



REVIEW

Homing, cell cycle kinetics and fate of transplanted hematopoietic stem cells

EF Srour^{1,2,3}, A Jetmore¹, FM Wolber¹, PA Plett¹, R Abonour¹, MC Yoder² and CM Orschell-Traycoff¹

Department of ¹Medicine and ²Pediatrics, Herman B Wells Center for Pediatric Research; and ³Department of Microbiology/Immunology, Indiana University School of Medicine, Indianapolis, IN, USA

Homing of transplanted hematopoietic stem cells to recipient bone marrow is a critical step in engraftment and initiation of marrow reconstitution. At present, only partial understanding of the cellular and molecular mechanisms governing homing exists. Likewise, only an incomplete list of adhesion molecules implicated in directing the trafficking of stem cells to the marrow microenvironment is available. Opposing hypotheses that attribute homing to an orderly and orchestrated cascade of events or to random migration of circulating cells find ample experimental support. Also unsettled is the fate of marrow-homed cells shortly after transplantation and the rapidity at which they begin to proliferate in their new marrow microenvironment. The limited number of studies in this field and disparities in their experimental design intensifies the confusion surrounding these critical aspects of stem cell biology. However, this area of research is moving forward rapidly and results capable of clarifying many of these issues are forthcoming.

Keywords: homing; trafficking; engraftment; cell cycle kinetics

Introduction

Following transplantation, hematopoietic stem cells (HSC) engraft and reconstitute normal bone marrow (BM) functions only if they home to and lodge in the specialized niches of the BM microenvironment. In many aspects, homing of transplanted HSC into the BM spaces mimics the natural movement of stem cells from one hematopoietic organ to another during ontogeny. It is believed that an intricate process involving interactions between adhesion molecules (AM) and their counter-receptors expressed on HSC and endothelium of different microenvironments directs cells during ontogeny from the yolk sac and/or para-aortic splanchnopleura, to the fetal liver, and finally to fetal BM^{1–4} where hematopoiesis is maintained throughout adult life. Whether homing is being considered in pre- or post-natal life, movement of HSC from one anatomical site to another requires trafficking and migration of these cells through the blood stream. In a sequence of events perhaps involving a reversal of the steps required for stem cell homing, these cells can be mobilized from the BM into the peripheral blood following administration of growth factors and/or chemotherapeutic agents. Despite the central role these phenomena play in stem cell biology and cancer therapy, processes governing trafficking, migration and homing of HSC to the BM, and their egress from the marrow into the periphery, both at the molecular and cellular levels, remain poorly understood. Equally ambiguous at present, is the immediate fate of stem cells arriving in the BM shortly after transplantation. In this review, we will debate our current

knowledge of homing of HSC to the BM and discuss the findings of the relatively few reports describing functional events of transplanted HSC shortly post-transplantation.

Homing as a specific process

That homing is a specific, multi-step process was first described in leukocytes.^{5,6} The classical lymphocyte re-circulation and leukocyte emigration paradigm describes an initial selectin-mediated interaction that promotes rolling of leukocytes along the endothelium, followed by $\beta 1$ or $\beta 2$ integrin-mediated firm adhesion to endothelial cells.⁷ Following extravasation, leukocytes attach to extracellular matrix molecules (ECM) in the underlying tissue and may anchor themselves to specific cellular elements. Homing of hematopoietic progenitor cells (HPC) may follow the same parameters described for lymphocytes and may involve many of the AM implicated in the homing and trafficking of lymphocytes.⁸ Many of these classes of AM play a role in anchoring of HPC within the marrow and in promoting differentiation.^{9,10} Direct involvement of particular AM in both homing and egress of HSC to and from the BM has been elucidated.^{11–16} Recently, Zanjani *et al*¹⁷ demonstrated that VLA-4 played a central role in homing and engraftment of transplanted human cells to the BM of sheep fetuses and that migration of transplanted cells to fetal liver in lieu of the BM could be positively modulated with anti- $\beta 1$ integrin antibody. Attraction of hematopoietic cells to the BM via specific chemoattractants, especially stromal cell-derived factor-1 (SDF-1) has also been implicated in selectively directing homing of primitive HPC to the BM.^{18,19} *In vitro* responsiveness to SDF-1 correlated with marrow engraftment potential presumably due to the specific ligand-receptor interaction required for the enhanced homing of these cells to the BM.²⁰ Furthermore, engraftment of human cells in NOD/SCID mice was prevented by treatment with antibodies to CXCR4.²⁰ On the other hand, Rosu-Myles *et al*²¹ demonstrated that CXCR4 expression on human hematopoietic cells was not required for effective stem cell repopulating function.

