

EDUCATION SESSION 12: CELLULAR THERAPY



New Strategies for Blood Progenitor Cell Mobilization

L. Bik To

After 20 years of painstaking clinical trials in peripheral blood stem cell mobilization and transplantation, a large database has accumulated on how to mobilize and to collect factors affecting mobilization, the progenitor cell dose effect on haemopoietic recovery, and even ambulatory transplant (To et al, 1997). Currently an increasing number of allogeneic transplants and most autologous transplants are performed using mobilized blood cells. Moreover analysis of the types of cells mobilized and mechanistic studies based on in vitro and animal models are contributing to developing new strategies of mobilization.

Current protocols of mobilization include G-CSF alone (autologous/allogeneic) or a combination of chemotherapy and haemopoietic growth factors such as G-CSF and GM-CSF (autologous). Adequate progenitor cell yields for single rescue (2×10^6 CD34+ cells/kg BW) can be achieved in most patients except those who have been heavily pretreated. The yield per apheresis can be increased by performing 3 to 4 blood volume aphereses without exhaustion of the circulating pool of progenitors or dangerous thrombocytopenia. However, up to 10% of allogeneic donors and 30% of autologous donors failed to mobilize sufficient progenitors and apheresis and cryopreservation put a demand on health care resources and may be uncomfortable for patients. The heterogeneity of mobilization response may well be due to polygenetic influences as seen in studies of different mouse strains (Roberts et al, 1997).

Three questions will be addressed: can we avoid apheresis altogether, how can we increase yield of mobilization and how can we develop better mobilization strategies.

Is apheresis necessary?

Brugger et al (1995) reported that ex vivo expansion of mobilized CD34+ cells using a cocktail of recombinant human haemopoietic growth factors and suggested that it may be possible to expand one-tenth of an apheresis product to provide sufficient cells for autotransplantation. This amount could also be obtained through 1 to 2 unit phlebotomy.

Uyl-de Groot et al (1999) compared the haemopoietic recovery between conventional autologous bone marrow

transplant and that with two 500 ml phlebotomy following IMVP + G-CSF mobilization. The cells were stored at 4°C and re-infused 24 hours after preconditioning with BAM (BCNU 300 mg/m² IV day 1, cytarabine 3000 mg/m² 12 hourly day 2, and melphalan 140 mg/m² day 3). G-CSF was administered post transplant. Neutrophil recovery were faster, associated with a 18% cost reduction compared with bone marrow transplantation. Platelet recovery was no faster.

Ex vivo expansion as a means of reducing apheresis requirement is probably not cost-effective unless one can demonstrate a purging advantage. A comparative study of haemopoietic reconstitution between leukapheresed cells and phlebotomy blood will need to be done although the restriction on the duration of preconditioning therapy to 3 days will limit the types of disease in which this approach can be tested. Furthermore, one leukapheresis can harvest 3-5 times the number of progenitors than two 500 ml phlebotomies, so it is unlikely that sufficient cells for rapid reconstitution can be obtained in all patients.

New agents: Improving progenitor yield

A number of new agents are currently tested for their ability to mobilize progenitor cells. TPO/MGDF was first cloned as a megakaryocytic lineage factor that ameliorates thrombocytopenia after chemotherapy. Recent data suggest that it has a proliferative and anti-apoptotic effect on primitive haemopoietic cells as well as mobilization. However, it was withdrawn from clinical trials because of the development of neutralising antibodies leading to severe thrombocytopenia. Flt-3 ligand has also been shown to have promising effect in non-human primates. A series of chimeric receptor agonists, e.g. of Flt-3 ligand-G-CSF and of IL-3-G-CSF, have also been shown to be potential mobilizing agents (MacVittie et al, 1998).

Improving the efficacy of mobilizing agents currently in use

G-CSF

The conventional dose of G-CSF used in mobilization is 10-12 µg/kg sc daily when used alone or 5 µg/kg sc daily

when used with chemotherapy. In allogeneic donors, further escalating G-CSF dose appears promising (Waller et al 1996). We found that 10 µg/kg twice daily increases the chance of harvesting > 5 x 10⁶ CD34⁺ cells from 70% to 90%. When used with chemotherapy, G-CSF also shows a dose related enhancement of mobilization (Lie et al 1998). For 10 consecutive patients who did not show a significant rise in blood progenitor cells within 14 days following chemotherapy and G-CSF, G-CSF dose was increased from 5 to 10 µg/kg/day (*n* = 9) or from 10 to 15 µg/kg/day (*n* = 1). As a result, there were significant increases in total yield as well as yield per apheresis of mononuclear cells, CD34⁺ cells and CFU-GM (*P* < 0.025, < 0.01 and < 0.005, respectively). After G-CSF dose escalation, six of the 10 patients had sufficient CD34⁺ cells for performing transplantation. These results demonstrate a dose-dependent response of progenitor cell mobilisation by G-CSF when used in combination with chemotherapy. Moreover, increasing the dose of G-CSF as late as the third week of mobilisation may still provide sufficient cell yield even with patients who did not show a significant mobilisation with conventional doses of G-CSF.

