Isolation and therapeutic potential of human haemopoietic stem cells

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Abstract

The haemopoietic stem cell (HSC) has long been regarded as an archetypal, tissue specific, stem cell, capable of completely regenerating haemopoiesis after myeloablation. It has proved relatively easy to harvest HSC, from bone marrow or peripheral blood. In turn, isolation of these cells has allowed therapeutic stem cell transplantation protocols to be developed, that capitalise on their prodigious self renewal and proliferative capabilities. *Ex vivo* approaches have been described to isolate, genetically manipulate and expand pluripotent stem cell subsets. These techniques have been crucial to the development of gene therapy, and may allow adults to enjoy the potential advantages of cord blood transplantation. Recently, huge conceptual changes have occurred in stem cell biology. In particular, the dogma that, in adults, stem cells are exclusively tissue restricted has been questioned and there is great excitement surrounding the potential plasticity of these cells, with the profound implications that this has, for developing novel cellular therapies. Mesenchymal stem cells, multipotent adult progenitor cells and embryonic stem cells are potential sources of cells for transplantation purposes. These cells may be directed to produce HSC, *in vitro* and in the future may be used for therapeutic, or drug development, purposes.

Introduction

Over the last 30 years, an increased knowledge of HSC biology has allowed us to isolate enriched populations of HSC from many sources and assay their functional capabilities, using *in vitro* and *in vivo* systems. Selected relevant advances are discussed.

Haemopoietic stem cell biology

HSC responsible for definitive adult haemopoiesis are derived from the ventrolateral mesoderm (Zon, 1995) and are first seen in humans 4–5 weeks post fertilization (Godin and Cumano, 2002). During ontogeny HSC can be found in the yolk sac, para-aortic splanchnopleura, aortogonadomesonephros (AGM) area, fetal liver (FL), bone marrow (BM) and circulating in the fetal blood. Post-natally they are present in cord blood, BM and peripheral blood (PB). Using, chromosomally

(Abramson et al., 1977), or retrovirally, marked (Jordan and Lemischka, 1990; Keller and Snodgrass, 1990) HSC, it has been demonstrated that single, pluripotent, cells are capable of repopulating the entire haemopoietic system of a mouse, after myeloablative injury. However, very small numbers of pluripotent, primitive stem cells exist and, in humans, the majority of these cells are normally quiescent (Graham and Pragnell, 1992; Ploemacher, 1999). Therefore, a functional stem cell compartment is conceived to exist, in which, at any one time, only a fraction of these HSC are actively cycling. It is these cycling, pluripotent, HSC that, while still capable of self renewal and in dynamic equilibrium with the quiescent pool, give rise to progressively more lineage restricted daughter cells, with increasing proliferative activity (Graham and Wright, 1997). Figure 1 illustrates this haemopoietic stem cell hierarchy.

Figure 1. Human haemopoietic stem cell hierarchy. Figure 1 illustrates the haemopoietic stem cell hierarchy. Quiescent pluripotent haemopoietic stem cells are in dynamic equilibrium with cycling pluripotent stem cells that in turn give rise to progressively more lineage restricted daughter cells. On this figure some of the characteristics that are used to isolate subpopulations of stem cells are shown. In addition, a schematic representation of which cells within the hierarchy are measured by the most common *in vitro* assays is included.

Abbreviations: PHSC = Pluripotent haemopoietic stem cell, CLP = common lymphoid precursor, CMP = common myeloid precursor, B/macrophage = cell with potential to divide into B lymphocyte of macrophage, SRC/CRU = SCID repopulating cell/competitive repopulation unit, E-LTCIC = extended longterm culture initiating cell, LTCIC = longterm culture initiating cell, CFU = colony forming unit, HPP = high proliferative potential, GM = granulocyte/monocyte.

Quiescence has been shown to be to be maintained, at least in part, by p21^{cip1/waf1} (Cheng et al., 2000), although many cell intrinsic (Morrison et al., 1997; Roeder and Loeffler, 2002) and extrinsic (Cashman et al., 2002; Lataillade et al., 2002) processes are likely to be involved in regulating the quiescent state. There is evidence that many of these cells do not predominantly exist in G₀, but rather, are progressing slowly through G₁ (Bradford et al., 1997; Cheshier et al., 1999). The relative rate of primitive stem cell cycling varies in a species dependent manner. In mice, primitive HSC enter cycle in an asynchronous manner and 95% of cells cycle over a 3 month period. While in humans and other primates, it has been estimated that, under steady state conditions, HSC divide approximately once per year (Mahmud et al., 2001; Rufer et al., 1999).

Although a subset of HSC can self renew for the lifetime of an animal, these cells do not appear to possess unlimited self renewal potential as witnessed, in murine models, by their eventual exhaustion in serial transplantation studies (Schofield, 1978). In addition, older mice have an increased proportion of stem cells that are cycling, possibly compensating for an age related reduction in the active stem cell pool (Morrison et al., 1996b). In humans, the self renewal capacity of stem cells has been reported to be reduced with age, especially under stress conditions (Chatta and Dale, 1996; Effros and Globerson, 2002; Oscier, 1987). The concept that HSC age has generated a lot of interest especially with reference to a possible telomere driven mitotic clock. Telomeres are sequences of repetitive DNA which protect the body of the chromosome from the degenerative consequences of replication (Greider, 1994). With each cycle of replication the telomere length shortens. This process can proceed until a critical telomere length, termed the Hayflick limit (Hayflick, 1992; Shay and Wright, 2000), is reached. At this point the cell leaves cell cycle, whereby replicative senescence is said to have occurred. Telomerase extends the telomere, by adding extra copies of the repetitive DNA sequence, and thus protects rapidly and continuously dividing cells from cell death or senescence (Lee et al., 1998). In the haemopoietic system telomerase activity is associated with self-renewal potential, with pluripotent stem cells expressing high levels (Morrison et al., 1996a). However, despite telomerase expression, telomeres in adult BM HSC are shorter than in HSC from cord-blood (CB), that are in turn shorter than those from FL HSC (Chiu et al., 1996; Engelhardt et al., 1997; Vaziri

et al., 1994). On average telomeres shorten by 50–100 bp per year. The most marked reduction occurs in early childhood, where there is a dramatic 30 fold increase in the rate of telomere shortening, as a result of high stem cell turnover. This settles to steady state by the end of the first year (Rufer et al., 1999).

In vitro assays

In vitro assays have been developed to identify and quantify cells at different levels within the stem cell compartment. The assays can be considered in two classes, quantitative, clonogenic assays, and assays of cells that initiate haemopoietic proliferation and differentiation on preformed stromal cultures. In clonogenic assays appropriate combinations of growth factors have been shown to stimulate cells to produce clonal progeny in semi solid culture conditions usually agar or methylcellulose (Graham and Wright, 1997; Holyoake et al., 1993). The relative position of the clonogenic cells quantified by these assays within the haemopoietic hierarchy is shown in Figure 1.

Stromal cell based culture conditions have been used to assay more primitive cells in the stem/progenitor cell compartment (Dexter et al., 1977). Stromal 'feeder' cells can be isolated from BM or other sites of haemopoiesis such as the fetal AGM area (Oostendorp et al., 2002) or fetal liver (Hackney et al., 2002; Punzel et al., 1999a). The source of these stromal cells has a profound influence on the haemopoietic cells that develop in these cultures (Charbord et al., 2002; Loeuillet et al., 2001; Punzel et al., 1999b; Takeuchi et al., 2002). In human BM, long-term culture initiating cells (LTCIC) have been identified at a frequency of 6 in 10⁴ cells (Sutherland et al., 1989) and an extended culture period has allowed analysis of more primitive human stem cells (E-LTCIC) (Hao et al., 1996). The myeloid-lymphoid initiating cell (ML-IC) assay is a culture system, based on a FL feeder cell layer, supplemented with Flt 3 ligand, SCF and IL-7, that has allowed the enumeration of primitive precursors. Primitive cells, whose progeny also possess the capacity to reinitiate long term multilineage haemopoiesis, can be assessed (Punzel et al., 1999c).

Xenografting approaches to analysing human HSC

The development of methods to transplant human haemopoietic cells into immune deficient mice, or preimmune fetal sheep, form the basis for *in vivo* human stem cell assays. These allow quantification of

the most primitive HSC with longterm lymphomyeloid repopulating ability. Initially, severe combined immunodeficiency (SCID) strains of mice, that lack B and T lymphocyte function, or beige/nude/xid (BNX) mice, were used (Turner et al., 1996). The BNX mice carry three recessively inherited genetic mutations. The nude component leads to thymic aplasia, the beige deficiency to lysosomal storage defects that affect cytotoxic T cells and granulocytes and the X-linked deficiency causes abnormal lymphokine activated killer cells and B cell abnormalities (Dick et al., 1992). Despite some success (Dao et al., 1998) rejection of systemically transplanted cells proves to be a problem as a result of innate immunity. A major advance in xenografting has been the generation of non-obese diabetic variants of the SCID mouse (NOD-SCID mice) (Larochelle et al., 1996; Shultz et al., 1995; Ueda et al., 2000). In addition to the SCID dependent B and T cell abnormalities, NOD/SCID mice have diminished natural killer (NK) cell activity, defects in complement and impaired macrophage function because of the NOD phenotype. Variants include NOD/SCID/Rag1^{null} mice, bred to overcome the problems that can occur in some NOD/SCID mice where a minor degree of lymphoid activity develops in older animals (Shultz et al., 2000) and the NOD/SCID/ β 2 macroglobulin^{null} mouse, that lacks any appreciable NK cell activity. Engraftment efficiency of human cells is significantly superior in NOD/SCID/ β 2m^{null} animals, compared to standard NOD-SCID mice (Ishikawa et al., 2002; Kollet et al., 2000). The NOD/SCID/ γ c^{null} variant has also recently been described to have no appreciable NK activity and engraftment rates that exceed those of NOD/SCID/ β 2m^{null} mice (Ito et al., 2002). Defects in dendritic cell function as a result of reduced cytokine production, in addition to the B,T and NK deficiencies, have been proposed as functionally important in NOD/SCID/ γ c^{null} mice. Cells capable of reconstituting haemopoiesis in these mouse models are termed SCID repopulating cells (SRC) or competitive repopulating units (CRU). Using limiting dilution analysis SRC/CRU can be quantified (Dick, 1996; Holyoake et al., 1999b).