Altogether, these data suggest that homing of HSC to the BM microenvironment following transplantation is a directed and specific phenomenon leading to the effective trafficking of these cells to the BM and eventual lodgment in specialized niches of hematopoiesis. In fact, directed trafficking to the BM of certain phenotypes of hematopoietic progenitor cells (HPC), but not others, has been documented²² and enhanced engraftment potential of candidate HSC expressing a particular repertoire of AM has been demonstrated.^{23,24} In addition, studies examining the functional repertoire of cells homing to the BM or spleen shortly after transplantation, revealed that, although both BM- and spleen-homed cells possessed long-term repopulating potential in secondary recipi-

Correspondence: EF Srour, Indiana University School of Medicine, 1044 West Walnut Street, R4–202, Indianapolis, IN 46202–5121, USA; Fax: 317–274–0396

Received 23 April 2001; accepted 19 June 2001

ents, kinetics of engraftment were quicker in recipients of cells that had been selected by a prior homing step to the spleen.²⁵ However, when homing of cells enriched for long-term engrafting potential was compared to that of populations of cells composed predominantly of non-engrafting cells,²⁴ a larger fraction of the former group of cells was recovered from the BM while non-engrafting cells were primarily found in the spleen (CM Orschell-Traycoff, manuscript in preparation). Collectively, these data can be interpreted to imply that either through directed and specific trafficking, or through selective lodgment in the BM microenvironment, primitive HPC arrive in and colonize the BM shortly after transplantation.

Homing as a random process

In discord with these studies are those which proclaim that homing is not specific and that trafficking of transplanted hematopoietic cells to the BM is random and incidental.^{26,27} These studies maintain that homing is a non-specific process resulting in the distribution of transplanted HSC in the host on the basis of organ mass and the complexity of the capillary bed of each organ.²⁶ As a result, distribution of infused cells shortly after transplantation results in non-specific seeding of cells in various tissues rather than a specific and directed trafficking of cells to hematopoietic sites.²⁷ In fact, several earlier studies^{28–30} noted the generalized distribution of transplanted cells in different organs of the recipient, challenging therefore the notion of definitive migration of transplanted cells to hematopoietic tissues. When the pattern of migration of prenatally transplanted hematopoietic cells was examined in a murine *in utero* transplantation model, Shaaban *et al*³¹ also concluded that homing to the fetal liver is non-selective. Given the developmental stage of recipient fetuses, this study was fundamentally different from other homing studies in that it combined the use of non-irradiated recipients and the examination of homing to the liver in the absence of other competing hematopoietic environments. It is important to stress at this point that none of these studies was designed to assess selective retention and/or lodgment of these cells in the marrow or to examine preferential survival and proliferation of specific groups of cells within the BM microenvironment.

We recently examined the issue of homing of both human and murine hematopoietic cells in appropriate hosts and investigated functional events early after arrival of these cells in the BM or spleen of recipient animals. In the first series, we examined and compared homing of fetal and adult hematopoietic cells to an adult hematopoietic microenvironment conditioned by lethal irradiation. Unfractionated cells from 12 to 18 days post-coitus murine fetal livers homed poorly to irradiated BM of adult hosts over a 4-h time period compared to adult murine bone marrow cells (FM Wolber, manuscript in preparation). Whereas between 2 and 4% of transplanted fetal liver cells from different gestational ages were detected in the BM of transplanted adult mice, in excess of 15% of transplanted adult BM cells homed to the marrow 4 h post-transplant. Similar disparities were observed for fetal liver vs adult bone marrow cells homing to the spleen of irradiated hosts. Interestingly, there were no significant differences in the proportions of days 12, 14, 16, or 18 fetal liver cells that homed to irradiated adult bone marrow (FM Wolber, manuscript submitted), arguing against the hypothesis that a particular phenotype, acquired during ontogeny, favored homing of hematopoietic cells from the liver to the marrow.

