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Reports of an ESO Task Force

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Clinical Utilisation of Human Haematopoietic Progenitors Elicited in Peripheral Blood by Recombinant Human Granulocyte Colony-stimulating Factor (rHuG-CSF)

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INTRODUCTION

IN ADULTS, haematopoietic progenitors circulate in the peripheral blood under physiological conditions. In these conditions, i.e. in the steady state, the compartment of circulating progenitors comprises granulocyte–macrophage, erythroid and bipotent colony-forming cells, as well as long-term culture initiating cells, at a frequency which is approximately 100-fold lower than that measured in normal bone marrow [1].

Cancer therapy with selected myelotoxic drugs induces a period of cytopenia of variable duration followed by rapid haematopoietic recovery. It has been demonstrated that haematopoietic progenitors increase in peripheral blood at the time of haematopoietic recovery which follows drug-induced cytopenia [2]. Treatment with clinically available haematopoietic growth factors, most notably recombinant human granulocyte colony-stimulating factor (rHuG-CSF) and recombinant human granulocyte–macrophage colony-stimulating factor (rHuGM-CSF), has been utilised to abbreviate the period of cytopenia after cancer therapy, delivered either at conventional or at high doses [3, 4]. Investigators have observed that while ameliorating myelotoxicity, growth factor treatment after chemotherapy is able to appreciably increase the number of haematopoietic progenitors circulating in peripheral blood. In addition, a short-term (i.e. 5–10 days) treatment with the same growth factors, of patients not previously treated with cancer therapy, is able *per se* to increase the circulation of haematopoietic progenitors.

Autologous bone marrow transplantation is a new treatment modality which allows dose escalation of selected cancer drugs without the limitations of haematopoietic toxicity. Both rHuG-CSF and rHuGM-CSF have been shown to convincingly accelerate neutrophil recovery following high-dose chemotherapy and autologous bone marrow support [5–7]. Despite the use of rHuG-CSF or rHuGM-CSF after transplantation, however, a period of absolute neutropenia persists for several days before evidence of engraftment. Moreover, platelet recovery following transplantation is not affected at all by treatment with presently available growth factors.

Circulating haematopoietic progenitors elicited by growth factor administration, preceded or not by chemotherapy, can be collected from peripheral blood on a large scale, cryopreserved and utilised to reconstitute the haematopoietic system of cancer patients treated with myeloablative cancer therapy. Utilisation of circulating progenitors after high-dose chemotherapy is associated with a faster recovery of leucocytes and platelets compared to bone marrow transplantation. Circulating progenitors can be reinfused in association with bone marrow cells, or utilised as the sole source of haematopoietic cells. Utilising circulating progenitors has the advantage of circumventing the need for general anaesthesia, and of allowing the collection of progenitors even if the bone marrow is damaged by previous radiotherapy or infiltrated with malignant cells. By far the most important advantage of this procedure compared to bone marrow transplantation is a faster recovery of all haematopoietic lineages, including platelets, after myeloablative cancer therapy.

rHuG-CSF has been successfully employed to elicit circulating progenitors in peripheral blood for autologous transplantation. This report will review the utilisation of rHuG-CSF for expansion, collection and clinical applications of circulating progenitors in cancer therapy.

rHuG-CSF AND rHuGM-CSF FOR ELICITING CIRCULATING PROGENITORS

The first observation that rHuG-CSF was able to elicit circulating progenitors originated from a phase I-II study in which rHuG-CSF was administered to 30 non-haematopoietic cancer patients, by either the subcutaneous or intravenous route, at doses ranging from 0.3 to 60 μg/kg. Peripheral blood progenitors were monitored by *in vitro* assays before and after the end of treatment [8]. The administration of rHuG-CSF was followed by a pronounced increase (up to 100-fold) in the number of circulating progenitors of myeloid, erythroid and megakaryocytic lineages at different maturation stages. Since successful

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haematopoietic reconstitution in cancer patients receiving myeloablative treatment has been reported when using progenitors collected from peripheral blood, it is suggested that rHuG-CSF might be useful for harvesting circulating progenitors for autologous transplantation.