As well as providing the means to assess normal human HSC function *in vivo*, these mice have allowed the development of murine models for the study of human haemopoietic malignancies. For instance, murine models of acute myeloid leukaemia (AML) (Bonnet and Dick, 1997), acute lymphoblastic leukaemia (ALL) (Nijmeijer et al., 2001, 2002), Chronic

myeloid leukaemia (CML) (Wang et al., 1998) multiple myeloma (Pilarski and Belch, 2002; Pilarski et al., 2002) and chronic lymphocytic leukaemia (CLL) (Bai et al., 2000) have been established.

An alternative xenografting system is the use of preimmune fetal sheep as recipients of human HSC (Civin et al., 1996; Traycoff et al., 1994; Zanjani et al., 1992). This system has the advantage of not requiring myeloablated hosts or exogenous cytokines to be administered and allows longterm follow up in a large animal model. The disadvantages are related to technical expertise in the transplantation procedure and cost implications.

Haemopoietic stem cell phenotype

Flow cytometric immunophenotypic analysis can be used to characterise the cell surface phenotype of subsets of HSC. Cells can then be sorted by phenotype using fluorescence activated cell sorting (FACS) protocols, although it should be pointed out that even highly enriched HSC populations may still be heterogeneous (Lemischka, 2001).

Human primitive stem cells have been found to be present in the lin⁻, Thy (CD90)⁺, ckit (CD117)^{lo/+}, Rh^{lo} fraction (Baum et al., 1992). The role of CD34 in defining human pluripotent stem cells has been an area of controversy (Goodell, 1999). Based on single cell culture experiments it was proposed that the most primitive human HSC were a subset of the CD34⁺CD38⁻ fraction of BM that accounted for 0.05% of BM mononuclear cells (MNC) (Terstappen et al., 1991). It was shown that LTCIC and HPP-CFC were present in this population (Prosper et al., 1996) and xenogenic transplantation studies in fetal sheep and NOD-SCID mice showed that the CD34⁺CD38⁻ population contained long term engrafting cells (Bhatia et al., 1997a; Civin et al., 1996). Human therapeutic stem cell transplantation was, therefore, undertaken using CD34 as a marker of stem cell dose and even selecting CD34 positive grafts.

The persuasive argument that CD34 was a defining marker for primitive HSC was challenged when a subfraction of CD34⁻ cells was found to be capable of generating lymphomyeloid progeny, and to recapitulate longterm multilineage haemopoiesis in NOD-SCID mice and fetal sheep models (Bhatia et al., 1998; Osawa et al., 1996). These CD34⁻ cells were found in a double-negative subpopulation of cells called the side population (SP) when dual wavelength flow cytometric analysis of hoechst 33342 stained BM cells was performed (Goodell et al., 1997). The as-

say for SP cells relies on the ability of primitive cells to excrete toxins and the hoescht 33342 dye. This is achieved by means of molecular efflux pumps such as the Bcrp1/ABCG2, a member of the ATP binding cassette superfamily, termed ABC transporters (Bunting, 2002; Zhou et al., 2001). SP cells are present in multiple species and can be sorted by FACS (Goodell et al., 1997).

One concern arising from these findings was that the most primitive stem cells were being discarded and the possibility of late graft failure occurring in transplant patients was raised. However, in mice, it has subsequently been shown that CD34⁺ stem cells harvested from 5FU treated animals can, upon engraftment in irradiated recipients and once steady state haemopoiesis has been established, generate pluripotent CD34⁺ cells (Sato et al., 1999). In addition, analysis of murine developmental biology has shown changes in CD34 expression by murine stem cells over time during development. Specifically, fetal stem cells are CD34⁺ and it is not until 10 weeks of age that substantial stem cell activity is demonstrated in the CD34⁺ fraction of BM (Ito et al., 2000). One unifying hypothesis is that pluripotent stem cells transit between CD34⁺ and CD34⁺ phenotype and that CD34 positivity reflects activation status (Goodell, 1999; Sato et al., 1999). It has yet to be proved whether or not the same argument holds true in humans.

A number of other cell surface molecules have been used to isolate human lymphomyeloid repopulating cells, these include, CD133 (formerly AC133), CD164, c-kit and KDR (VEGF-R). The CD133⁺ cell population includes CD34⁺ and CD34⁺ cells and can successfully support serial engraftment in fetal sheep transplantation models (Bhatia, 2001; Gallacher et al., 2000). In addition to HSC, purified CD133⁺ cells, isolated from human peripheral blood stem cell collections, have been reported to contain a population of extremely primitive adherent cells. These cells become CD164 positive and give rise to multiple, non adherent, primitive CD34⁺ cells with lymphomyeloid SRC ability in long term culture (Handgretinger; published online November 2002). As such, these cells appear to be precursor cells to HSC, as they are conventionally defined and have similarities with stromal or mesenchymal cells. Cells capable of durable long term repopulation in preimmune fetal sheep (Kawashima et al., 1996) are enriched in the CD34⁺ c-kit^{low} fraction. In addition, SP cells that are CD34⁺ are c-kit⁺, again suggesting that the most primitive, or quiescent, stem cells are c-kit negative, but when cells

are actively cycling they express c-kit and are responsive to SCF. Cells capable of long-term haemopoietic repopulation of NOD-SCID mice and fetal sheep have been reported to be enriched in the CD34 + KDR + fraction (Peschle et al., 2001; Ziegler et al., 1999).

The full phenotypic profile of human haemopoietic stem cells remains to be absolutely defined. Figure 1 summarises the expression profile of some of the important molecules.

Isolation of haemopoietic stem cells

It has proved possible to isolate stem cells from BM, CB and PB. Although these collections contain only small percentages of HSC, unmanipulated grafts are able to regenerate the haemopoietic system of patients, myeloablated with chemoradiotherapy. It is possible to collect stem cells from fetal sources, but, while research is ongoing, HSC from these sites are not viable options in the clinic at present.

Large scale isolation of clinically relevant dose of HSC

Unmanipulated BM was the first stem cell source used for transplantation purposes and has now been used therapeutically for 25 yr. 1–3% of BM MNC are CD34⁺ and this fraction has been shown to be capable of lymphomyeloid repopulation (Berenson, 1988; Andrews, 1992). In practice, bone marrow harvesting under general anaesthesia is still a major way of isolating pluripotent stem cells in large enough quantities for clinical applications.

CD34⁺ cells are also found normally in the peripheral blood but are extremely rare accounting for 0.01–0.05% of PB MNC. However, it has been shown that in the recovery phase following myelosuppressive cytotoxic chemotherapy, or after the administration of cytokine/chemokine combinations, there is a transient 15–25 fold increase in circulating CD34 positive cells (Hattori et al., 2002; Hess et al., 2002; Korbling, 1997; Lane et al., 1999; Moore et al., 2001). This rise allows adequate numbers of CD34 positive cells, for transplantation purposes, to be collected, from the peripheral blood, using apheresis techniques. The ultimate CD34 cell dose harvested can be predicted based on of the pre-apheresis peripheral blood CD34⁺ cell count and a threshold in the range of 20–40 CD34 positive cells per microlitre is used as a trigger to commence apheresis (Alcorn et al., 2000).

The agent of choice to mobilise peripheral blood stem cells (PBSC), in healthy human donors, has been G-CSF, because it appears to be potent and non toxic (Molineux et al., 1990). The molecular basis underlying stem cell mobilisation has not been fully elucidated. In murine models, expression of the G-CSF receptor on HSC is not required for their mobilisation by G-CSF (Liu et al., 2000), but the expression of this receptor in the marrow microenvironment is essential for mobilisation via G-CSF, cytotoxic chemotherapy or IL-8 (Liu et al., 1997). In non human primates and mice, antibodies to VLA-4 or its receptor VCAM1 can mobilise stem cells into the PB (Papayannopoulou and Nakamoto, 1993) and it has been shown that VCAM1 can be cleaved by neutrophil proteases in the bone marrow, released by the effect of G-CSF on BM neutrophils. This has been proposed to be part of the mechanism by which G-CSF stimulates release of HSC into the blood (Levesque et al., 2001). However, this is not the whole answer as the initiation of VLA4 mediated mobilisation is enhanced when concomitant $\beta 2$ integrin receptor blockade is utilised (Papayannopoulou et al., 2001) and the process seems to depend on an intact SCF/c-kit pathway (Papayannopoulou et al., 1998).

