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Haematopoietic Growth Factors and Peripheral Blood Stem Cells as Supportive Agents in Dose Intensification

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We have studied the requirements that have to be met to combine effective cancer chemotherapy with the mobilisation of peripheral blood stem cells (PBSC). We have shown that there is a differential induction of high numbers of PBSC following standard-dose chemotherapy (VIP) plus treatment with colony-stimulating factors. The combined sequential administration of interleukin 3 (IL-3) plus granulocyte–macrophage colony-stimulating factor (GM-CSF) induced maximal numbers of PBSC, including colony-forming unit-granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte (CFU-GEMM) and colony-forming unit-megakaryocyte (CFU-Meg), compared with the application of GM-CSF, granulocyte colony-stimulating factor (G-CSF) or chemotherapy alone. The number of CD34+ cells was highly variable depending on the prior treatment given to the patients. Mobilised CD34+ cells—depending on the cytokines used for recruitment—had a varying cloning efficiency, and were heterogeneous as to their level of commitment. Retransfusion of G-CSF-primed progenitor cells to pilot patients following high-dose chemotherapy demonstrated that PBSC recruited by standard-dose chemotherapy plus G-CSF accelerated both neutrophil and platelet recovery.
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INTRODUCTION

HIGH-DOSE chemotherapy is potentially curative in some chemosensitive tumours and a relationship between dose intensity and tumour response has been defined for several malignancies. The average relative dose intensity received is probably a major factor determining the outcome of chemotherapy [1]. Dose escalation, however, is limited mostly by non-haematological organ toxicity as well as by myelosuppression.

To facilitate the use of dose-intensive regimens, we have studied the requirements necessary to support patients with autologous peripheral blood stem cells (PBSC).

AUTOGRAFTING WITH PERIPHERAL BLOOD STEM CELLS

Peripheral blood stem cell autografting has been used with increasing frequency following high-dose therapy for malignancies as an alternative to the use of marrow stem cells. Successful transplantation with complete and sustained engraftment has been shown in various disorders, including acute non-lymphocytic leukaemia, lymphoma, neuroblastoma, breast cancer and other solid tumours [2–9].

When compared with autologous bone marrow transplantation, possible advantages of this modality are as follows:

- Collection of PBSC can take place in an outpatient setting without the need for general anaesthesia.
- A more rapid restoration of neutrophils and platelets can be achieved [3, 6, 7], probably due to the high number of committed progenitor cells infused.
- Possibility of autografting when bone marrow aspiration is hampered by tumour cell infiltration, fibrosis or hypoplasia following radiotherapy.
- Possibility of reduced contamination with malignant cells in disseminated cancer [9]; however, the actual level of malignant cell contamination is a matter of debate [10].

RECRUITMENT OF PERIPHERAL BLOOD STEM CELLS

Several ways to mobilise PBSC into circulation have been described. During rapid haematopoietic recovery following chemotherapy-induced myelosuppression, progenitor cells can be detected in the peripheral blood, particularly after cyclophosphamide treatment [11–14]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) as well as granulocyte colony-stimulating factor (G-CSF) also expand the pool of circulating haematopoietic progenitors [15–19]; this effect is potentiated when the colony-stimulating factors (CSF) are given following high-dose cyclophosphamide [15, 20].

Colony-stimulating factor-exposed circulating progenitors provide rapid recovery and sustained haematopoiesis not only when both bone marrow and peripheral blood stem cells are reinfused [20], but also when given alone to patients following high-dose therapy [16, 21–24].

To recruit PBSC in patients eligible for high-dose chemotherapy, at our institution, a standard dose chemotherapy regimen is used followed by the application of CSF.

A 1-day course of VIP (VP-16 = etoposide: 500 mg/m^2, ifosfamide: 4 g/m^2, cisplatinum: 50 mg/m^2) followed by the individual or combined sequential administration of different growth factors were applied to a total of 91 patients. Patients were sequentially assigned to receive GM-CSF (n=18), G-CSF (filgrastim) (n=40) and interleukin 3 (IL-3) plus GM-CSF (n=18) (Behringwerke Marburg, Germany). 15 patients were not treated with CSFs (controls). The cytokines were applied once daily subcutaneously for 14 days at a dose of 250 μg/m^2,

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starting 24 h after the end of chemotherapy. In the group of patients given IL-3 plus GM-CSF, IL-3 was applied from days 1–5, followed by the administration of GM-CSF on days 6–14. Control patients ($n=15$) were treated without cytokines. The rationale to combine IL-3 plus GM-CSF in our study is based on *in vitro* as well as animal studies, indicating that these cytokines act synergistically to stimulate progenitor cells, particularly when given in a sequential mode [25, 26]. Moreover, this combination might retain the beneficial effects of IL-3 on thrombopoiesis and of GM-CSF on neutrophil production.

Although this was not a randomised study, age of patients, tumour type and type of prior treatment were balanced. Patients with bone marrow infiltration were excluded.