Transplantation of human BM- and mobilized peripheral

blood (MPB)-derived CD34⁺ cells or fractions of CD34⁺ cells into conditioned NOD/SCID mice revealed that infused cells colonize the BM and spleen 40 h post-transplant in a non-specific manner.³² To examine whether any preferential homing of CD34⁺ cells to the BM can be documented, grafts containing between 40% and 60% CD34⁺ cells were transplanted and cells arriving in the BM 40 h later were immunophenotyped for the expression of CD34. These analyses indicated that the phenotypic profile of cells recovered from the BM reflected the original composition of the transplanted graft, suggesting that CD34⁺ cells contained in the graft did not selectively home to the marrow microenvironment. We also compared the homing pattern of two fractions of MPB CD34⁺ cells isolated based on their position in cell cycle. We previously demonstrated that BM and MPB CD34⁺ cells in G₀ (G₀CD34⁺ cells) and cells in G₁ (G₁CD34⁺ cells) differ drastically in their capacity to support human chimeric hematopoiesis in the BM of conditioned NOD/SCID recipients.³³ G₀CD34⁺ and G₁CD34⁺ cells isolated from human MPB homed with equal efficiencies to the BM of recipient animals demonstrating that engrafting cells (G₀CD34⁺ cells) did not selectively home to the BM in numbers significantly higher than those observed for non-engrafting cells (G₁CD34⁺ cells).³² These and similar studies outlined above, present a substantial volume of work arguing against the existence of selective homing mechanisms that govern trafficking of transplanted cells to hematopoietic sites. However, these studies do not rule out the presence of selective and discriminating interactions between cells trafficking to the BM and elements of the marrow microenvironment that are essential for the retention and lodgment of homed cells in these sites.

Fate of transplanted hematopoietic cells

Upon trafficking to and lodgment in the BM microenvironment of irradiated recipients, transplanted HSC are believed to undergo massive cell proliferation resulting in the establishment and maintenance of a new hematopoietic system. When the number of circulating hematopoietic cells required to reverse the level of cytopenia resulting from myeloablation is considered, immediate and substantial proliferation of HSC arriving in the BM is a reasonable prediction. Such a fate of transplanted HSC has been supported by several studies. Transplantation of murine HSC in non-ablated hosts documented that as early as 12 h post-transplant, in excess of 50% of long-term engrafting cells enter active phases of cell cycle.³⁴ Studies with the fluorescent marker PKH-26 led Hendriks and coworkers³⁵ to conclude that none of the transplanted cells failed to proliferate shortly after transplantation. Cui *et al*²⁷ examining cell cycle status of transplanted cells in irradiated and non-irradiated recipients, observed rapid proliferation of cells in irradiated mice but not in non-ablated hosts. These observations are consistent with the anticipated immediate proliferation of transplanted cells that may be required to establish hematopoiesis in the host.

On the other hand, a substantial number of studies have demonstrated that transplanted cells trafficking to the BM remain dormant for a considerable duration and do not exhibit signs of proliferation until several hours post-transplant. Lanzkron *et al*³⁶ could not detect any proliferation of transplanted purified progenitor cells homing to the BM 48 h post-transplant beyond that observed at time 0. Similarly, Oostendorp *et al*³⁷ reported that in excess of 90% of cells detected in the BM 1 day post-transplant had remained dormant

although cells trafficking to other tissues proliferated more extensively. Even in embryonic environments supporting rapid proliferation of hematopoietic cells such as fetal liver, prenatally transplanted cells did not begin cycling until 12 to 24 h post-transplant.³¹ Again, some of the observations of Cui *et al*²⁷ partially support the absence of immediate proliferation of transplanted cells in nonmyeloablated hosts.

