Standards for PBSC mobilisation and collection

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Introduction

Standards or target thresholds for the number of cells mobilised and collected for stem cell transplantation are essential if high dose therapy with blood cell rescue is to be carried out safely. Many assays are available for measuring different but often overlapping haemopoietic cell populations at different stages of the differentiation pathway (Figure 1), but all the in vitro assays are surrogate measurements. The ultimate end point is durable, multilineage engraftment and any parameter used as a standard must ultimately be related to its ability to predict haemopoietic recovery.

Progenitor cell measurement

There is a large body of evidence to suggest that different classes of progenitor/stem cells give rise to different phases of haemopoietic reconstitution. 'Late' progenitors such as those measured in colony forming assays appear to give rise to rapid engraftment whereas longer more durable engraftment appears to arise from more primitive cells.

Jones and colleagues clearly demonstrated in the mouse that the cells giving rise to long term repopulation were distinct from those giving rise to short term haemopoietic recovery [1]. Kaizer and colleagues found that removal of CFC (but not earlier cells) from the infused human marrow by incubation with 4-hydroxycyclophosphamide resulted in delayed haematological regeneration [2] and Robertson et al. found that purging with anti-CD33 which removed GM-CFC and BFU-E but not LTCIC resulted in a similar delay [3].

In the majority of clinical situations in which PBSC 'rescue' is used, the high dose chemotherapy employed causes only temporary myelosuppression and the autograft is only required to provide relatively short term support. It thus seems reasonable in these circumstances to use assays of relatively late cells for standardisation.

The ease and reliability of the different assays must also be taken into account. Colony forming assays are robust within individual centres but there is often marked variability between centres and quality assurance exercises are difficult. Fresh stem cell preparations are ideally required for quality control purposes because if frozen aliquots are used, this introduces the large potential variability of freeze-thaw recoveries. The flow cytometric measurement of cells expressing the CD34 antigen correlates reasonably well with measures of GM-CFC and providing standardised staining and analysis protocols are used it is possible to obtain good concordance between different laboratories [4, 5]. However, the lowest levels of CD34+ cells in the blood, at which PBSC may still be worthwhile, are below 0.1% which can be challenging for a routine laboratory. The major advantage of CD34+ cell measurements is that they can be done within an hour or so and can be used to determine when to commence apheresis and to determine on the same day whether an apheresis collection was adequate.

At University College London Hospitals a large number of patients with relapsed or resistant malignant lymphomas have been mobilised with cyclophosphamide 1.5/m² followed by daily G-CSF [6]. A white blood cell count nadir occurs at day 8 and the white blood cell count rises rapidly after this time. Step wise analysis of these patients has identified the first day on
which the post nadir white blood cell count exceeds 5.0 x 10⁹/l to be the optimum time to harvest PBSC [7] as determined by assays of GM-CFC and CD34+ cells. This agrees very closely with the data of Haas [8] who found peak circulating progenitor levels commencing when the recovery WBC reached 4.75 x 10⁹/l.

In an initial cohort of 81 patients with lymphoma treated by high dose BEAM chemotherapy nearly all had at least two apheresis collections although only one was returned in many patients. A wide range of cell numbers and progenitor cell numbers was thus returned after the high dose therapy making it possible to address the issue of progenitor cell thresholds required for engraftment. All these patients recovered their neutrophil counts to >0.5 x 10⁹/l within 28 days but a proportion of patients had delayed platelet recovery. A threshold effect was clearly discernible with no patients receiving either more than 3.5 x 10⁸/kg MNC, 3.5 x 10⁹/kg CD34+ cells or 3.5 x 10⁹/kg GM-CFC having delayed platelet recovery (Table 1) [7]. Thirty three patients received less than 3.5 x 10⁹/kg CD34+ cells and analysis of this informative group revealed that the GM-CFC count was more informative than the CD34+ count. Furthermore, the number of apheresis collections required to reach a given number of cells and whether or not the patient had previously received intensive salvage therapy also influenced recovery suggesting that progenitor cell 'quality' is important as well as quantity. It is noteworthy that Schneider and colleagues have found that progenitor cell 'quality' as determined in 'pre-CFU-assays' also varied according to the therapy given prior to stem cell mobilisation [9].

This implies that threshold requirements might vary in different clinical situations, although there is in general good agreement with the UCH data [10].

It must be emphasised that the BEAM regimen is not myeloablative and endogenous recovery would ultimately occur in most patients without stem cell support. With myeloablative regimens such as total body irradiation or high dose busulphan, attention must also be paid to long-term engraftment. From the limited experience with myeloablative regimens it has been found that if there are adequate progenitor cells in an unmanipulated stem cell infusion for rapid engraftment then stable engraftment is almost invariably [11]. This may not be the case if new mobilising regimens are developed or the PBSC are subjected to in vitro culture. Under these circumstances it would be essential to demonstrate the presence of reasonable numbers (precise threshold required unknown) of LTCIC of CAFC or equivalent before proceeding to clinical studies. The presence of such cells may, however, still not ensure stable engraftment.

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References


Table 1. Data is on 81 patients except for MNC where the six patients receiving purified CD34+ cells are excluded.

