Introduction

Peripheral blood progenitor cell transplantation: Where do we stand?
Chairman's summary of the European School of Oncology Task Force Meeting 'Peripheral Blood Progenitor Cells' held September 29–30, 1995


Summary

Whether or not peripheral stem cells have an unlimited capacity for self renewal is debated. However, everyday haematopoietic requirements are met by progenitors; and it seems that few 'real' stem cells are needed. Although we may not yet have identified these 'true' stem cells, for practical purposes the long term culture-initiating cells (LTC-ICs) are a close approximation. To date, experience in peripheral blood progenitor cell (PBPC) transplantation is largely confined to non-ablative regimens. It is therefore difficult to determine the number of PBPCs needed to effect long-term reconstitution. The number of tumour cells present among mobilised PBPCs can be reduced using the CD34 affinity column and by positive purging methods. The ex vivo expansion of CD34 cells also has the effect of diluting tumour cell concentration.

In clinical use, PBPC transplantation has a proven role in support of high dose chemotherapy in certain haematological and oncological malignancies but the concept of dose intensification is not universally accepted. With the exception of leukaemia, lymphoma, myeloma or relapsed testicular cancer and possibly some subgroups of breast cancer; high dose chemotherapy does not demonstrate a survival benefit. For patients with CML, autografting with Ph+ cells appears to become a useful alternative to allogeneic BMT. Allogeneic PBPC transplantation may have potential, though work is preliminary. Cord blood transplantation between matched siblings is viable, but it is not yet clear whether this source will increase the donor pool for adults needing allogeneic transplantation. For gene therapy using haematopoietic cells to be effective, a greatly increased rate of transduction will be needed.

Meeting in Paris in September 1995, a European School of Oncology Task Force considered a number of important questions relating to peripheral blood progenitor cell (PBPC) physiology and transplantation. This review is a brief account of their conclusions.

Key words: chemotherapy, cord blood, cytokines, gene therapy, granulocyte growth factors, peripheral blood progenitor cells, purging, review, transplantation

Graft composition and engineering

The classical definition of stem cells requires that they have the ability to self-renew, that they have the potential to differentiate and that they have extensive proliferative capacity. There is an inverse relationship between proliferation and differentiation.

The true stem cell cannot be defined precisely. However, for practical purposes, the long term culture-initiating cells (LTC-ICs) are a sufficiently close approximation, and their assay is anchored in solid experimental data [1]. For working protocols, we can rely on LTC-ICs as indicative of the stem cell compartment even though this kind of cell may not fully represent it. In mice, it appears that around 5–130 and in man 10–30,000 such cells are needed to reconstitute the haematopoietic system [2]. Factors adversely influencing LTC-IC generation include the nature and extent of previous chemotherapy. In poor mobilisers, cell harvest may be improved by growth factors such as the combination of SCF and G-CSF.

Immunophenotyping of stem cells has shown that primitive progenitors express the phenotype CD34+, lineage negative, Thy 1+, CD38+. FACS analysis of cells selected from mobilised peripheral blood using a CD34 affinity column shows that the great majority are committed progenitors expressing both CD38 and HLA-DR. However, the purified sample also contains more primitive cells: among the selected cells, roughly one in 240 are LTC-ICs [3].

The role of the telomere

For many years, it has been known that serial passage of bone marrow eventually results in failed engraftment. This 'decline', which shows that stem cells have only a finite capacity for self-renewal, is now known to have a clear-cut physiological basis in the telomere. These terminal components of all linear chromosomes have a tandem repeat in man of TTAGGG. They are responsible for alignment during each mitosis. In normal cord blood cells there are approximately 15,000 base pairs on the telomere, but 40–200 base pairs are lost during each mitotic cycle. When these base pairs
are used up, i.e., at the Hayflick limit, the cell is effectively senescent [4].

Measuring telomere length may eventually provide a simple way of predicting the proliferation capacity of haematopoietic cells and therefore the likely quality of long-term engraftment. However, it seems that a subset of CD34 cells expresses telomerase, an enzyme capable of repairing the telomere. This may be the physiological basis of stem cells' capacity for self renewal. Whatever the basis of that capacity, we know that haematopoietic cells do not expand logarithmically. On average, it seems that each stem cell gives rise to one stem cell and one differentiated cell. This is the basis of the renewal probability of 0.5 seen in adult steady-state haematopoiesis.

How many cells are needed?

The bulk of PBPC transplant experience to date is with non-ablative regimens. In this setting, various degrees of endogenous recovery occur, and it is therefore misleading to argue that we have data on long-term engraftment. In terms of long-term recovery, quality control will be particularly important, especially in relation to allogeneic programmes. However, standards are difficult to recommend until major centres produce large series using good assays.