We also examined in both xenogeneic and syngeneic transplantation models the immediate fate of transplanted hematopoietic cells recovered from the BM of recipient mice a few hours post-transplant. When total human BM or MPB CD34⁺ cells were transplanted into conditioned NOD/SCID mice, cells recovered from the BM up to 40 h post-transplant were predominantly in G₀/G₁ phases of cell cycle. Assuming that the xenogeneic marrow microenvironment of NOD/SCID mice is not conducive for the proliferation of human cells, recipient animals were injected with a cocktail of recombinant human cytokines before, during and after transplantation of human CD34⁺ cells. However, no significant enhancement of proliferation of cells trafficking to the BM of treated mice was observed suggesting that an active proliferation inhibition mechanism in the marrow microenvironment precludes transplanted cells from entering active phases of cell cycle immediately after transplantation. We also demonstrated that cells isolated in S/G₂+M phases of cell cycle (isolated by cell sorting using Hoechst 33342 staining) continue their progression through the cell cycle upon seeding the BM of recipient NOD/SCID mice only to be arrested again in G₁. Interestingly, transplanted G₀CD34⁺ and G₁CD34⁺ cells behaved similarly in recipient animals in that both phenotypes remained dormant up to 40 h post-transplant demonstrating that proliferation of cells seeding the marrow after transplantation is specifically inhibited regardless of their hematopoietic potential.

From the standpoint of what is required from HSC after their arrival in the BM, observations documenting lack of cellular proliferation immediately post-transplant may be considered counterintuitive. However, when one considers the time required before hematopoietic cells commence cell division *in vitro* in response to supraphysiologic doses of exogenous cytokines, an *in vivo* delay of up to 40 h before cells begin to proliferate may not be very surprising. We questioned the biologic significance of proliferation inhibition in the context of outcomes of stem cell transplantation. It was hypothesized that cycling cells might be more adversely affected by an unscheduled cell cycle arrest than quiescent cells. Transplanted cells in G₀ and early G₁ may remain quiescent in the BM microenvironment until appropriate signals and conditions are conducive for their proliferation while interruption of cell cycle progression of cycling cells may lead to apoptosis. We transplanted conditioned NOD/SCID mice with cells in G₀/G₁ or S/G₂+M isolated by Hoechst 33342 staining and flow cytometric cell sorting and at 40 h post-transplant, cells seeding the BM were recovered and analyzed for the expression of annexin V.³² The number of S/G₂+M cells expressing annexin V was more than double that detected among cells in G₀/G₁. Interestingly, the percentage of S/G₂+M cells expressing annexin V exceeded one-third of all cells recovered from the marrow demonstrating that cell cycle arrest of these cells in BM of recipient mice was detrimental to their long-term survival.³²

It may therefore be possible that proliferation inhibition in the BM of recipient animals may serve as a selection mechanism for the type of cells suited for lodgment and colonization of the marrow and initiation of hematopoiesis. In

response to an unscheduled arrest of their cell cycle progression, transplanted cycling cells begin to undergo apoptosis and are as such eliminated from the pool of surviving cells seeding the marrow. On the other hand, their mitotically quiescent counterparts can remain dormant in the marrow without adverse effects on their survival. This hypothesis may explain why predominantly cycling cells generated in *ex vivo* expansion cultures or during *in vitro* manipulation of cells for the purpose of gene transfer fail to efficiently engraft conditioned animals and may account for why we previously observed that MPB CD34⁺ cells traversing in short-term culture from G₀ into G₁ fail to engraft NOD/SCID recipients.³³

Conclusions

This brief overview of the limited volume of information we currently have regarding homing of transplanted HSC and early functional events following transplantation of these cells leaves us with a quandary of conflicting observations. On the issue of whether homing of HSC to the BM is specific or random, sufficient convincing data on both sides of the argument can be found. However, it should be noted that almost all of the existing data do not distinguish between directed specific trafficking of cells to the BM and preferential lodgment and survival of cells in the marrow microenvironment. Similarly, whether cells begin to proliferate immediately after trafficking to the marrow or remain dormant for a considerable period of time remains a source of contention. The number of studies examining this issue is rather limited. Further confusion arises from the dissimilarities in the transplantation models used by different investigators making it rather difficult to deduce any meaningful conclusions. It remains to be emphasized, however, that many of these studies, whether addressing the specificity of homing or the behavior of cells shortly after transplantation challenge well-established dogmas in this field. That homing is a specific well-orchestrated phenomenon has long been considered the doctrine governing trafficking of transplanted cells to the BM. Yet, many recent studies suggest that homing is neither selective nor specific. Similarly, although HSC have always been expected to proliferate rapidly after arriving in the BM, new information suggests that these cells remain dormant for a number of hours following their seeding of the BM. It is evident that more detailed investigations are required to better understand these important facets of stem cell biology and transplantation.