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<td>&gt; 3.5 % Risk</td>
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<tr>
<td>MNC 10⁹/kg</td>
<td>57/0/43 [0%]</td>
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<tr>
<td>CD34 10⁹/kg</td>
<td>51/1/41 [2%]</td>
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<td>GM-CFC 10⁷/kg</td>
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Purging of peripheral blood progenitor cells

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Key words: breast neoplasms, CD34+ cells, chemotherapy, cytokines, dose-intensity, ex vivo purging, in vivo purging, peripheral blood progenitor cells, small cell lung cancer, transplantation

Introduction

Peripheral blood progenitor cells (PBPCs) have replaced bone marrow (BM) cells for autografting after high-dose chemotherapy. As compared with autologous BM, PBPCs permit a faster lympho-haematopoietic recovery and can be collected in an outpatient setting. Moreover, PBPC preparations are contaminated less frequently with malignant cells [1], although several investigators have shown that tumour cells can also be detected in PBPC preparations, particularly in patients with neuroblastoma, breast cancer, small cell lung cancer, and follicular lymphoma [2-6]. In addition, gene marking studies demonstrated that contaminating tumour cells might contribute to relapse after autologous BM transplantation [7-9]. These findings suggest the application of purging strategies to minimize the number of contaminating tumour cells in the autograft. Up to now, however, no formal proof has been provided that reinfusion of tumour cells upon PBPC transplantation actually leads to higher relapse rates or impaired overall survival.

This report briefly summarizes our approach to PBPC purging, i.e., 1) an in vivo purging upon effective cytoreductive chemotherapy prior to PBPC harvest and 2) an ex vivo purging consisting of two steps, the positive selection of CD34+ cells and finally the ex vivo expansion of CD34+ cells in cytokine-supported liquid cultures.

In vivo purging

We have recently analysed peripheral blood samples from 46 solid tumour patients undergoing PBPC mobilization after conventional-dose VIP-(E) chemotherapy and G-CSF administration for the presence of cytokeratin (CK) and/or HEA125-positive tumour cells [4]. Prior to chemotherapy, we found that 4 of the 46 newly diagnosed patients with stage IV breast cancer and small cell lung cancer contained CK-positive tumour cells in the peripheral blood, whereas in the bone marrow, 8 of the 46 patients were CK-positive. These data demonstrated a higher percentage of patients being CK-positive in the marrow as compared to the peripheral blood. After one cycle of VIP-(E) chemotherapy and G-CSF administration, a total of 13 patients contained CK-positive epithelial tumour cells in the peripheral blood, i.e., tumour cells were actually mobilized concomitantly to normal haematopoietic cells in 9 additional patients [4]. The highest risk of concomitantly mobilizing PBPCs and malignant epithelial tumour cells was observed in patients with bone marrow micrometastases, particularly in patients with stage IV breast cancer (7/7) as well as in patients with extensive-stage small cell lung cancer (SCLC) (5/10). However, we have shown that one additional cycle of the same chemotherapy regimen resulted in a clearance of the tumour cells - as analysed with a detection limit of 1 in 400,000 nucleated cells - in the majority of the patients, i.e., in 5/7 patients with metastatic breast cancer as well as in all patients with extensive-stage SCLC [4]. This observation suggests that effective cytoreductive chemotherapy prior to PBPC harvest is critical to minimize the risk of tumour cell contamination. Based on these observations, we would recommend to perform at least two cycles of induction chemotherapy prior to PBPC harvest (in vivo purging). However, considering the observation that both the quality as well as the quantity of mobilized PBPCs declines with multiple cycles of prior chemo- or radiotherapy, PBPC harvest should not be considered too late in the management of the disease [10,11].

Although in vivo purging with two cycles of VIP-E chemotherapy prior to PBPC harvest is a highly effective anti-tumour treatment in a variety of malignancies (e.g., high-grade non-Hodgkin's lymphoma, Hodgkin's disease, breast cancer, small cell lung cancer, soft tissue sarcomas), the transplantation of unmanipulated PBPCs still carries the possible risk of retransplanting residual tumour cells. Therefore, we would prefer to further ex vivo manipulate PBPC preparations to ensure an additional reduction of contaminating tumour cells.

Positive selection of CD34+ PBPCs

One approach to further reduce the number of contaminating tumour cells is the positive selection of...
CD34+ cells from unmanipulated PBPC preparations. This procedure results in an about 2-4 log depletion of non-CD34+ cells including tumour cells [12, 13]. In addition, our group has shown that the CD34+ cell selection does not reduce the number of more primitive progenitor/stem cells, such as the long-term culture initiating cells (LTC-ICs) [14]. These data suggest that the long-term capabilities of the CD34+ cell product should not be impaired. By limiting dilution analyses, we calculated that approximately 0.5% of the CD34+ selected cells are LTC-ICs [14]. Transplantation of positively selected CD34+ cells into cancer patients resulted in identical haematological recovery patterns when compared to patients receiving unmanipulated PBPC transplants, thereby demonstrating the feasibility of this approach [12, 15]. Moreover, we have shown in our study [15] that the positive selection of CD34+ cells results in a 470 fold T-cell depletion and thus enables this procedure for the use in allogeneic CD34+ cell transplants [16]. Finally, CD34+ cell selection reduces the volume of the frozen product and thus reduces the side effects related to the infusion of larger volumes of cryoprotectant and debris of lysed red cells.