Peripheral blood stem cells were evaluated by the expression of the CD34 antigen as well as their colony forming capacity *in vitro* [colony-forming unit-granulocyte–macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E), colony-forming unit-granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte (CFU-GEMM), colony-forming unit-megakaryocyte (CFU-Meg)].

We have preferred to use a standard-dose combination chemotherapy (VIP) instead of high-dose cyclophosphamide, because this regimen, which displays broad antitumour activity, obviates the occurrence of severe thrombocytopenia. High-dose regimens might induce thrombocytopenia concurrent with maximal PBSC levels.

RECRUITMENT OF PERIPHERAL BLOOD STEM CELLS BY VIP-CHEMOTHERAPY PLUS COLONY-STIMULATING FACTORS

The use of VIP plus different CSF resulted in the mobilisation of high numbers of PBSC within a narrow time period after chemotherapy. It is of particular importance that the use of different cytokines not only induced different numbers of total progenitors, but also resulted in a differential recruitment of committed clonogenic cells. The highest number of circulating PBSC (median, 24 000 total progenitors/ml) were recruited by the sequential administration of IL-3 plus GM-CSF, which particularly stimulated the mobilisation of CFU-GEMM (median, 840/ml blood; range, 160–1020) and CFU-Meg (median, 620/ml; range, 0–4490). The median numbers of myeloid and erythroid precursor cells were 10 490 CFU-GM/ml (range, 1000–24 300) and 10 660 BFU-E/ml (range, 3870–24 300), respectively [27]. When compared to the total number of clonogenic progenitors, the number of CD34+ cells did not differ between the IL-3/GM-CSF, GM-CSF and G-CSF treated group of patients, with a median of approximately 450 000 CD34+ cells/ml. This observation suggests that IL-3 plus GM-CSF-induced CD34+ cells have the highest clonogenic capacity.

The calculated cloning efficiency of CD34+ cells in the IL-3 plus GM-CSF treated group of patients was 5.6%, which means that one out of 18 CD34+ cells gave rise to colonies. This suggests that more than 90% of CD34+ cells mobilised either represent more mature precursors without clonogenic capacity *in vitro* and/or very immature progenitors which do not proliferate *in vitro* in response to the culture conditions used. Interestingly, the cloning efficiency was highest (median, 10%) in patients who did not receive any cytokines following VIP chemotherapy.

Correlation analyses between CD34+ cells and clonogenic progenitors revealed that rising CD34+ cell levels did not reflect the number of clonogenic progenitor cells, with the exception of myeloid progenitors (CFU-GM) in the GM-CSF and G-CSF treated groups of patients, and megakaryocytic progenitors (CFU-Meg) in the patients given IL-3 plus GM-CSF ($r = 0.89$).

This observation indicates that the number of CD34+ cells *per se* does not predict their capacity to form myeloid, erythroid, megakaryocytic or multilineage colonies; rather, the way these cells have been mobilised has to be considered in detail.

Colony-forming unit-granulocyte–macrophage correlated well with peripheral blood mononuclear cells independent of any cytokine administration ($r > 0.7$). This finding may be helpful in defining the time of optimal PBSC harvest for haematopoietic support following high-dose chemotherapy [28].

Dual colour flow cytometry analyses of CD34+ cells revealed that most of these progenitors simultaneously stained positive for 'later' markers such as CD33, CD38, and HLA-DR, although the percentage of CD34+/HLA-DR− cells was 6% (median value; range, 0.3–21%), indicating that CSF-induced CD34+ cells include early haematopoietic progenitors.

There was a substantial variation among patients in the yield of CD34+ cells as well as clonogenic precursors, which could be attributed to prior chemotherapy and/or radiotherapy. Those patients who received the most intensive therapy before entering the study had the lowest number of circulating progenitors. Thus, it may be important to harvest patients considered for dose intensification as early as possible.

HIGH-DOSE CHEMOTHERAPY (3-DAY VIP REGIMEN) SUPPORTED BY PERIPHERAL BLOOD STEM CELLS

Our approach in high-dose chemotherapy is first to treat patients eligible for dose intensification with a standard dose VIP protocol, followed by the application of CSF in order to combine a treatment regimen with broad antitumour activity with the simultaneous mobilisation of PBSC. These cells can be harvested by leukapheresis, frozen and reinfused into the patients after high-dose intensification chemotherapy (Fig. 1).

We have now started to support patients following 3-day high-dose VIP chemotherapy with G-CSF primed progenitor cells, recruited in a preceding 1-day VIP cycle. The 3-day high-dose VIP regimen applies cumulative doses of 1.5 g/m^2 etoposide, 12 g/m^2 ifosfamide and 150 mg/m^2 cisplatinum. This regimen was adapted from the studies of Neidhart *et al.* [29], substituting ifosfamide for cyclophosphamide. Ifosfamide shows broad antitumour activity, particularly for sarcomas, germ cell tumours, lymphomas, lung cancer and ovarian tumours [30].

19 patients received a 3-day VIP high-dose chemotherapy course; the first 6 patients were treated without PBSC while all subsequent patients were supported by PBSC. The patients in this study—with advanced malignancies—had all responded to preceding VIP chemotherapy.