Acknowledgements

This work was supported by National Institutes of Health grants RO1 HL55716 and RO1 HL62200 to EFS and NIDDK Center for Excellence in Molecular Hematology grant P50 DK49218.

References

- 1 Sanchez M-J, Holmes A, Miles C, Dzierzak E. Characterization of the first definitive hematopoietic stem cells in the AGM and liver of the mouse embryo. *Immunity* 1996; 5: 513–525.
- 2 Yoder M, Hiatt K, Dutt P, Mukherjee P, Bodine D, Orlic D. Characterization of definitive lymphohematopoietic stem cells in the day 9 murine yolk sac. *Immunity* 1997; 7: 335–344.
- 3 Delassus S, Cumano A. Circulation of hematopoietic progenitors in the mouse embryo. *Immunity* 1996; 4: 97–106.

- 4 Tavassoli M. Embryonic and fetal hemopoiesis: an overview. *Blood Cells* 1991; **1**: 269–281.
- 5 Springer T. Adhesion receptors of the immune system. *Nature (Lond.)* 1990; **346**: 425–434.
- 6 Springer T. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994; **76**: 301–314.
- 7 Kansas GS. Selectins and their ligands: current concepts and controversies. *Blood* 1996; **88**: 3259–3287.
- 8 Simmons PJ, Zannettino A, Gronthos S, Leavesley D. Potential adhesion mechanisms for localisation of haemopoietic progenitors to bone marrow stroma. *Leuk Lymphoma* 1994; **12**: 353–363.
- 9 Long M. Blood cell cytoadhesion molecules. *Exp Hematol* 1992; **20**: 288–301.
- 10 Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994; **84**: 2068–2101.
- 11 Papayannopoulou T, Nakamoto B. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci USA* 1993; **90**: 9374–9378.
- 12 Hardy CL. The homing of hematopoietic stem cells to the bone marrow. *Am J Med Sci* 1995; **309**: 260–266.
- 13 Dercksen MW, Gerritsen WR, Rodenhuis S, Dirkson MK, Slaper-Cortenbach IC, Schaasberg WP, Pinedo HM, von dem Borne AE, van der Schoot CE. Expression of adhesion molecules on CD34+ cells: CD34+ L-selectin+ cells predict a rapid platelet recovery after peripheral blood stem cell transplantation. *Blood* 1995; **85**: 3313–3319.
- 14 Jacobsen K, Kravitz J, Kincade PW, Osmond DG. Adhesion receptors on bone marrow stromal cells: *in vivo* expression of vascular cell adhesion molecule-1 by reticular cells and sinusoidal endothelium in normal and gamma-irradiated mice. *Blood* 1996; **87**: 73–82.
- 15 Papayannopoulou T, Craddock C. Homing and trafficking of hematopoietic progenitor cells. *Acta Haematol* 1997; **97**: 97–104.
- 16 Prosper F, Stroncek D, McCarthy JB, Verfaillie CM. Mobilization and homing of peripheral blood progenitors is related to reversible downregulation of alpha4-beta1 integrin expression and function. *J Clin Invest* 1998; **101**: 2456–2467.
- 17 Zanjani ED, Flake AW, Almeida-Porada G, Tran N, Papayannopoulou T. Homing of human cells in the fetal sheep model: modulation by antibodies activating or inhibiting very late activation antigen-4-dependent function. *Blood* 1999; **94**: 2515–2522.
- 18 Aiuti A, Webb IJ, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Exp Med* 1997; **185**: 111–120.
- 19 Imai K, Kobayashi M, Wang JX, Shinobu N, Yoshida H, Hamada J, Shindo M, Higashino F, Tanaka J, Asaka M, Hosokawa M. Selective secretion of chemoattractants for haemopoietic progenitor cells by bone marrow endothelial cells: a possible role in homing of haemopoietic progenitor cells to bone marrow. *Br J Haematol* 1999; **106**: 905–911.
- 20 Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 1999; **283**: 845–848.