SCF has therefore been added to mobilisation protocols. In mice the combination of SCF and G-CSF synergistically increased the number of short and long term repopulating HSC (Molineux et al., 1991; Yan et al., 1995). These murine HSC, mobilised with SCF plus G-CSF, supported serial transplantation whereas G-CSF mobilised stem cells showed limited secondary transplantation potential (Yan et al., 1995). In non-human primates it was shown that HSC could be mobilised using SCF plus G-CSF (Andrews et al., 1994) and these studies were, therefore, extended into phase I/II trials in humans. Good collections were obtained and rapid engraftment was seen in heavily pretreated patients (Facon et al., 1999; Glaspy et al., 1997; Moskowitz et al., 1997). In humans, the addition of SCF to G-CSF and cyclophosphamide for stem cell harvesting resulted in significantly more LTC-IC activity and $CD34^+$ cell numbers being collected (Weaver et al., 1996). Recently, the characteristics of human stem cell populations collected using SCF and G-CSF, compared to G-CSF alone, in healthy donors, have been further investigated (Hess et al., 2002). The authors found that twice as many mononuclear cells and five times as many $CD34$ positive cells were collected using SCF in addition to G-CSF. Interestingly, while the frequency of SCID repopulating cells (SRC)

was the same in both harvests (1 SRC per 8×10^6 MNC), subfractionation revealed that the $CD34$ positive fraction of SCF and G-CSF mobilised cells was a relatively poor source of SRC, in contrast to G-CSF mobilised collections, where this fraction contained the majority of SRC activity. The authors suggested, that the increase in the number of primitive $CD34^-$, $CD38^+$, Lin^- , $AC133^+$ cells, collected by combining SCF and G-CSF, compensated for the reduction in SRC seen in the $CD34^+$ fraction of these collections.

Purification of stem cell subsets

It has become possible to isolate pure populations of cells for clinical and experimental purposes from large scale unmanipulated stem cell collections. This has involved using either a unique phenotypic marker or a functional property such as dye efflux or RNA expression. The two main techniques use either monoclonal antibodies complexed to magnetic beads or FACS (Table 1). The main drawback with FACS scanning was the speed of sorting. At 4000 cells per second this technology could not be used for clinical purposes. However, new technology allows upwards of 70 000 cells per second to be sorted, making this a viable, though expensive and not readily available, clinical option. Two representative examples, $CD34^+$ selection and isolation of quiescent stem cells are discussed.

Purification of $CD34^+$ stem cells

The debate regarding $CD34^-$ cells notwithstanding, evidence has been presented that suggests that functionally the $CD34^+CD38^-CD90^+$ (Thy1⁺) subset of human BM cells contains large numbers of primitive haemopoietic precursors (Baum et al., 1992; Craig et al., 1993; Murray et al., 1995). Furthermore, cell cycle analysis has shown that the replicative stress following bone marrow transplantation (BMT) is borne by cells within this subset (Thornley et al., 2001). Follow up studies have shown that $CD34^+$ cell dose predicts time to engraftment, survival and post transplant morbidity following allogeneic stem cell transplantation (Mavroudis et al., 1996; Pecora et al., 1998; Powles et al., 2000), while only the dose of $CD34^+Thy-1^+$ cells transplanted correlates with durable engraftment (Pratt et al., 2001).

There are several other theoretical and practical advantages of purifying $CD34^+$ cells from more mature progeny in unmanipulated stem cell collections. Firstly, in the autologous transplant setting, for dis-

Table 1. Summary of haemopoietic stem cell selection technologies

Brand	Source	Technology	Methodology	Comment
Positive selection				
Isolex 300i	Baxter Healthcare	Anti-CD34 antibody. Magnetic microspheres	Anti-CD34 labelled cells, rosetted with magnetic microspheres (3–4 μ M), are retained in a magnetic field. Cells released from microapheres by PR34 peptide	Automated; 50% recovery of input CD34+ cells
CliniMACS	Miltenyi Biotech	Anti-CD34 antibody. Paramagnetic microbeads	Anti-CD34 labelled cells indirectly conjugates to iron-dextran microbeads (50–100 nM); labelled cells released on switching off the magnetic field	High purity of CD34-enriched product biodegradable microbeads not removed, no apparent detriment to sell function; 50% recovery of input CD34+ cells
		Anti-AC133 antibody	Antibody to CD133 expressed on early subset of CD34+ progenitors; cells selected as with CD34 kit (above)	
Fluorescence activated cell sorting				
FACS Vantage	Becton Dickinson		Viable, CD34+ cells sorted automatically from CD34– cells based on fluoreescence of bound anti-CD34 antibody	High yields and purities; but not easily scaled up for clinical transplantation (4000 cells/second)
MoFlo	DakoCytomation	As above	Sort rate of 70 000 cells/second; >99% purities	
Negative selection				
StemSep	Stem Cell Technologies	Bispecific (lineage markers and dextran) tetrameric antibodies	Lineage committed cells labelled with a cocktail of tetrameric complexes and incubated with colloidal dextran-iron. Lineage negative cells collected in column effluent.	High purity of CD34-enriched product

eases such as breast cancer, multiple myeloma, lymphoma and neuroblastoma, where the malignant cell type is not CD34⁺, enrichment can form the basis of a tumour cell purging strategy. Secondly, relative T-cell depletion through CD34 selection may be advantageous in reducing the risk of graft-versus-host disease (GvHD) in allogeneic transplants. Thirdly, stem cell enrichment is the starting point for stem cell expansion protocols and finally, CD34⁺ enrichment strategies reduce the volume of cells and DMSO to be stored, which is especially important in paediatric practice.

Isolation of quiescent stem cells

A FACS based technique has been used to isolate quiescent stem cells for further study. It is based on the

staining of both DNA and RNA content of cells using Hoechst 33342 and Pyronin Y, respectively (Holyoake et al., 1999a). Quiescent cells have diploid DNA with restricted RNA transcription, which enables gating by flow cytometry on the viable (PI[−]) CD34⁺ Hoechst^{lo} Pyronin^{lo} population (Figure 2a). Post stem cell transplantation, probably as a result of the survival of a small population of quiescent malignant stem cells, relapse remains a significant risk. Clearly quiescent malignant stem cells would evade the lethal effects of cell cycle dependent therapies and would not be destroyed by pre-transplant conditioning. In CML, deeply quiescent leukaemic (Ph⁺) stem cells have been proven to exist in all patient samples studied in the stable chronic phase of the disease (Graham et

al., 2002; Holyoake et al., 1999a, 2001). Moreover, these cells are resistant to the tyrosine kinase inhibitor imatinib mesylate (formerly STI571), indeed the drug itself causes a relative accumulation of viable quiescent Ph^+ CD34^+ cells *in vitro* (Graham et al., 2002). The latter research was made possible by developing FACS technology using the vital fluorescent dye CFSE (carboxyfluorescein succinimidyl ester) to track cell divisions. Quiescent cells retain maximal fluorescence as intensity halves with each division that forms two daughter cells (Figure 2b). The ability to isolate quiescent leukaemic stem cells will facilitate research into methods to modulate their quiescence to render them sensitive to therapies such as imatinib and ultimately achieve the goal of a non-transplant cure for diseases such as CML (Jørgensen et al., 2002a–c).

Therapeutic uses of haemopoietic stem cells

Established therapies

Stem cell transplantation

Established therapeutic procedures use either BM, PBSC or cord blood, as the source of stem cells. Transplantation of HSC from other sources, such as, fetal liver, mesenchymal stem cells (MSC), embryonic stem (ES) cells or pluripotent adult type stem cells are at present experimental.

HSC transplantation, after myeloablative chemoradiotherapy, in the autologous or allogeneic setting, has become an established therapeutic option in the management of haematological malignancies, breast cancer, thalassaemia, sickle cell disease, and aplastic anaemia (Tabbara et al., 2002; Vassiliou et al., 2001). Autoimmune disease is a rapidly expanding indication for autologous stem cell transplantation and this has recently been extensively reviewed in the context of inducing tolerance in these diseases (Burt et al., 2002; Gratwohl et al., 2001). Allogeneic donors are usually HLA matched siblings, or volunteer unrelated donors, although innovative strategies to allow transplantation across HLA barriers are being increasingly investigated (Aversa et al., 2001; Henslee-Downey, 2001).

For allogeneic stem cell transplantation, the choice of stem cell source, either BM or PBSC, has important implications, as many post-transplantation outcome parameters, such as overall survival in disease subsets and chronic GvHD, may be influenced by the source of HSC used for transplantation. The extent to

which this occurs has been the subject of much debate over the last few years (Korbling and Anderlini, 2001; Verfaillie et al., 2000).

Traditional conditioning regimes for allogeneic transplantation are extremely toxic and have precluded the use of this treatment modality in older patients or those with poor performance status. Novel transplant protocols have, therefore, been devised and are now established in clinical practice, that reduce the toxicity of the initial conditioning therapy, so called non-myeloablative transplantation. These protocols are primarily immunomodulatory and allow donor chimerism to be obtained via a period of tolerance induction and mixed chimerism (Chakraverty et al., 2002; Childs et al., 1999; Maris et al., 2001; Slavin et al., 1998; Storb et al., 1999). These approaches rely on immunological graft versus disease effects as the mainstay of therapy and are discussed in more detail in the article by Childs in this journal.