Nevertheless, available experience may provide useful clinical guidance in relation to short-term recovery. Patients receiving >3.5 x 10⁸ mononuclear or 2 x 10⁹ CD34 positive cells per kg, receive an adequate but perhaps not an optimal graft. Delayed platelet recovery is more common in patients who receive fewer than 2.0–3.5 x 10⁸ CD34 cells per kg, or fewer than 3.5–4.0 x 10⁹ GM-CFCs per kg [5]. However, the latter two variables seem to add little additional predictive information, in many cases, over the number of mononuclears. The number of leukaphereses required to achieve adequate harvest may be an important factor.

Among patients who had low levels of GM-CFCs transplanted, platelet counts recovered more quickly in people who had had a single apheresis than in those in whom two aphereses were required (see D. Linch, this issue).

In determining the adequacy of a graft, it is probably easier to measure CD34 levels than those of GM-CFCs: CD34 assays can be standardised between laboratories in a way that culture assays cannot. However, even the CD34 assay is difficult at low levels. With a regimen that is familiar and standardised, the simple measurement of mononuclear cell count may provide sufficient predictive information. In interpreting the outcome of PBPC transplantation, differences in patient selection are likely to be far more important than variation in cell numbers transplanted.

There is some evidence to suggest that basal CD34 count relates to mobilisation capacity, but more work is needed to confirm this relationship. Good predictors of poor mobilisation are urgently needed so that protocols can be adapted accordingly [6].

Preliminary data suggest that the mobilisation of progenitor cells may be caused by the release of metalloproteinases from neutrophils. In the microenvironment, stem cells are attached by proteoglycans, which are split by proteinases. Studies that will test this idea are in progress [7].

Purging and expansion

The mobilisation of PBPCs has the potential also to mobilise tumour cells. According to recent data 30% to 50% of solid tumour patients have evidence of malignant cells in peripheral blood following growth factor supported, chemotherapeutic mobilisation, even if only a minority of them had been positive beforehand [8]. However, the addition of a course of chemotherapy prior to mobilisation – i.e., in vivo purging – may prevent this co-mobilisation.

Both positive and negative purging methods are available. Positive selection using the CD34 column is popular and ensures a 2–4 log depletion of tumour cells in the enriched CD34 cell fraction. However, the additional use of negative selection techniques, which achieve a 4–5 log depletion of tumour cells, may be necessary. Gene marking studies have shown that malignant cells remaining in the graft may contribute to relapse, although such graft contamination may be less important in the clinical setting [9]. Nevertheless, if PBPC transplantation is to be used to support dose-intensified chemotherapy, we must ensure that clonogenic malignant cells are not transplanted along with the graft.

The rationale for ex vivo expansion of CD34 progenitor cells is that it may further decrease overall tumour cell contamination while also reducing the volume that has to be collected from the patient. In vitro expansion of progenitors can be achieved using cocktails of growth factors. Using SCF, IL-1β, IL-6, IL-3, and erythropoietin, the Freiburg group have achieved a median 190 fold expansion of total colony forming cells, while levels of tumour cells remained undetectable [10]. The use of ex vivo expanded CD34 cells has been investigated in a phase I trial [3]. The FLK-2 ligand and thrombopoietin are promising useful additions to the agents available for ex vivo expansion [11].

Ex vivo expansion of LTC-ICs has not been achieved in adults. This may be because of the use of stroma-free systems, or because of prior chemotherapy, or because of the cells' limited proliferation potential. However, 20–40 fold expansion of the LTC-IC population in cord blood has been demonstrated [12].

PBSC transplantation in clinical use

Support for high dose chemotherapy

The case of PBPC transplantation as a support for dose intensification in lymphomas and high risk leukaemias...
has been made [13]. In multiple myeloma, survival is only marginally improved, but there may be useful palliation. Among the solid tumours, the use of dose intensification with stem cell support has become increasingly popular. However, until now it has only found a role in relapsed testicular cancer.

There may also be subgroups of patients with breast cancer who benefit. In this disease, retrospective analysis has suggested that there is a distinct relationship between the dose of chemotherapy actually administered and the response achieved. The results of studies which pushed the dose of chemotherapy 5–10 times that normally used in standard metastatic disease have not in themselves been encouraging [14]. However, these studies were in relatively poor risk patients and there may be a case for dose intensification as consolidation therapy following initial induction in high risk patients, as in the haematological malignancies.

In a series of around twenty such studies, 6%–40% of patients who were non-responders at induction were converted into CRs, and overall CRs of 30%–100% have been achieved. Overall, no survival benefit was seen. Nevertheless, of the subgroups of patients already in CR post induction, i.e., in women with chemosensitive disease, 45% survived more than three years.