The efficacy of rHuG-CSF mobilised progenitors to ameliorate neutrophil and platelet recovery after high-dose chemotherapy has been investigated [9]. 17 patients with non-myeloid malignancies received rHuG-CSF, 12 μg/kg/day for 6 days, by continuous subcutaneous infusion. Granulocyte–macrophage progenitors in peripheral blood increased by a median of 58-fold over pretreatment values, and erythroid progenitors increased by a median of 24-fold, with substantial variations in the yield among patients. Three leucapheresis procedures collected a mean total of 33×10^4 granulocyte–macrophage progenitors per kg body weight, and these cells were reinfused with bone marrow cells after high-dose busulphan/cyclophosphamide treatment. Platelet recovery occurred significantly earlier in these patients than in control patients transplanted with bone marrow without circulating progenitors, reaching $50 \times 10^3/\mu l$ at a median of 15 days after transplantation compared with 39 days in controls. It was concluded that rHuG-CSF-mobilised progenitors successfully reduced thrombocytopenia and neutropenia following high-dose chemotherapy; a neglected issue was the ability of circulating progenitors to reconstitute haematopoiesis in the long term, since bone marrow cells were infused as well.

In another study, four consecutive groups of patients with Hodgkin's disease, relapsing after or resistant to primary chemotherapy, some with bone marrow involvement, were treated with cyclphosphamide, etoposide and either total body irradiation or carmustine, followed by autologous stem cell transplantation [10]. Group I received a bone marrow graft without growth factor. Groups II and III received no growth factor prior to or during collection of circulating progenitors, but did receive rHuG-CSF or rHuGM-CSF, respectively, after transplantation. Group IV received rHuG-CSF for mobilisation of haematopoietic progenitors, and after infusion of peripheral blood and bone marrow cells. In this latter group, myeloid recovery after transplantation was significantly faster than in the other groups (10 versus 12 days), and stable platelet recovery to 20 000/μl without need for further transfusions was surprisingly rapid (13 versus 30 days in non-mobilised groups). An analysis of medical costs comparing groups III and IV demonstrated a 45% decrease in costs in group IV receiving rHuG-CSF-mobilised progenitors, due to early platelet engraftment and earlier discharge from hospital. It was concluded that rHuG-CSF-mobilised progenitors were very useful for early myeloid and platelet recovery, shortening hospitalisation and decreasing costs.

An interesting comparison between the effects of rHuG-CSF and rHuGM-CSF on eliciting circulating progenitors for autologous transplantation was carried out by Peters and colleagues [11]. Two groups of patients, mostly with stage II to IV breast cancer, were treated with 6 μg/kg/day subcutaneous rHuG-CSF for 8 days, or with 8–16 μg/kg/day intravenous rHuG-CSF for 8 days. Circulating cells were harvested by leucapheresis at days 6, 8 and 9 of cytokine infusion, and reinfused with autologous bone marrow after a high-dose chemotherapy programme consisting of cyclophosphamide, cisplatin and carmustine. Both rHuG-CSF and rHuGM-CSF resulted in the collection of large amounts of committed haematopoietic progenitors that were capable of accelerating haematopoietic recovery, compared with bone marrow alone or bone marrow with either rHuG-CSF or rHuGM-CSF. In addition, the use of rHuG-CSF-primed progenitors was associated with a significant reduction in platelet transfusion requirements and in-patient hospital charges.

Overall, these data demonstrate that a substantial amelioration of haematological toxicity due to high dose cancer therapy can be achieved by the use of rHuG-CSF-primed circulating progenitors. In comparison to the use of cytokines after transplantation, not only neutropenia but also thrombocytopenia is reduced with use of cytokine-elicited circulating progenitors. A possible explanation of this important effect is that the cells collected after cytokine priming are enriched with large numbers of committed progenitors, of both myeloid and megakaryocytic lineages, which are able to differentiate to mature cells within a few days. It is worth noting that rHuG-CSF elicits not only myeloid-committed progenitors, as one could expect on the basis of its lineage specificity, but also progenitors of other lineages (erythroid and megakaryocytic) [12]. A problem which is still open, and has not been addressed by any of these studies, is the capability of the circulating progenitors to sustain life-long haematopoiesis, or in other words, whether they comprise truly undifferentiated stem cells.

Another problem which is peculiar to the utilisation of rHuG-CSF without preceding chemotherapy is the variable yield of circulating progenitors collected, i.e. the content in progenitors enumerated as colony forming units (CFU)-GM is variable from 0 to over 10^6/kg body weight depending on whether the patient has been heavily pretreated, has bone marrow involvement or on some other unknown variable. Accordingly, a variable number of leucaphereses (three or four in most studies) is needed to collect the optimal dose of circulating progenitors.

rHuG-CSF AND rHuGM-CSF IN ASSOCIATION WITH CHEMOTHERAPY FOR ELICITING CIRCULATING PROGENITORS

Collection of circulating progenitors after chemotherapy, either at conventional dose or at high dose, followed by rHuG-CSF takes advantage of the additional mobilising effect of chemotherapy. As a consequence, the yield of the collection is more predictable, and less leucapheresis procedures are needed to harvest the optimal number of circulating progenitors. As the peak of circulating progenitors may vary in different patients, this approach achieves best results when a daily monitoring of haematopoietic progenitors (e.g. determination of CD34-positive cells) is conducted [13].