In a murine model, taking this approach to the extreme, Hayashi et al. (2002) have performed *in utero* haemopoietic stem cell transplantation followed by post natal donor lymphocyte infusion (DLI). This approach capitalises on the pre-immune status of the fetus to generate tolerance and mixed chimerism, that is then converted to full donor chimerism post natally. Theoretically, in the future, this approach could be used to treat many diseases diagnosed *in utero* by facilitating cellular or organ transplantation. In humans, *in utero* transplantation has been performed in 40–50 cases (Flake and Zanjani, 1997, 1998, 1999; Hayward et al., 1998; Jones et al., 1996). However, in practice, in humans, competition between host and donor stem cells and the inherent capacity of the human fetus to reject allogeneic grafts, remain major stumbling blocks to this approach. However, there is optimism that the recent murine experiments, converting low level mixed chimerism achieved *in utero* to full donor chimerism, using DLI post-natally, may be translated into the human setting (Flake, 2001).

In the context of stem cell transplantation it is important to question the self renewal capacity of HSC. Following allogeneic stem cell transplantation there is rapid telomere shortening equivalent to about 15 yr of normal ageing (Wynn et al., 1998, 1999). Coupled with the observations that telomere shortening is a feature of bone marrow failure syndromes (Ball et al., 1998), that clonal stem cell disorders increase dramatically as a function of age (Goldstone et al., 2001), and that telomere length is often significantly shorter in patients with neoplastic conditions, particularly as their

Figure 2. (A) Isolation of quiescent (G₀) CD34⁺ stem cells. Cells that are out of cycle can be identified and sorted from their cycling counterparts by FACS by virtue of their low RNA content (Pyronin^{low} staining) and diploid DNA content. (B) Tracking of stem cell divisions using the vital fluorescent stain CFSE. Cells are labelled with the vital fluorescent stain, carboxy fluorescein succinimidyl ester (CFSE) and sorted by FACS using narrow channels to ensure homogeneity of staining. The cell cycle arresting agent, colcemid, is used to identify the position in the FACS histogram of the peak of maximum fluorescence. Thus, CFSE⁺ cells that remain undivided may be identified owing to their maximum fluorescence. These cells are indicated by arrow in Figure 2B. With each division the daughter cells fluoresce at half the intensity of the parent.

diseases progress (Brummendorf et al., 2000, 2001b), these findings have led to speculation that, post stem cell transplantation, stem cell disorders may be increased. In long term survivors, either bone marrow failure, due to stem cell exhaustion, or an increase in secondary malignancies, as a result of chromosomal instability, secondary to shortened telomeres might be expected (Engelhardt and Finke, 2001; Shay, 1998). Some studies do seem to support this concept. For instance, myelodysplasia has been observed to complicate up to 20% of autografts (Friedberg et al., 1999) and, *in vitro*, a decrease in CFU and LTCIC activity has been documented post transplant (Podesta et al., 1997, 1998). Theoretically, these effects would be most pronounced in older patients, with older donors, who experienced problems such as GVHD or infections post transplant, resulting in increased stem cell turnover.

However, this remains an area of controversy and there is significant evidence from animal models and human studies to suggest that HSC reserve actually exceeds the demands put upon it post transplantation. In murine serial transplantation experiments stem cell reserve, though finite, seems to significantly exceed the lifetime of individual animals (Harrison, 1979).

While, in humans, Mathioudakis et al. (2000) report that allogeneic BM graft recipients studied 20–30 yr post transplant show polyclonal haemopoiesis with only moderate telomere shortening in terms relative to that required to produce senescence. Rufer et al. (2001) further suggest that the accelerated telomere shortening post HSC transplantation is limited to the first year post transplantation and equates to a transplantation induced loss of only 20% of the telomere reserve (Brummendorf et al., 2001a).

Gene therapy

The aim of gene therapy, with reference to HSC, is to harvest the stem cells, genetically manipulate them *ex vivo* and return them to the body to correct the genetic abnormality. This is particularly likely to be achieved where only small amounts of a substance are required to make a significant impact on the severity of a disorder. For example, converting severe haemophilia (<1% normal factor) to moderate disease (1–5% normal factor). Alternatively, genetically manipulated stem cells are likely to be selected if the genetic manipulation confers a survival advantage on the transduced cells, as is the case in SCID patients. There has been intense interest in this field

and this has been extensively reviewed recently (Günzberg et al., 2002; Tisdale and Wilson, 2002; Woods et al., 2002). Modified viral and synthetic vectors, that either insert into the genome, or are maintained episomally, have been described. Problems that are encountered clinically include low transfection efficiencies and downregulation of gene expression over time. However, in 2000 a French group successfully corrected the deficiency in expression of the γ -c cytokine subunit, common to IL-2, -4, -7, -9 and -15, in ten children affected by this form of SCID, using an oncoretroviral vector (Cavazzana-Calvo et al., 2000). This was a major breakthrough, proving, as it did, that gene therapy could be clinically efficacious. Encouraging results have also been reported in patients with the ADA-deficient form of SCID (Aiuti et al., 2002) and, to a lesser degree, in haemophilia B (Kay et al., 2000; Monahan and White, 2002).

Unfortunately, longterm follow up of the children successfully treated for the γ -c cytokine subunit deficiency, has shown that one child has developed a leukaemic disease, probably as a result of insertional mutagenesis (Marshall, 2002a, b). What is not known is whether retroviral vectors have a predilection for insertional mutagenesis of key oncogenes, or if the insertion site was chosen by the virus entirely at random. A database of all known human DNA integration sites is being established to help answer this question. The problem had not been recognized despite extensive pre-clinical animal experimentation.

Experimental therapies

Ex vivo expansion as a precursor to transplantation

The potential benefits of *ex vivo* expansion of stem cells in the clinical setting are vast. For instance, the use of umbilical cord blood or fetal liver stem cells in transplantation protocols in adults has many theoretical benefits (Gluckman, 2001; Gluckman et al., 1997) but in practice these options are severely limited by the small number of stem cells obtained in CB/FL donations (Migliaccio et al., 2000; Rubinstein et al., 1998). Theoretically, expansion of cells capable of short-term engraftment after myeloablative therapy would increase speed of initial engraftment, with reduction in the time to neutrophil and platelet recovery, while expansion of the most primitive cells would ensure durability of engraftment and reduce the replicative stress on the repopulating cells. Furthermore, many

current gene transfer systems require stem cells to be cycling to incorporate the gene of interest, so driving stem cells to self renew, in this setting, would enhance transfection rates and efficiency of gene transfer.

In the human setting many combinations of growth factors have been used to expand pluripotent stem cells (Alcorn and Holyoake, 1996; Holyoake, 1996; Holyoake et al., 1997; Holyoake et al., 1996; Petzer et al., 1996; Piacibello et al., 1997) with (Breems et al., 1998; Moore et al., 1997) or without (Bhatia et al., 1997b) stromal cells. This has been the subject of a recent review (Heike and Nishikawa, 2002). Recently *in vitro* maintenance and expansion of human haemopoietic repopulating cells in a serum and stromal free medium has been described (Almeida-Porada et al., 2000; Bhardwaj et al., 2001; Bhatia et al., 1997b, 1999). In this context bone morphogenetic proteins (BMPs) (Bhatia et al., 1999), Hedgehog proteins (Hhs) (Bhardwaj et al., 2001), Wnts (Van den Berg et al., 1998), TGF β , fibroblast growth factors (Le Bousse-Kerdiles et al., 1996), Notch and Notch ligands (Kojima and Griffin, 2001; Milner and Bigas, 1999) have all been shown to have direct and indirect effects, via stromal cells, on pluripotent HSC self renewal potential and may become incorporated into future *ex vivo* expansion protocols.

In all *ex vivo* protocols described, significant numbers of primitive stem cells are lost to differentiation and no studies have been published that show a clinically significant expansion in the most primitive HSC. In addition, studies in mice and non human primates have revealed that, as cells enter cell cycle, as a consequence of *ex vivo* expansion the cells exhibit a transient reduction in their ability to home to the bone marrow and also acquire other defects limiting short and long term reconstitutive properties (Szilvassy et al., 2000).

Novel therapeutic strategies dependent on stem cell plasticity

There are two populations of multipotent stem cells that can be isolated from the bone marrow, a) HSCs and b) MSC. It has been suggested that both may arise from a common multipotent adult progenitor cell (Jiang et al., 2002; Hall et al., 2001). The explosion of experimental interest and the vast therapeutic potential of these stem cells, are both critically dependent on their 'plasticity'.