- 21 Rosu-Myles M, Gallacher L, Murdoch B, Hess DA, Keeney M, Kelvin D, Dale L, Ferguson SSG, Wu D, Fellows F, Bhatia M. The human hematopoietic stem cell compartment is heterogeneous for CXCR4 expression. *Proc Natl Acad Sci USA* 2000; **97**: 14626–14631.
- 22 Kollet O, Peled A, Lapidot T. Exclusive homing of human CD38–/low CXCR4+ stem cells to the spleen and bone marrow of immune deficient mice within 1–16 hrs. *Blood* 1999; **94**: 390a (Abstr.).
- 23 van der Loo JCM, Xiao XL, McMillin D, Hashino K, Kato I, Williams DA. VLA-5 is expressed by mouse and human long-term repopulating hematopoietic cells and mediates adhesion to extracellular matrix protein fibronectin. *J Clin Invest* 1998; **102**: 1051–1061.
- 24 Orschell-Traycoff CM, Hiatt K, Dagher RN, Rice S, Yoder M, Srour EF. Homing and engraftment kinetics of Sca-1+lin– cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood* 2000; **96**: 1380–1387.
- 25 Szilvassy SJ, Bass MJ, Van Zant G, Grimes B. Organ-selective homing defines engraftment kinetics of murine hematopoietic stem cells and is compromised by *ex vivo* expansion. *Blood* 1999; **93**: 1557–1566.
- 26 van Hennik PB, de Koning AE, Ploemacher RE. Seeding efficiency of primitive human hematopoietic cells in nonobese diabetic/severe combined immune deficiency mice: Implications for stem cell frequency assessment. *Blood* 1999; **94**: 3055–3061.
- 27 Cui JS, Wahl RL, Shen TL, Fisher SJ, Recker E, Ginsburg D, Long MW. Bone marrow cell trafficking following intravenous administration. *Br J Haematol* 1999; **107**: 895–902.
- 28 Lahiri SK, Van Putten J. Distribution and multiplication of colony forming units from the bone marrow and spleen after injection of irradiated mice. *Cell Tissue Kinet* 1969; **2**: 21–24.
- 29 Kretschmar AL, Conover WR. Colony-forming cells in the spleen: determination of the fraction transplanted. *Transplantation* 1969; **8**: 576–581.
- 30 Vos O, Buurman WA, Ploemacher RE. Mobilization of haemopoietic stem cell (CFU) into the peripheral blood of the mouse: effects of endotoxin and other compounds. *Cell Tissue Kinet* 1972; **5**: 467–479.
- 31 Shaaban AF, Kim HB, Milner R, Flake AW. A kinetic model for the homing and migration of prenatally transplanted marrow. *Blood* 1999; **94**: 3251–3257.
- 32 Jetmore A, Plett PA, Tong X, Wolber FM, Breese R, Abonour R, Orschell-Traycoff CM, Srour EF. Homing efficiency, cell cycle kinetics and survival of quiescent and cycling human Cd34+ cells transplanted into conditioned NOD/SCID recipients. *Blood* (submitted).
- 33 Gothot A, van der Loo JCM, Clapp DW, Srour EF. Cell cycle-related changes in repopulating capacity of human mobilized peripheral blood CD34+ cells in non-obese diabetic/severe combined immune-deficient mice. *Blood* 1998; **92**: 2641–2649.
- 34 Nilsson SK, Dooner MS, Quesenberry PJ. Synchronized cell-cycle induction of engrafting long-term repopulating stem cells. *Blood* 1997; **90**: 4646–4650.
- 35 Hendriks PJ, Martens ACM, Hagenbeek A, Keij JF, Visser JWM. Homing of fluorescently labeled murine hematopoietic stem cells. *Exp Hematol* 1996; **24**: 129–140.
- 36 Lanzkron SM, Collector MI, Sharkis SJ. Hematopoietic stem cell tracking *in vivo*: a comparison of short-term and long-term repopulating cells. *Blood* 1999; **93**: 1916–1921.
- 37 Oostendorp RAJ, Ghaffari S, Eaves CJ. Kinetics of *in vivo* homing and recruitment into cycle of hematopoietic cells are organ-specific but CD44-independent. *Bone Marrow Transplant* 2000; **26**: 559–566.