Brugger and colleagues [14] have treated cancer patients with conventional dose etoposide, ifosfamide and cisplatin, followed by subcutaneous rHuG-CSF, 5 μg/kg/day for 14 days. Circulating progenitors were collected by a single 2-h leucapheresis on the day of peak levels of progenitor cells (median 11 days after chemotherapy), and median yields of CFU-GM and CD34-positive cells in the leucapheresis were 8×10^6 and 3.4×10^8, respectively. Patients were then treated with high dose etoposide, ifosfamide and cisplatin, with or without circulating progenitors. All patients received recombinant human interleukin 3 (rHuIL-3) and rHuGM-CSF after high dose chemotherapy. Platelet and neutrophil recovery were shown to be significantly faster in patients receiving circulating progenitors.

Gianni and colleagues have focused on the application of high dose single agent chemotherapy followed by cytokine treatment, both as a novel treatment modality for chemosensitive cancers, and as a means for collecting circulating progenitors for autologous transplantation. High dose cyclophosphamide (HD-CTX) is an alkylating agent with dose-related cytotoxic activity in a

broad spectrum of human tumours. At doses up to 7 g/m^2 it is associated with marked antitumour responses in Hodgkin's disease, non-Hodgkin's lymphoma, small cell lung cancer, ovarian cancer, breast cancer and multiple myeloma. In addition, HD-CTX expands a circulating progenitor cell pool which is highly effective in accelerating haematopoietic recovery following myeloablative chemoradiotherapy. HD-CTX-mobilised progenitors, when administered with autologous bone marrow cells following high dose chemotherapy, reduced the period of neutropenia from 17 to 9.5 days, and of platelet transfusion dependence from 24 to 9 days compared to historical controls receiving bone marrow alone [2]. rHuGM-CSF, while ameliorating HD-CTX-induced myelotoxicity, further increases circulation of haematopoietic progenitors [15–17]. Administration of rHuG-CSF after HD-CTX in a relapsed Hodgkin's disease patient induced a dramatic mobilisation of circulating progenitors, which were collected and transplanted after total body irradiation and high-dose melphalan [18]. In this patient, the period of absolute neutropenia (<500 neutrophils/μl) following autografting was 5 days, and the period of thrombocytopenia (<20 000/μl) was 6 days.

High dose etoposide (HD-VP16), like HD-CTX, has a marked antitumour activity and a remarkable mobilising capacity of circulating progenitors, which is enhanced by either rHuGM-CSF or rHuG-CSF treatment. Both these cytokines reduced the period of absolute neutropenia following HD-VP16 from 8 to 3 days, while no effect was observed on platelet counts. In addition, the median increase of circulating progenitors was 7.4-fold after HD-VP16 alone, and 22-fold after cytokine administration following HD-VP16 [4].

CHARACTERISATION OF CIRCULATING PROGENITORS ELICITED BY rHuG-CSF

rHuG-CSF treatment increases the frequency of granulocyte–macrophage, erythroid, megakaryocytic and mixed myeloid–erythroid progenitors in comparison to the steady state peripheral blood. This increase affects all types and maturational stages of progenitor cells measurable *in vitro*, and is already demonstrable at low doses of rHuG-CSF [8]. This phenomenon is typically transient, dynamic and strictly dependent on cytokine administration; the frequency of circulating progenitors rapidly decreases after discontinuation of the treatment.

Circulating progenitors can also be distinguished by flow cytometry based on expression of surface membrane molecules referred to as CD34 and CD33 antigens, similar to their bone marrow counterparts. Cells expressing the CD34 antigen comprise virtually all haematopoietic progenitors forming myeloid, erythroid, megakaryocytic, mixed and blast cell colonies. CD34-positive cells transiently circulate in the peripheral blood of patients treated with HD-CTX with or without rHuGM-CSF [17], and their frequency correlates well with that of myeloid progenitors detected by *in vitro* colony assay [13], so that daily determinations of peripheral blood CD34-positive cells can guide on a real-time basis the timing and the yield of leucaphereis procedures (Fig. 1).