Plasticity within the haemopoietic system has been shown in experiments demonstrating that cells ap-

parently committed to one particular fate can be re-programmed to differentiate along a separate lineage pathway by modulation of specific transcription factors. As shown in Figure 3, this process may be achieved by either trans-differentiation, that is the direct re-programming of one cell type to another; or by de-differentiation and subsequent re-differentiation along the alternative pathway. The elegant studies of the Enver group upon which this figure is based, have demonstrated that murine GM-CFU isolated from BM have no intrinsic erythroid potential. However, when GATA1-ER fusion protein was activated by the addition of tamoxifen, 85% of these cells were re-programmed and formed colonies containing erythrocytes, basophils and eosinophils. These experiments failed to show any de-differentiation on transient activation of GATA1 and thus it was concluded, that the transition from G/M to erythroid lineages, mediated by GATA1, was most likely to be due to direct trans-differentiation (Heyworth et al., 2002). Other experiments have demonstrated that the balance between mutually antagonistic transcription factors can induce and maintain differentiation and be manipulated to re-program other haemopoietic lineages; these findings have recently been reviewed in detail (Orkin and Zon, 2002).

Recent research has not only suggested that there is potential plasticity in haemopoietic lineage choice, within the haemopoietic system, but has suggested that HSC exhibit multi-lineage stem cell plasticity, as such HSC have the theoretical potential to be used as regenerative cellular therapy to treat many diseases. In this context, multi-lineage stem cell plasticity is defined as the capacity of tissue specific stem cells to give rise to functional end cells of a separate lineage not normally present at the site of origin of the stem cell. This property has been identified for HSC and for stem cells derived from many other tissues, including brain, skeletal muscle and liver. It is impossible to discuss in depth here all the issues that currently surround the plasticity of HSC. Instead the most recent key papers are discussed and the reader is directed to a number of excellent reviews (Forbes et al., 2002b; Graf, 2002; Hirschi and Goodell, 2002; Krause, 2002; McKay, 2002; Orkin and Zon, 2002; Sanchez-Ramos, 2002; Tsai et al., 2002).

There are a number of contentious issues to address when determining whether stem cell plasticity is a naturally occurring phenomenon. Many laboratories have reported studies purporting to show that HSCs may give rise to mature cells of other lineages. The

most notable of these observations are that HSCs can contribute to the liver (Forbes et al., 2002a; Lagasse et al., 2000), CNS (Jiang et al., 2002; Mezey et al., 2000), endothelia (Grant et al., 2002; Jiang et al., 2002) and skeletal muscle (Bittner et al., 1999). However, the balance of evidence is that very rare circulating HSCs do not, in steady state have the capacity to contribute to other lineages in the absence of stress or injury to the recipient tissue. The group of Wiessman et al. have recently demonstrated that single HSCs could 'robustly reconstitute peripheral blood leukocytes', however, they were unable to find any evidence for contribution to other tissues. Even in parabiotic animal studies, where the peripheral circulation a GFP expressing transgenic mouse was conjoined with that of a wild type animal, no GFP positive cells were found in any tissue other than the blood (Wagers et al., 2002). However, neither the donor, nor the recipient mouse, had received any conditioning prior to parabiosis and the numbers of PBSC circulating in peripheral blood were likely to be low under these conditions. It would have been interesting to observe the possibility of plasticity in these experiment if larger doses of previously harvested PBSC were re-infused. Additionally, in these experiments the recipient animal had not been injured so did not have any requirement for tissue regeneration. This is an important caveat, as most of the situations in which the plasticity of stem cells has been convincingly demonstrated, have been in the setting of disease, or injury, for example, in mouse models of muscular dystrophy (MDX mice) (Bittner et al., 1999; Corti et al., 2002; Mackenzie et al., 2002), metabolic liver disease (FAH^{-/-} mice) (Lagasse et al., 2000), after whole body irradiation; or in human recipients of BM or PBSC transplants that had subsequently undergone injury or infective insult to the liver (Korbling et al., 2002). Thus, it remains unclear how great the potential for HSCs to generate new tissues may be, under non-steady state conditions. Such models are important as the majority of patients who may require therapeutic donation of plastic stem cells would exhibit tissue damage/injury.

A number of concerns need to be addressed to enable correct interpretation of experiments designed to demonstrate plasticity. The main criteria by which plasticity should be defined are that reconstitution of both expected and unexpected tissues should be *robust*; there should be *direct evidence* of the adopted program; and that the potential for *self-renewal* should be maintained in the unexpected tissue.

Figure 3. Alternative routes for reprogramming committed precursor cells. The established route of linear differentiation is indicated by the solid arrows and alternative routes by broken arrows.

Abbreviations: Stem cell (SC), Monocyte (M), Granulocyte (G) Erythroblast (Eryth), Myeloid precursor (G/M).

A number of concerns have been raised in attempts to prove the existence of multi-lineage somatic stem cells, foremost of which are whether the apparent plasticity is the result of:

- (a) *Fusion* of donor and recipient cells such that a marked donor stem cell fuses with a differentiated recipient cell thus, transferring the marker gene to the recipient tissue and apparently demonstrating that the stem cell has given rise to this committed cell. Such a process would result in a fused cell with a nuclear content of $4n$ comprised of DNA from both cells. It has been possible to demonstrate *in vitro* that ES cells can undergo fusion with either BM or CNS stem cells (Terada et al., 2002; Ying et al., 2002), however this occurs at a very low frequency of approximately 1 in 1×10^5 – 10^6 . Also, a number of *in vivo* studies, most notably those of Korbliing (Korbliing et al., 2002) and Okamoto (Okamoto et al., 2002) have failed to show hyperploidy or in the case of sex mismatched transplants, the presence of multiple sex chromosomes.
- (b) *Contamination* of somatic stem cell populations with cells of other lineage which may be possible as tissues are excised and prepared from complex structures. This possibility was highlighted in the experiments from the Goodell group that initially seemed to show that a population of muscle derived stem cells could reconstitute the haemopoietic system (Jackson et al., 1999). However, on closer examination it was found that the reconstituting population was of haemopoietic and not muscle origin (McKinney-Freeman et al., 2002), thus no reprogramming of lineage demonstrated in these experiments.

stituting population was of haemopoietic and not muscle origin (McKinney-Freeman et al., 2002), thus no reprogramming of lineage demonstrated in these experiments.

In addition the ability to reproduce these results in disparate laboratories will be critical. There have been many reports of HSCs contributing to other tissues that have based their conclusions on the presence of 1% or less donor cells in the recipient tissue and in a number of cases different outcomes have been reported from various laboratories, for example in the case of HSC to neural tissue (Castro et al., 2002; Sanchez-Ramos, 2002). Thus it is necessary to establish standard protocols by which robust reconstitution can be repeatedly reproduced before plasticity can be accepted.

MSC

MSC are a population of cells, adherent in culture, that have the potential to generate skeletal muscle, cartilage (chondrocytes), bone (osteoblasts), fat (adipocytes) and fibroblasts (Caterson et al., 2002; Dennis et al., 2002; Erices et al., 2000; Jiang et al., 2002; Pittenger et al., 1999; Prockop, 1997; Reyes and Verfaillie, 2001). These cells form the stroma in which the bone marrow HSCs reside and can form a functional haemopoietic microenvironment *in vitro*. Markers for these cells are poorly defined and to date the best description is that these cells are adherent in culture and specifically express the cell surface antigen STRO-1 (Dennis et al., 2002), in addition to Sca1 and CD106

(V-CAM1). In humans, no subset of the adherent cell population has, as yet, been shown to have the potential to produce blood cells; but corresponding murine multipotent adult progenitor cells (mMAPC) have been shown to be capable of differentiating into almost any somatic cell type, including haemopoietic cells (Jiang et al., 2002; Reyes et al., 2001; Reyes and Verfaillie, 2001).

In clinical medicine isolated human MSCs have been used to treat Osteogenesis imperfecta (Brittle Bone Disease). After transplantation, bone density and growth rates were increased and fracture occurrence significantly reduced (Horwitz, 2001; Horwitz et al., 1999, 2001, 2002). These promising results show in this instance that the plasticity of MSCs may be exploited directly to improve specific disease states.

The identification of this population of human cells and their isolation offers a number of potential clinical benefits. Firstly, the capacity of MSCs to regenerate the haemopoietic microenvironment suggests that the co-administration of either newly isolated or *ex vivo* expanded MSCs with HSCs may offer support to the HSCs and improve engraftment and haemopoietic recovery of the recipient. Such trials are currently being undertaken and preliminary results suggest that haemopoietic recovery may indeed be improved; the results and potential of these studies have been reviewed in detail by Koc and Lazarus (Koc and Lazarus, 2001). In the allogeneic setting, transplants using MSC revealed an unexpected property of these cells. MSCs were found to down regulate allo-immune responses, a finding that has huge implications for the field of allograft rejection and GVHD. In a non-human primate skin graft model infusion of *ex-vivo* expanded MSCs prolonged the time to rejection of histo-incompatible skin grafts. Even when 'third-party' skin grafts, belonging to neither recipient, nor MSC donor, were used allo-reactivity appeared to be significantly reduced (Bartholomew et al., 2002). A multi-centre phase 1 trial is currently being undertaken in which allogeneic MSCs are concomitantly transfused into patient with haematological disorders receiving BM or PBSC transplants. Preliminary results compare favorably with historical controls (Koc and Lazarus, 2001).