SCF as an adjunct to G-CSF

SCF enhances progenitor mobilization by G-CSF in patients with breast cancer, ovarian cancer and non-Hodgkin's lymphoma (Glaspy et al, 1995; Weaver et al, 1996; Begley et al, 1997; Moskowitz et al, 1997; Stiff et al, 1997). In previously untreated patients with breast cancer higher levels and more prolonged mobilization occurred (Begley et al, 1997; Roberts et al, 1999). Studies of CD34⁺ cells mobilised did not reveal any difference in maturity as defined with CD38, Thy-1 & MDR-1 co-expression but they showed a lower c-kit co-expression (Roberts et al, 1999).

The mechanism(s) for the synergistic effect of SCF on G-CSF is still unclear, but Roberts et al (1999) postulated that it may be related to its effect on adhesion molecule function, its ligand relation with c-kit, or synergism with G-CSF on proliferation. SCF's effect on the SDF-1/CXCR4 ligand pair may also contribute to its mobilising effect.

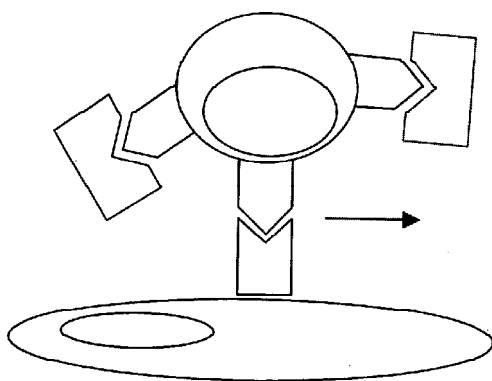


Figure 1. Mobilization Hypothesis

Investigating the mechanism of mobilization

Mobilization is the opposite of homing during ontogeny or following transplantation. The reduced bone marrow haemopoiesis in SDF-1-deficient mice and the chemotactic effect of SDF-1 on CD34⁺ cells suggests strongly that the SDF-1/CXCR4 ligand pair is likely to be involved in the trafficking of haemopoietic progenitor and stem cells (Mohle et al, 1998).

Another line of evidence suggests that the disruption of the β-1 integrin-mediated adhesive interaction between haemopoietic progenitors and the bone marrow microenvironment is a key step of mobilization (Fig 1). It is also known that Ca⁺⁺ are necessary for integrin activity and that G-CSF administration has been associated with osteopenia. Takamatsu et al has investigated whether G-CSF may modulate integrin adhesion through bone breakdown and local release of Ca⁺⁺ (1998). An increased bone turn-over as measured by elevated urinary deoxypyridinoline and reduced serum osteocalcin was demonstrated during short term administration of G-CSF to human and the changes were correlated to CD34⁺ cell mobilization. In murine studies, G-CSF administration also led to increased osteoclast number. However, G-CSF did not inhibit osteocalcin synthesis by osteoblast and there was no evidence that G-CSF could activate osteoclasts directly. Furthermore pamidronate, an inhibitor of osteoclasts, did not inhibit mobilization. Hence, even though G-CSF administration leads to stimulation of osteoclasts and bone resorption and inhibition of osteoblasts, these are parallel but dissociable events from progenitor cell mobilization.

An alternative hypothesis for the mobilization effect of G-CSF is that neutrophil activation by G-CSF may lead to metalloprotease like activity that disrupts the adhesive interaction between progenitors and the microenvironment (Figure 2). This may explain the rapid mobilization seen in the murine IL-8 model because IL-8 activates neutrophils (Laterveer et al 1996). Current work in a number of centres are addressing this issue.

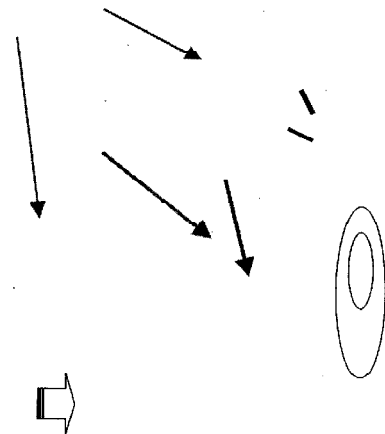


Figure 2. New Hypothesis

Conclusions

1. G-CSF dose escalation, addition of synergistic cytokines, and large volume apheresis increase yield.
2. The role of venesection compared with apheresis is uncertain, probably limited.
3. The progenitor cell/stromal cell adhesion interaction, especially the integrin-fibronectin pathway, is the rational target for developing more effective mobilization strategy.
4. Neutrophils may be critically involved in modulation the adhesive interaction leading to mobilization.
5. Deciphering polygenic influence on mobilization may increase understanding of and ability to improve mobilization.