Accordingly, CD34-positive progenitors circulate in the peripheral blood of cancer patients treated with standard dose chemotherapy and rHuG-CSF [14]. Dual colour flow cytometry analysis of collected CD34-positive cells indicated that 93% co-expressed the lineage-associated marker CD38, and 89% co-expressed HLA-DR. A small proportion of these cells (7–11%) was lineage-negative, and probably comprised undifferentiated pluripotent stem cells.

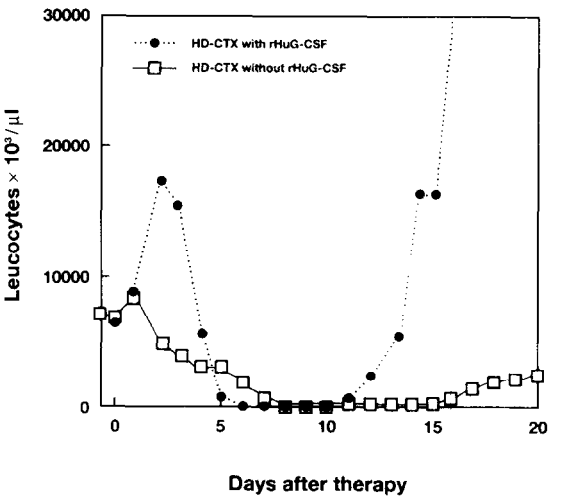

Fig. 1. Leucocyte recovery after high dose cyclophosphamide (HD-CTX) cancer therapy, followed or not followed by rHuG-CSF. The median day of peak of CD34-positive cells per microlitre of peripheral blood occurred on day +18 after HD-CTX without rHuG-CSF (CD 34+ cells/μl = 68), and on day +13 after HD-CTX with rHuG-CSF (CD 34+ cells/μl = 539) (data from the Istituto Nazionale Tumori, Milan, Italy).

The capability of rHuG-CSF-elicited circulating CD34-positive cells to sustain long-term haematopoietic reconstitution after myeloablative cancer therapy has not been adequately addressed so far. Shpall and colleagues [19] reinfused circulating CD34-positive cell-enriched suspensions to breast cancer patients following high-dose chemotherapy. In this small series of patients, short-term haematopoietic recovery was indistinguishable from that of patients transplanted with both circulating plus bone marrow CD34-positive cells, and substantially faster than that achieved with bone marrow CD34-positive cells. Even if preclinical as well as clinical data suggest that durability of engraftment with circulating progenitors should not be a problem, clinical long-term results from ongoing studies are awaited [20].

PROSPECTS FOR UTILISATION OF CIRCULATING PROGENITORS IN CANCER THERAPY

Utilisation of rHuG-CSF-elicited circulating haematopoietic progenitors in the clinical practice of cancer therapy will profoundly change current treatment strategies. In particular, the substantial reduction in haematopoietic and related toxicities granted by circulating progenitors will permit the design of novel treatment modalities for chemosensitive tumours, with higher initial dose size and dose intensity.

Problems still unresolved with the procurement and utilisation of circulating progenitors include an optimal rHuG-CSF mobilising protocol, with or without chemotherapy, the optimal dose of rHuG-CSF and the utilisation of rHuG-CSF after transplantation of circulating progenitors. The question of whether or not it is necessary to transplant both circulating and bone marrow progenitors is directly related to the long-term engraftment potentiality of circulating progenitors, which is now being addressed both preclinically and clinically by several studies. Technology is now available for selecting haematopoietic progenitors from the bulk of the leucapheresis product, which might be useful both for eliminating contaminating tumour cells from the graft and for further manipulation. In this regard, circulating progenitors appear to be a good target for

genetic manipulation, particularly for retroviral-mediated gene transfer [21].

Another potential application of circulating progenitors could be in allogenic transplantation, provided that technology for elimination of contaminating T cells is available, and long-term engraftment capability is demonstrated. A short treatment of the donor with rHuG-CSF followed by leucapheresis would be more acceptable than bone marrow harvest, and would expand the recruitment to unrelated donors.

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Growth Factors in Haematology: Prophylactic Versus Interventional Use

Marc A. Boogaerts

GROWTH FACTORS have drastically changed haematology. They have not only improved the understanding of haematopoiesis and leukaemogenesis, but they have also provided new therapeutic approaches to a variety of deficiency states and/or malignant conditions.

Granulocyte growth factors can be used in two ways in haematology: (1) prophylactic, i.e. to prevent infections post-chemotherapy, postbone marrow transplantation or in immunodeficiency states (e.g. HIV infection or chronic lymphocytic leukaemia), and (2) interventional, i.e. therapeutic, to treat