Embryonic stem (ES) cells

ES cell lines are derived from the inner cell mass (ICM) of the blastocyst and are equivalent to epiblasts. They have been isolated from multiple species in-

cluding, most recently, humans (Evans and Kaufman, 1981; Nichols, 2001; Thomson et al., 1998; Thomson and Marshall, 1998). In murine studies, when injected, *ex vivo*, into blastocysts from normal mice and reimplanted, then these cells contribute to all intraembryonic tissues and this technology forms the basis for transgenic research (Hole and Graham, 1997). *Ex vivo*, using appropriate culture conditions, it is possible to routinely generate primitive and definitive erythroid cells, myeloid, lymphoid, endothelial cells and haemangioblasts from ES cells. Furthermore, in murine models, cells capable of long term *in vivo* multilineage repopulation have also been produced in culture (Hole et al., 1996; Keller and Snodgrass, 1999; Keller, 1995; Odorico et al., 2001; Palacios et al., 1995; Potocnik et al., 1994; Risau et al., 1988; Robertson, 2001; Wiles and Keller, 1991; Yamashita et al., 2000). Theoretically, ES cells could, in the future, be used as a renewable source of large numbers of stem cells, either for direct replacement therapy, or to serve as vehicles for gene therapy. In addition, differentiated ES cells might provide large numbers of phenotypically identical HSC that may allow *in vitro* analysis of HSC responses to new drugs and allow collection of toxicity data *ex vivo*.

Murine ES cells have been extensively studied while human cell lines have not and there is a temptation to assume that human ES cells will always behave in exactly the same way as murine cells, when patently this may not be the case. This has recently been reviewed (Smith, 2001) and inter species differences have been found. In contrast to murine ES cells, human ES cells are not maintained in a pluripotent state in the presence of LIF, a process that in the mouse is mediated via gp130, although they do express Oct-4. As a result of genetic heterogeneity human ES cells may show large individual differences in the ability to establish and maintain cell lines. The kinetics of development of genetic, or epigenetic, instability may vary between species and there is a lack of good model systems for identifying these changes early in the human. One practical problem, is that, in murine models, it is often only during *in vivo* experimentation, that these phenomena are uncovered and such experiments are unethical in the human setting. Four other problems require to be addressed for this technology to be a viable transplantation option (Grompe, 2002; Orkin and Morrison, 2002). The first is that ES cells are immunogenic and, as such, incompatible with the host cells. The second is that undifferentiated murine ES cells have been shown to produce teratomas in trans-

plant recipients and, as such, human cells used for cellular therapies will require to be completely differentiated. Thirdly, little evidence exists to support the longterm functional capabilities of ES cells post transplantation, although one recent study, in a murine model of Parkinson's disease, is very encouraging in this respect (Kim et al., 2002). Finally, there are huge ethical questions to be addressed. In the U.S.A. this has meant that there has been no public funding for human ES cell research (Robertson, 2001). This has not however stopped private companies investigating and indeed patenting ES cell lines (Marshall, 2000).

Concluding remarks

Stem cell transplantation has become an established therapeutic intervention for many diseases and the applications of this therapy are likely to increase in the next few years with older patients suitable for non myelosuppressive therapy and *in utero* transplantation perhaps becoming a viable therapeutic option. *Ex vivo* expansion may allow cord blood to become a viable source of stem cells for transplantation purposes and replacement therapy using genetically modified stem cells is an exciting area of research. The explosion of interest in stem cell plasticity is likely to be sustained and effort will be directed to verifying the remarkable results obtained in recent years at the single cell level. Finally, the use of adult pluripotent stem cells or ES cells as a source for the *in vitro* generation of HSC for cellular therapies holds great promise if the ethical dilemmas can be resolved.

Addendum

After a first case of childhood leukemia found in a recipient of the Paris gene therapy trial, as reported above, a second case of leukemia was observed, apparently due to the insertion of the retrovirus DNA near a gene called LMO2, which is known to cause childhood leukemia. A third case of such insertion was found, with no apparent leukemic development. For these reasons such trial has been suspended and in any other case exceptional caution and restraint are advocated. (Check, Nature, (2003) 421, 678).

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References

- Abramson S, Miller RG & Phillips RA (1977) The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. *J Exp Med* 145: 1567–1579.
- Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, Morecki S, Andolfi G, Tabucchi A, Carlucci F, Marinello E, Cattaneo F, Vai S, Servida P, Miniero R, Roncarolo MG & Bordignon C (2002) Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 296: 2410–2413.
- Alcorn MJ, Farrell E, Barr J, Pearson C, Green R & Holyoake T (2000) The number of CD34+ cells mobilized into the peripheral blood can predict the quality of subsequent collections. *J Hematother Stem Cell Res* 9: 89–93.
- Alcorn MJ & Holyoake TL (1996) *Ex vivo* expansion of haemopoietic progenitor cells. *Blood Rev* 10: 167–176.
- Almeida-Porada G, Brown RL, MacKintosh FR & Zanjani ED (2000) Evaluation of serum-free culture conditions able to support the *ex vivo* expansion and engraftment of human hematopoietic stem cells in the human-to-sheep xenograft model. *J Hematother Stem Cell Res* 9: 683–693.
- Andrews RG, Briddell RA, Knitter GH, Opie T, Bronsden M, Myerson D, Appelbaum FR & McNiece IK (1994) *In vivo* synergy between recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in baboons enhanced circulation of progenitor cells. *Blood* 84: 800–810.
- Aversa F, Velardi A, Tabilio A, Reisner Y & Martelli MF (2001) Haploidentical stem cell transplantation in leukemia. *Blood Rev* 15: 111–119.
- Bai L, Kon K, Tatsumi M, Ito H, Hayashi S & Brautigan M (2000) A human B-cell CLL model established by transplantation of JOK-1 cells into SCID mice and an anti-leukemia efficacy of fludarabine phosphate. *Oncol Rep* 7: 33–38.
- Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC & Gordon-Smith EC (1998) Progressive telomere shortening in aplastic anemia. *Blood* 91: 3582–3592.
- Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A & Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. *Exp Hematol* 30: 42–48.
- Baum CM, Weissman IL, Tsukamoto AS, Buckle AM & Peault B (1992) Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci USA* 89: 2804–2808.
- Bhardwaj G, Murdoch B, Wu D, Baker DP, Williams KP, Chadwick K, Ling LE, Karanu FN & Bhatia M (2001) Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. *Nat Immunol* 2: 172–180.
- Bhatia M (2001) AC133 expression in human stem cells. *Leukemia* 15: 1685–1688.
- Bhatia M, Bonnet D, Murdoch B, Gan OI & Dick JE (1998) A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat Med* 4: 1038–1045.