References

- Begley CG, Basser R, Mansfield R, Thomson B, Parker WRL, Layton J, To LB, Cebon J, Sheridan WP, Fox RM, Green MD. Enhanced levels and clonogenic capacity of blood progenitor cells following administration of stem cell factor plus G-CSF to humans. *Blood* 1997;90:3378-3389.
- Brugger W, Heimfeld S, Berenson RJ, Mertelsmann R, Kanz L. Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated ex-vivo. *N Engl J Med* 1995;333:283.
- Glasy J, LeMaistre CF, Lill M, Jones A, Moore R, Briddell D, Menchaca, Turner S, Shpall EJ. Dose-response of seven day administration of recombinant methionyl human stem cell factor in combination with filgrastim for progenitor cell mobilisation in patients with stage II-IV breast cancer. *Blood* 1995;86(1):463a.
- Laterveer L, Lindley IJD, Heemskerk DPM, Camps JAJ, Pauwels EKJ, Willemze R, Fibbe WE. Rapid mobilisation of hematopoietic progenitor cells in rhesus monkeys by a single intravenous injection of interleukin-8. *Blood* 1996;87:781.
- Lie AKW, Hui CH, Rawling T, Dyson PG, Thorp D, Benic J, Rawling CM, Toogood I, Horvath N, Simmons PJ, To LB. Granulocyte colony-stimulating factor (G-CSF) dose-dependent efficacy in peripheral blood stem cell mobilisation in patients who had failed initial mobilisation with chemotherapy and G-CSF. *Bone Marrow Transplantation* 1998;22:853-857.
- MacVittie TJ, Farese AM, Dacquel Smith L, Casey DB, Smith WG, Woulfe SL, Streeter PR, McKearn JP. Members of the progenipointins, a family of chimeric FLT-3 ligand and G-CSF receptor agonists mobilise hematopoietic progenitor cells with different kinetics and efficiency in non-human primates. *Blood* 1998;92:682a.
- Moskowitz CH, Stiff P, Gordon MS, McNiece I, Ho AD, Costa JJ, Broun ER, Bayer RA, Wyres M, Hill J, Jelaca-Maxwell K, Nichols CR, Brown SL, Nimer SD, Gabrilove J. Recombinant methionyle human stem cell factor and filgrastim for peripheral blood progenitor cell mobilisation and transplantation in non-Hodgkins lymphoma patients – results of a phase I/II trial. *Blood* 1997;89:3136-3147.
- Roberts AW, Foote S, Alexander WS, Scott C, Robb L, Metcalf D. Genetic influences determining progenitor cell mobilisation and leukocytosis induced by granulocyte colony-stimulating factor. *Blood* 1997;89(8):2736-2744.
- Roberts MM, Swart BW, Simmons PJ, Basser RL, Begley CG, To LB. Prolonged release and C-Kit expression of haemopoietic precursor cells mobilised by stem cell factor and granulocyte colony stimulating factor. *B J Haematol* 1999;104:778-784.
- Stiff P, Grinrich R, Luger S, Brown RA, LeMaistre CF, Perry D, Schenkein D, List A, Mason JR, Bensinger W, Wheeler CA, Freter C, Murphy-Filkins R, Wyres M, Parker W, Emmanouilides C. Improved PBPC collection using stem cell factor and filgrastim (G-CSF) compared to G-CSF alone in heavily pretreated lymphoma and Hodgkins disease patients. *Blood* 1997;90:591a.
- Takamatsu Y, Simmons PJ, Moore RJ, Morris HA, To LB, Lévesque J-P. Osteoclast-mediated bone resorption is stimulated during short-term administration of granulocyte colony-stimulating factor but is not responsible for hematopoietic progenitor cell mobilisation. *Blood* 1998;92(9):3465-3473.
- To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. *Blood* 1997;89(7):2233-2258.
- Uyl-de Groot CA, Ossenkoppele GJ, Buijt I, Huijgens PC. Costs of peripheral blood progenitor cell transplantation using whole blood mobilised by filgrastim as compared with autologous bone marrow transplantation in Non-Hodgkin's lymphoma. *Pharmacoeconomics* 1999;15(3):305-311.
- Weaver A, Ryder D, Crowther D, Dexter TM, Testa NG. Increased numbers of long-term culture-initiating cells in the apheresis product of patients randomised to receive increasing doses of stem cell factor administered in combination with chemotherapy and a standard dose of granulocyte colony-stimulating factor. *Blood* 1996;88:3323-3328.