**Ex vivo expansion of CD34+ PBPCs**

The demonstration of a non-impeded functional integrity of positively selected CD34+ cells allowed us to study whether or not these cells can be expanded *ex vivo* for possible clinical application. Successful expansion of PBPC *ex vivo* would considerably reduce the patients’ blood volume that has to be processed for transplantation, thereby further decreasing the overall tumour cell load in the final autograft. Moreover, this method may circumvent the need for leukapheresis.

We have demonstrated that a combination of stem cell factor (SCF), interleukin-1β (IL-1), IL-3, IL-6 and erythropoietin (Epo) effectively mediates the *ex vivo* expansion of committed progenitor cells [17] and maintains the number of more primitive LTC-ICs [14]. The reason for the observation that LTC-ICs are not expanded significantly in the presence of SCF, IL-1, IL-3, IL-6 and Epo-supported liquid cultures may be due to the fact that a non-physiological stroma-free culture system is used, or to their inherently limited proliferation potential [18]. Moreover, there is evidence that prior chemotherapy negatively influences the potential for *ex vivo* expansion [19 and own unpublished observations].

**Ex vivo expansion of CD34+ PBPCs in the presence of cytokine-supported liquid cultures, however, would only be advantageous, if potentially contaminating tumour cells would not be expanded concomitantly. This possibility cannot be excluded completely. Therefore, to address the question whether or not contaminating tumour cells are expanded concomitantly to CD34+ PBPCs, we have looked by immunocytochemistry for such tumour cells following expansion of CD34+ PBPCs taken from 10 consecutive stage II—III breast cancer patients, and we have so far failed to find any residual tumour cells, with a detection level of one cell per $4 \times 10^5$ nucleated cells [20]. Thus, to answer the fundamental question, we co-cultured CD34+ PBPCs in medium to which defined quantities of primary or xenograft-derived tumour cells were added. Over a period of 14 days, we found a 2-log increase in haematopoietic progenitor cells, but we did not find a net amplification of tumour cell numbers [20]. However, the tumour cells present were still viable and were able to regrow when maintained in serum-containing cultures for longer periods. These data suggest that cytokine-supported short term *ex vivo* expansion of CD34+ PBPCs contaminated with epithelial tumour cells can confer a proliferative advantage of haematopoietic cells without an apparent risk of concomitantly expanding residual tumour cells.

Based on these findings, we recently undertook a phase I/II trial in 10 solid tumour patients with *ex vivo* expanded positively selected CD34+ PBPCs [21]. This study was performed primarily to answer the question as to whether or not such *ex vivo* expanded cells would successfully mediate haematopoietic recovery after high-dose chemotherapy. The transplantation potential of *ex vivo* expanded cells was tested after high-dose VICE chemotherapy (VP16 1500 mg/m², ifosfamide 12,000 mg/m², carboplatin 750 mg/m², and epirubicin 150 mg/m²). All patients showed rapid haematopoietic engraftment comparable to historical control patients receiving either unseparated PBPCs or positively selected CD34+ cells [21]. The results do not allow firm conclusions about the long-term *in vivo* capabilities of the CD34+ cells cultured *ex vivo*. The introduction of a genetic marker into the transplanted cells or the use of allogeneic cells may clarify this issue [21]. However, based on limiting dilution analyses of LTC-ICs after *ex vivo* expansion, we detected approximately 70,000–100,000 LTC-ICs within these cultures. Extrapolation from experiments in mice indicates that a total of 30,000 LTC-ICs may suffice for long-term reconstitution after 'myeloablative' therapy [14, 21]. We are currently performing transplants of *ex vivo* expanded CD34+ PBPCs into transgenic SCID-mice in order to get further insight into the biological capabilities of the cells generated *ex vivo* [22].

In conclusion, starting from a small number of CD34+ cells, which corresponds to less than 10% of the CD34+ cells normally present within our 2-hour leukapheresis preparation, *ex vivo* expansion enables a considerable reduction of the patients' blood volume that has to be processed for transplantation. By using this method, a total of 100–200 ml of blood at the time of maximal progenitor cell mobilization yields sufficient CD34+ cell numbers for transplantation, thereby minimizing the overall tumour cell number in the final autograft.

In summary, our studies demonstrate the feasibility of a three step purging strategy (Figure 1) consisting of...
an in vivo purging by induction chemotherapy with subsequent PBPC collection, followed by the selection of CD34+ cells and finally their ex vivo expansion. This multi-step procedure results in an estimated total 6–7 log reduction of tumour cells in the final graft, thereby minimizing the patients’ risk for a transplant-mediated relapse. We are now focusing on ex vivo expansion of antigen-presenting cells from CD34+ PBPCs which might be used clinically to treat minimal residual disease after high-dose chemotherapy [23].

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References

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