- Bhatia M, Bonnet D, Wu D, Murdoch B, Wrana J, Gallacher L & Dick JE (1999) Bone morphogenetic proteins regulate the developmental program of human hematopoietic stem cells. *J Exp Med* 189: 1139–1148.
- Bhatia M, Wang JC, Kapp U, Bonnet D & Dick JE (1997a) Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci USA* 94: 5320–5325.
- Bhatia R, McGlave PB, Miller JS, Wissink S, Lin WN & Verfaillie CM (1997b) A clinically suitable *ex vivo* expansion culture system for LTC-IC and CFC using stroma-conditioned medium. *Exp Hematol* 25: 980–991.
- Bittner RE, Schofer C, Weipoltshammer K, Ivanova S, Streubel B, Hauser E, Freilinger M, Hoger H, Elbe-Burger A & Wachtler F (1999) Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. *Anat Embryol (Berl)* 199: 391–396.
- Bonnet D & Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3: 730–737.
- Bradford GB, Williams B, Rossi R & Bertoncello I (1997) Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol* 25: 445–453.
- Breems DA, Blokland EA, Siebel KE, Mayen AE, Engels LJ & Ploemacher RE (1998) Stroma-contact prevents loss of hematopoietic stem cell quality during *ex vivo* expansion of CD34+ mobilized peripheral blood stem cells. *Blood* 91: 111–117.
- Brummendorf TH, Holyoake TL, Rufer N, Barnett MJ, Schulzer M, Eaves CJ, Eaves AC & Lansdorp PM (2000) Prognostic implications of differences in telomere length between normal and malignant cells from patients with chronic myeloid leukemia measured by flow cytometry. *Blood* 95: 1883–1890.
- Brummendorf TH, Rufer N, Baerlocher GM, Roosnek E & Lansdorp PM (2001a) Limited telomere shortening in hematopoietic stem cells after transplantation. *Ann N Y Acad Sci* 938: 1–7; discussion 7–8.
- Brummendorf TH, Rufer N, Holyoake TL, Maciejewski J, Barnett MJ, Eaves CJ, Eaves AC, Young N & Lansdorp PM (2001b) Telomere length dynamics in normal individuals and in patients with hematopoietic stem cell-associated disorders. *Ann N Y Acad Sci* 938: 293–303; discussion 303–304.
- Bunting KD (2002) ABC transporters as phenotypic markers and functional regulators of stem cells. *Stem Cells* 20: 11–20.
- Burt RK, Slavin S, Burns WH & Marmont AM (2002) Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: Getting closer to a cure? *Blood* 99: 768–784.
- Cashman J, Clark-Lewis I, Eaves A & Eaves C (2002) Stromal-derived factor 1 inhibits the cycling of very primitive human hematopoietic cells *in vitro* and in NOD/SCID mice. *Blood* 99: 792–799.
- Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H & Shine HD (2002) Failure of bone marrow cells to transdifferentiate into neural cells *in vivo*. *Science* 297: 1299.
- Caterson EJ, Nesti LJ, Danielson KG & Tuan RS (2002) Human marrow-derived mesenchymal progenitor cells: isolation, culture expansion, and analysis of differentiation. *Mol Biotechnol* 20: 245–256.
- Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P, Deist FL & Fischer A (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 288: 669–672.
- Chakraverty R, Peggs K, Chopra R, Milligan DW, Kottaridis PD, Verfuert S, Geary J, Thuraiandaram D, Branson K, Chakrabarti S, Mahendra P, Craddock C, Parker A, Hunter A, Hale G, Waldmann H, Williams CD, Yong K, Linch DC, Goldstone AH & Mackinnon S (2002) Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood* 99: 1071–1078.
- Charbord P, Oostendorp R, Pang W, Herault O, Noel F, Tsuji T, Dzierzak E & Peault B (2002) Comparative study of stromal cell lines derived from embryonic, fetal, and postnatal mouse blood-forming tissues. *Exp Hematol* 30: 1202.
- Chatta GS & Dale DC (1996) Aging and haemopoiesis. Implications for treatment with haemopoietic growth factors. *Drugs Aging* 9: 37–47.
- Cheng T, Rodrigues N, Shen H, Yang Y, Dombkowski D, Sykes M & Scadden DT (2000) Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science* 287: 1804–1808.
- Cheshier SH, Morrison SJ, Liao X & Weissman IL (1999) *In vivo* proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc Natl Acad Sci USA* 96: 3120–3125.
- Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, Read EJ, Carter C, Bahceci E, Young NS & Barrett AJ (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: Full donor T-cell chimerism precedes alloimmune responses. *Blood* 94: 3234–3241.
- Chiu CP, Dragowska W, Kim NW, Vaziri H, Yui J, Thomas TE, Harley CB & Lansdorp PM (1996) Differential expression of telomerase activity in hematopoietic progenitors from adult human bone marrow. *Stem Cells* 14: 239–248.
- Civin CI, Almeida-Porada G, Lee MJ, Olweus J, Terstappen LW & Zanjani ED (1996) Sustained, retransplantable, multilineage engraftment of highly purified adult human bone marrow stem cells *in vivo*. *Blood* 88: 4102–4109.
- Corti S, Strazzer S, Del Bo R, Salani S, Bossolasco P, Fortunato F, Locatelli F, Soligo D, Moggio M, Ciscato P, Prella A, Borsotti C, Bresolin N, Scarlato G & Comi GP (2002) A subpopulation of murine bone marrow cells fully differentiates along the myogenic pathway and participates in muscle repair in the mdx dystrophic mouse. *Exp Cell Res* 277: 74–85.
- Craig W, Kay R, Cutler RL & Lansdorp PM (1993) Expression of Thy-1 on human hematopoietic progenitor cells. *J Exp Med* 177: 1331–1342.
- Dao MA, Shah AJ, Crooks GM & Nolte JA (1998) Engraftment and retroviral marking of CD34+ and CD34+CD38– human hematopoietic progenitors assessed in immune-deficient mice. *Blood* 91: 1243–55.
- Dennis JE, Carbillet JP, Caplan AI & Charbord P (2002) The STRO-1+ marrow cell population is multipotential. *Cells Tissues Organs* 170: 73–82.
- Dexter TM, Moore MA & Sheridan AP (1977) Maintenance of hematopoietic stem cells and production of differentiated progeny in allogeneic and semiallogeneic bone marrow chimeras *in vitro*. *J Exp Med* 145: 1612–1616.
- Dick JE (1996) Normal and leukemic human stem cells assayed in SCID mice. *Semin Immunol* 8: 197–206.
- Dick JE, Sirard C, Pflumio F & Lapidot T (1992) Murine models of normal and neoplastic human haematopoiesis. *Cancer Surv* 15: 161–181.
- Effros RB & Globerson A (2002) Hematopoietic cells and replicative senescence. *Exp Gerontol* 37: 191–196.
- Engelhardt M & Finke J (2001) Does telomere shortening count? *Blood* 98: 888–890.
- Engelhardt M, Kumar R, Albanell J, Pettengell R, Han W & Moore MA (1997) Telomerase regulation, cell cycle, and telomere stability in primitive hematopoietic cells. *Blood* 90: 182–193.

- Erices A, Conget P & Minguell JJ (2000) Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 109: 235–242.
- Evans MJ & Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292: 154–156.
- Facon T, Harousseau JL, Maloisel F, Attal M, Odriezola J, Alegre A, Schroyens W, Hulin C, Schots R, Marin P, Guilhot F, Granena A, De Waele M, Pigneux A, Meresse V, Clark P & Reiffers J (1999) Stem cell factor in combination with filgrastim after chemotherapy improves peripheral blood progenitor cell yield and reduces apheresis requirements in multiple myeloma patients: A randomized, controlled trial. *Blood* 94: 1218–1225.
- Flake AW (2001) *In utero* transplantation of haemopoietic stem cells. *Best Pract Res Clin Haematol* 14: 671–683.
- Flake AW & Zanjani ED (1997) *In utero* hematopoietic stem cell transplantation. A status report. *Jama* 278: 932–937.
- Flake AW & Zanjani ED (1998) *In utero* transplantation for thalassemia. *Ann N Y Acad Sci* 850: 300–311.
- Flake AW & Zanjani ED (1999) *In utero* hematopoietic stem cell transplantation: Ontogenic opportunities and biologic barriers. *Blood* 94: 2179–2191.
- Forbes SJ, Poulsom R & Wright NA (2002a) Hepatic and renal differentiation from blood-borne stem cells. *Gene Ther* 9: 625–630.
- Forbes SJ, Vig P, Poulsom R, Wright NA & Alison MR (2002b) Adult stem cell plasticity: New pathways of tissue regeneration become visible. *J Clin Sci (Lond)* 103: 355–369.
- Friedberg JW, Neuberg D, Stone RM, Alyea E, Jallow H, LaCasce A, Mauch PM, Gribben JG, Ritz J, Nadler LM, Soiffer RJ & Freedman AS (1999) Outcome in patients with myelodysplastic syndrome after autologous bone marrow transplantation for non-Hodgkin's lymphoma. *J Clin Oncol* 17: 3128–3135.
- Gallacher L, Murdoch B, Wu DM, Karanu FN, Keeney M & Bhatia M (2000) Isolation and characterization of human CD34(–)Lin(–) and CD34(+)Lin(–) hematopoietic stem cells using cell surface markers AC133 and CD7. *Blood* 95: 2813–2820.
- Glaspy JA, Shpall EJ, LeMaistre CF, Briddell RA, Menchaca DM, Turner SA, Lill M, Chap L, Jones R, Wiers MD, Sheridan WP & McNiece IK (1997) Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients. *Blood* 90: 2939–2951.
- Gluckman E (2001) Hematopoietic stem-cell transplants using umbilical-cord blood. *N Engl J Med* 344: 1860–1861.
- Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, Ortega J, Souillet G, Ferreira E, Laporte JP, Fernandez M & Chastang C (1997) Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 337: 373–381.
- Godin I & Cumano A (2002) The hare and the tortoise: An embryonic haematopoietic race. *Nat Rev Immunol* 2: 593–604.
- Goldstone AH, Burnett AK, Wheatley K, Smith AG, Hutchinson RM & Clark RE (2001) Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. *Blood* 98: 1302–1311.
- Goodell MA (1999) Introduction: Focus on hematology. CD34(+) or CD34(–): does it really matter? *Blood* 94: 2545–2547.
- Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC & Johnson RP (1997) Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 3: 1337–1345.
- Graf T (2002) Differentiation plasticity of hematopoietic cells. *Blood* 99: 3089–3101.
- Graham GJ & Pragnell IB (1992) The haemopoietic stem cell: Properties and control mechanisms. *Semin Cell Biol* 3: 423–434.
- Graham GJ & Wright EG (1997) Haemopoietic stem cells: Their heterogeneity and regulation. *Int J Exp Pathol* 78: 197–218.
- Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L & Holyoake TL (2002) Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. *Blood* 99: 319–325.
- Grant MB, May WS, Caballero S, Brown GA, Guthrie SM, Mames RN, Byrne BJ, Vaught T, Spoerri PE, Peck AB & Scott EW (2002) Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. *Nat Med* 8: 607–612.
- Gratwohl A, Passweg J, Gerber I & Tyndall A (2001) Stem cell transplantation for autoimmune diseases. *Best Pract Res Clin Haematol* 14: 755–776.
- Greider CW (1994) Mammalian telomere dynamics: Healing, fragmentation shortening and stabilization. *Curr Opin Genet Dev* 4: 203–211.
- Grompe M (2002) Adult versus Embryonic Stem Cells: It's Still a Tie. *Mol Ther* 6: 303.
- Gunzburg W, Hemiston T, Seymour L (eds) (2002) Gene transfer and cell based therapies. Current opinion in molecular therapeutics 4: 281–407.
- Hackney JA, Chabord P, Brunk BP, Stoeckert CJ, Lemischka IR & Moore KA (2002) A molecular profile of a hematopoietic stem cell niche. *Proc Natl Acad Sci USA* 99: 13061–13066.
- Hall FL, Han B, Kundu RK, Yee A, Nimni ME & Gordon EM (2001) Phenotypic differentiation of TGF-beta1-responsive pluripotent premesenchymal prehematopoietic progenitor (P4 stem) cells from murine bone marrow. *J Hematother Stem Cell Res* 10: 261–271.
- Hao QL, Thiemann FT, Petersen D, Smogorzewska EM & Crooks GM (1996) Extended long-term culture reveals a highly quiescent and primitive human hematopoietic progenitor population. *Blood* 88: 3306–3313.
- Harrison DE (1979) Mouse erythropoietic stem cell lines function normally 100 months: loss related to number of transplantations. *Mech Ageing Dev* 9: 427–433.
- Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, Hicklin DJ, Zhu Z, Bohlen P, Witte L, Hendriks J, Hackett NR, Crystal RG, Moore MA, Werb Z, Lyden D & Rafii S (2002) Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med* 8: 841–849.
- Hayashi S, Peranteau WH, Shaaban AF & Flake AW (2002) Complete allogeneic hematopoietic chimerism achieved by a combined strategy of in utero hematopoietic stem cell transplantation and postnatal donor lymphocyte infusion. *Blood* 100: 804–812.
- Hayflick L (1992) Aging, longevity, and immortality *in vitro*. *Exp Gerontol* 27: 363–368.
- Hayward A, Ambruso D, Battaglia F, Donlon T, Eddelman K, Giller R, Hobbins J, Hsia YE, Quinones R, Shpall E, Trachtenberg E & Giardina P (1998) Microchimerism and tolerance following intrauterine transplantation and transfusion for alpha-thalassemia-1. *Fetal Diagn Ther* 13: 8–14.
- Heike T & Nakahata T (2002) *Ex vivo* expansion of hematopoietic stem cells by cytokines. *Biochim Biophys Acta* 1592: 313–321.
- Henslee-Downey PJ (2001) Allogeneic transplantation across major HLA barriers. *Best Pract Res Clin Haematol* 14: 741–754.
- Hess DA, Levac KD, Karanu FN, Rosu-Myles M, White MJ, Gallacher L, Murdoch B, Keeney M, Ottowski P, Foley R, Chin-Yee I & Bhatia M (2002) Functional analysis of human hema-