A second group in whom intensive chemotherapy may be beneficial is those with inflammatory T4 disease or more than ten positive axillary nodes. In the latter group, reports from Duke University [15] and Milan [16] suggest high dose chemotherapy with haematopoietic support improves survival relative to historical controls from the same institutions. Nevertheless, a clear case for dose intensification has still to be made by randomised prospective studies.

This conclusion is also justified in the case of ovarian and lung cancer where trials to date have not been promising.

The possible long-term side effects of PBSC transplantation in support of high dose chemotherapy must be considered. However, the incidence of secondary AML and MDS seems to be 1%–5%; and this is not much different from that expected with other therapies. Indeed, there is reason to suggest that a single exposure to high dose chemotherapy plus PBSCs early in the course of disease may reduce the risk of secondary leukaemia by lessening the need for multiple courses of chemotherapy later on.

CML

Philadelphia-negative cells have been shown to persist in patients with CML. It appears that around half of all patients with Ph" cells can be autografted with Ph-progenitors, and this figure rises to two-thirds in patients who have not been heavily pre-treated with interferon. A third of patients maintain Ph negativity after grafting, on a regimen of interferon and low dose IL-2.

The experience from Genoa on which these conclusions are based emphasises the importance of early treatment [17]. Among newly diagnosed patients treated only with hydroxyurea, there is considerable potential for lenograstim supported leukapheresis to serve as the source of primitive normal cells for therapeutic use. Mobilisation early in CML is safer, less expensive, more effective and induces the highest frequency of Ph-cells. In untreated patients, the numbers of CD34, GM-CFU and LTC-ICs obtained are not dissimilar from values in normal allotransplant donors. PBPCs may therefore represent an alternative to allogeneic BMT in patients with CML.

A look to the future

**Allogeneic PBPC transplantation**

Allogeneic PBPC transplantation – either as an adjunct to BMT or alone – is promising. Although the long-term effects of allografting in good risk patients remain to be established, CD34-selected PBSCs of donor origin appear able completely to restore haematopoiesis. Early data suggest that patients treated with allo PBPCs achieve the level of 100 neutrophils/μl 4–5 days faster than those treated with BMT, and this may enable earlier hospital discharge.

The protocol now used by the Hannover group [18] uses CD34 selection and aims to achieve a minimum of 4 x 10^6 CD34 cells per kg in patients transplanted with PBPCs alone. However, the minimum and optimal number of progenitor cells still needs to be clearly established, as does the benefit, or otherwise, of CD34 cell selection.

The role of T-cell depletion also needs to be reconsidered now that data suggest it is helpful in reducing the incidence of GvHD. It may be that 1 × 10^5 T-cells are sufficient to achieve a graft versus leukaemia effect while not increasing the risk of GvHD. Work in progress in Hannover, together with that by groups in Seattle, Houston and Basel (who have not used T-cell depletion in PBSC transplantation) should provide further information.

According to the Hannover experience, the effects of PBSC mobilisation and leukapheresis on the donor appear mild: a dose reduction of G-CSF was required in only one of ten cases, and the plasma levels measured were within the range of endogenous G-CSF seen in people with fever. In the donor, it is estimated that only 1% of the total number of stem cells is mobilised.

**Cord blood transplantation**

Transplantation of cord blood obtained from siblings is feasible, although existing data relate to fewer than 100 cases, most of them children under 40 kg [19]. The median cell dose transplanted, at around 5 x 10^7 per kg, was a log lower than is usual with marrow recipi-
ents. All cases showed satisfactory and sustained engraftment. However, the fact that the median time to platelet recovery was about 50 days suggests that the number of cells involved may be close to the minimum required. It is too early to say whether the transplantation of ‘immunologically naïve’ lymphocytes may reduce the incidence of GvHD following a mismatched donation. The implications for any graft versus leukaemia effect also need to be monitored.

It is not possible at present to determine whether there are sufficient cells present in cord blood, either with or without expansion, for transplantation into adults. The long-term question of whether use of cord blood can extend the donor pool for adults requiring allogeneic transplantation therefore remains unanswered. However, current international efforts aimed at setting up a bank of cryopreserved cord blood are worthwhile as a prelude to well controlled clinical studies.

**Gene therapy**

Currently we are able to transduce 20%, at most, of stem cells exposed to a retroviral vector. Given a self renewal probability of 0.5, the chance that a single transduced stem cell will give rise only to stem cells over ten population doublings is only one in a thousand. The great bulk of cells will undergo irreversible differentiation. It is therefore not surprising that current efforts at gene therapy have met with little success. The aim should be to transplant into patients, populations of cells that have been 100% transduced. The FLK-2 ligand is promising as a means of inducing the cell cycling necessary for transduction in primitive cells [20]. However, it will probably be necessary to use positive selection to achieve a sufficiently high proportion of transduced cells in any graft.

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