinfusion of autologous bone marrow because it was massively infiltrated by tumor. At that time, it was the general belief that the BEAM regimen widely used for autografting patients with NHL was myeloablative. The patient, however, recovered hemopoiesis within 4 weeks and is still alive and well in complete remission of his disease, possibly cured. Four additional patients⁷ were treated in similar conditions, until in 1993 we decided to administer BEAM followed by the combination of G-CSF, GM-CSF and erythropoietin (EPO) to further patients with NHL fulfilling all criteria to be autografted but with no available safe autograft free of residual tumor cells on histology. We reported recently on a series of 25 patients with NHL treated by BEAM followed by this cytokine cocktail instead of an autograft, who recovered their hemopoiesis within 3 weeks.⁸

In the past 6 months, we have made an interesting observation which further extends this concept to *in vivo* expansion of an autograft, in a 20-year-old patient with progressing stage III Bb Hodgkin's disease who was

Figure 1 Kinetics of white blood cell (WBC) and polymorphonuclear (PMN) after transplant No. 1.

admitted to the cell therapy unit of our Department for double intensification with a double autograft. The patient had received six courses of MOPP-ABV chemotherapy as first line, and three courses of MINE salvage second line chemotherapy; he was in partial remission at the time when leukaphereses were done yielding a total of 6.5×10^6 CD34⁺ cells/kg and 16×10^5 CFU-GM/kg of body weight. A first intensification by CBV (cyclophosphamide 120 mg/kg, BCNU 400 mg/m², VP-16 1200 mg/m²) followed by the reinfusion of half of the dose of peripheral blood stem cells collected was administered. Recovery to 0.5×10^9 /l polymorphonuclear cells (PMN) occurred on day 12 (Figure 1), and recovery to 50×10^9 /l platelets on day 11 post graft. The patient entered into complete remission.

A month later, a second intensification was given with busulfan 16 mg/kg and cyclophosphamide 120 mg/kg (BU-CY 2), and the second half of the autograft infused.

While under busulfan therapy, the patient developed epilepsy, fell from his bed and sustained a large facial injury with massive bleeding which necessitated surgery. On an emergency basis, he received from day 1 post transplant the combination of G-CSF (5 µg/kg/day), GM-CSF (5 µg/kg/day) and EPO (150 U/kg/day), to accelerate as much as possible hemopoietic recovery. Neutropenia below 0.5×10^9 /l lasted only 4 days and recovery of PMN occurred on day 9 (Figure 2). Recovery of platelets to 10×10^9 /l occurred on day 30. At the time of writing, the patient is in complete remission.

Combinations of cytokines so far have not been administered *in vivo* to patients for several reasons: one is that few cytokines are licensed for clinical use and cocktails such as those used *in vitro* cannot presently be used *in vivo*. G-CSF, GM-CSF and EPO are the only available cytokines

Figure 2 Kinetics of white blood cell (WBC) and polymorphonuclear (PMN) after transplant No. 2.

outside prospective studies and SCF is the only new one announced to be licensed soon, but only for mobilization of stem cells. We set up a 2 week liquid culture serum-free system containing stem cell factor with CD34⁺ marrow cells. All combinations of G-CSF, GM-CSF and EPO were tested. At day 14, the number of CFU-GM and cells scored was significantly higher ($P \leq 0.03$), with the combination of the three cytokines than with two or one cytokine. The level of CFU-GM expansion achieved with the three cytokines was four times higher than with any combination of two of them.⁸

In patients submitted to a double autograft program, the duration of the neutropenia after the second autograft is usually longer than during the first autograft. Also, as opposed to the BEAM and the CBV regimen, the BU-CY 2 regimen is severely myeloablative and spontaneous hemopoietic recovery, even after administration of G-CSF has never been observed. Finally, kinetics of recovery of hemopoiesis have been linked to the dose of stem cells infused evaluated as CD34⁺ cells/kg. Doses above 2.5, 5, 10 and lastly 15×10^6 CD34⁺ cells/kg have been successively recommended to ensure most rapid hemopoietic recovery, beyond which no further improvement can be expected.

Our observation of very fast hemopoietic recovery after a fully myeloablative regimen followed by the infusion of a small number of CD34⁺ cells and, further, of a recovery faster after a second autograft than after a first one, strongly suggests that the cytokine cocktail that has been administered has indeed induced *in vivo* expansion with a reduction in the neutropenia duration.

Of course, further observations are needed and randomized trials testing cytokine combinations *vs* single cyto-

kine administration such as a G-CSF of GM-CSF may be warranted. The use of such a cytokine combination may bring benefit to patients autografted or allografted with a small number of stem cells, such as adult recipients of unrelated cord blood and to victims of radiation accidents. With regard to the enormous research developed for *in vitro* stem cell expansion, we propose that the alternative approach of *in vivo* expansion be seriously considered.

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