All patients—whether supported by PBSC or not—received IL-3 plus GM-CSF following dose intensification. These cyto-

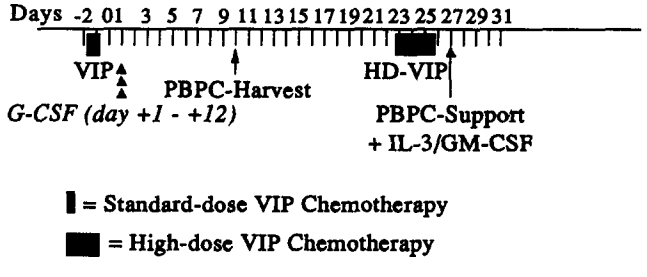

Fig. 1. Regimen used to recruit peripheral blood stem cells.

kines were chosen because—based on *in vitro* data with the CSF available for clinical use—these growth factors are more likely to stimulate proliferation and differentiation of both the myeloid and megakaryocytic precursor cells used for retransfusion.

Impressive enhancement of haematological recovery was observed in the patients supported with autologous PBSC [24]. Figure 2 illustrates the time course of neutrophils and platelets in a patient who did not receive PBSC following high-dose chemotherapy (a), and in another patient supported by G-CSF-primed progenitor cells (b). Both patients had small cell lung cancer and were comparable in terms of age and extent of prior treatment. There was a prolonged period of pancytopenia, with 10 days of < 100 neutrophils and 8 days < 20000 platelets, although the patient was given IL-3 plus GM-CSF (Fig. 2a). As can be seen in Fig. 2b, haematopoietic recovery was significantly shortened in the patient supported by G-CSF-mobilised PBSC. This was particularly impressive for platelets.

DISCUSSION

We have studied the requirements that have to be met to combine an effective cancer chemotherapy with PBSC mobilisation. We have demonstrated a differential induction of high numbers of PBSC following standard-dose chemotherapy plus treatment with CSF. Chemotherapy plus CSF was employed for the mobilisation of PBSC instead of CSF alone for the following reasons:

- This procedure allows cytotoxic treatment to be given early during the management of patients and treatment is not therefore preceded by a period where CSF are given only to recruit PBSC.
- Since the question of tumour cell contamination in harvested cell suspensions is still being debated [10], it might be advantageous to precede the harvest by a therapeutic chemotherapy regimen.

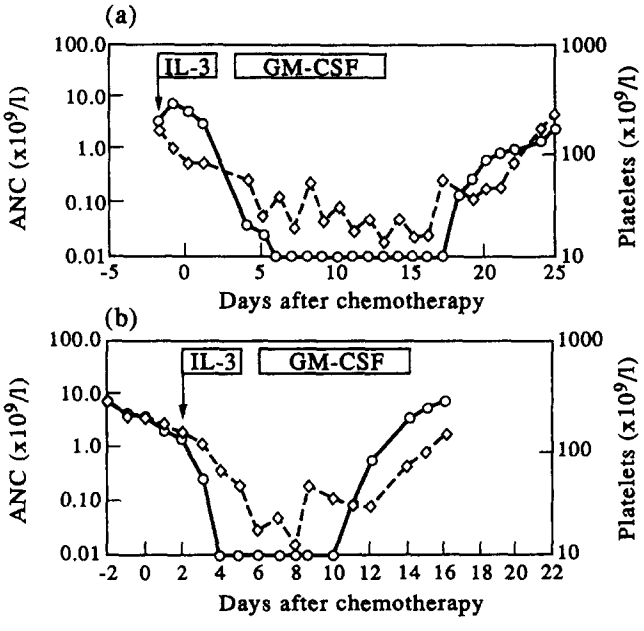

Fig. 2. Neutrophil and platelet levels in 2 patients with small cell lung cancer following high-dose VIP chemotherapy. (a) Patient not receiving peripheral blood stem cells (PBSC) following high-dose chemotherapy; (b) patient supported by granulocyte colony-stimulating factor (G-CSF)-primed progenitor cells. ◇, platelets; ○, absolute neutrophil count (ANC). Il-3, interleukin 3; GM-CSF, granulocyte-macrophage colony-stimulating factor.

- The ability to harvest relatively pure mononuclear cell preparations of PBSC is facilitated because at the time of maximal progenitor cells levels, neutrophil levels are not too high.

The potential of other growth factors not studied clinically so far, such as stem cell factor, with respect to the enhancement of PBSC in humans needs to be defined. Moreover, it is essential to determine whether tumour cells might also be recruited during PBSC mobilisation, thus raising the possibility of contaminated leukapheresis preparations. In this context, the use of purified CD34+ cells, instead of unseparated progenitor cell suspensions, could result in depletion of tumour cells potentially present in the cell suspension after leukapheresis.

Finally, the *ex vivo* expansion of purified progenitor cells could provide large numbers of clonogenic progenitors for repetitive clinical use and as targets for gene transfer [31].

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