- topoietic repopulating cells mobilized with granulocyte colony-stimulating factor alone versus granulocyte colony-stimulating factor in combination with stem cell factor. *Blood* 100: 869–878.
- Heyworth C, Pearson S, May G & Enver T (2002) Transcription factor-mediated lineage switching reveals plasticity in primary committed progenitor cells. *Embo J* 21: 3770–3781.
- Hirschi KK & Goodell MA (2002) Hematopoietic, vascular and cardiac fates of bone marrow-derived stem cells. *Gene Ther* 9: 648–652.
- Hole N & Graham GJ (1997) Use of embryonal stem cells in studies of molecular haemopoiesis. *Baillieres Clin Haematol* 10: 467–483.
- Hole N, Graham GJ, Menzel U & Ansell JD (1996) A limited temporal window for the derivation of multilineage repopulating hematopoietic progenitors during embryonal stem cell differentiation *in vitro*. *Blood* 88: 1266–1276.
- Holyoake T, Jiang X, Eaves C & Eaves A (1999a) Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood* 94: 2056–2064.
- Holyoake TL (1996) Cytokines at the research-clinical interface: Potential applications. *Blood Rev* 10: 189–200.
- Holyoake TL, Alcorn MJ, Richmond L, Farrell E, Pearson C, Green R, Dunlop DJ, Fitzsimons E, Pragnell IB & Franklin IM (1997) CD34 positive PBPC expanded *ex vivo* may not provide durable engraftment following myeloablative chemoradiotherapy regimens. *Bone Marrow Transplant* 19: 1095–1101.
- Holyoake TL, Freshney MG, Konwalinka G, Haun M, Petzer A, Fitzsimons E, Lucie NP, Wright EG & Pragnell IB (1993) Mixed colony formation *in vitro* by the heterogeneous compartment of multipotential progenitors in human bone marrow. *Leukemia* 7: 207–213.
- Holyoake TL, Freshney MG, McNair L, Parker AN, McKay PJ, Steward WP, Fitzsimons E, Graham GJ & Pragnell IB (1996) *Ex vivo* expansion with stem cell factor and interleukin-11 augments both short-term recovery posttransplant and the ability to serially transplant marrow. *Blood* 87: 4589–4595.
- Holyoake TL, Jiang X, Jorgensen HG, Graham S, Alcorn MJ, Laird C, Eaves AC & Eaves CJ (2001) Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth *in vitro* in association with up-regulation of expression of interleukin-3. *Blood* 97: 720–728.
- Holyoake TL, Nicolini FE & Eaves CJ (1999b) Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. *Exp Hematol* 27: 1418–1427.
- Horwitz EM (2001) Marrow mesenchymal cell transplantation for genetic disorders of bone. *Cytotherapy* 3: 399–401.
- Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L & Hofmann T (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci USA* 99: 8932–8937.
- Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyritz RE & Brenner MK (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 5: 309–313.
- Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyritz RE & Brenner MK (2001) Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 97: 1227–1231.
- Ishikawa F, Livingston AG, Wingard JR, Nishikawa S & Ogawa M (2002) An assay for long-term engrafting human hematopoietic cells based on newborn NOD/SCID/beta2-microglobulin(null) mice. *Exp Hematol* 30: 488–494.
- Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, Ueyama Y, Koyanagi Y, Sugamura K, Tsuji K, Heike T & Nakahata T (2002) NOD/SCID/gamma(c)(null) mouse: An excellent recipient mouse model for engraftment of human cells. *Blood* 100: 3175–3182.
- Ito T, Tajima F & Ogawa M (2000) Developmental changes of CD34 expression by murine hematopoietic stem cells. *Exp Hematol* 28: 1269–1273.
- Jackson KA, Mi T & Goodell MA (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* 96: 14482–14486.
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA & Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418: 41–49.
- Jones DR, Bui TH, Anderson EM, Ek S, Liu D, Ringden O & Westgren M (1996) *In utero* haematopoietic stem cell transplantation: current perspectives and future potential. *Bone Marrow Transplant* 18: 831–837.
- Jordan CT & Lemischka IR (1990) Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev* 4: 220–232.
- Jørgensen H, Allan E, Graham S, Richmond L, Godden J & Holyoake T (2002a) Will drug combinations effectively eradicate quiescent leukaemic stem cells in Chronic Myeloid Leukaemia. *Experimental Hematology* 30: 73.
- Jørgensen HG, Elliott MA, Allan EK, Carr CE, Holyoake TL & Smith KD (2002b) Alpha-1-acid glycoprotein expressed in the plasma of chronic myeloid leukaemia patients does not mediate significant *in vitro* resistance to ST1571 (Glivec). *Blood* 99: 713–715.
- Jørgensen HG, Elliott MA, Paterson S, Holyoake TL & Smith KD (2002c) Further observations on the debated ability of AGP to bind imatinib. *Blood* 100: 368–369.
- Kawashima I, Zanjani ED, Almada-Porada G, Flake AW, Zeng H & Ogawa M (1996) CD34+ human marrow cells that express low levels of Kit protein are enriched for long-term marrow-engrafting cells. *Blood* 87: 4136–4142.
- Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW & High KA (2000) Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat Genet* 24: 257–261.
- Keller G & Snodgrass HR (1999) Human embryonic stem cells: The future is now. *Nat Med* 5: 151–152.
- Keller G & Snodgrass R (1990) Life span of multipotential hematopoietic stem cells *in vivo*. *J Exp Med* 171: 1407–1418.
- Keller GM (1995) *In vitro* differentiation of embryonic stem cells. *Curr Opin Cell Biol* 7: 862–869.
- Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sanchez-Pernaute R, Bankiewicz K & McKay R (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 418: 50–56.
- Koc ON & Lazarus HM (2001) Mesenchymal stem cells: Heading into the clinic. *Bone Marrow Transplant* 27: 235–239.
- Kojika S & Griffin JD (2001) Notch receptors and hematopoiesis. *Exp Hematol* 29: 1041–1052.
- Kollet O, Peled A, Byk T, Ben-Hur H, Greiner D, Shultz L & Lapidot T (2000) beta2 microglobulin-deficient (B2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. *Blood* 95: 3102–3105.

- Korbling M (1997) Peripheral blood stem cells: A novel source for allogeneic transplantation. *Oncologist* 2: 104–113.
- Korbling M & Anderlini P (2001) Peripheral blood stem cell versus bone marrow allotransplantation: Does the source of hematopoietic stem cells matter? *Blood* 98: 2900–2908.
- Korbling M, Katz RL, Khanna A, Ruitfrok AC, Rondon G, Albitar M, Champlin RE & Estrov Z (2002) Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 346: 738–746.
- Krause DS (2002) Plasticity of marrow-derived stem cells. *Gene Ther* 9: 754–758.
- Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL & Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat Med* 6: 1229–1234.
- Lane TA, Ho AD, Bashey A, Peterson S, Young D & Law P (1999) Mobilization of blood-derived stem and progenitor cells in normal subjects by granulocyte-macrophage- and granulocyte-colony-stimulating factors. *Transfusion* 39: 39–47.
- Larochelle A, Vormoor J, Hanenberg H, Wang JC, Bhatia M, Lapidot T, Moritz T, Murdoch B, Xiao XL, Kato I, Williams DA & Dick JE (1996) Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: Implications for gene therapy. *Nat Med* 2: 1329–1337.
- Lataillade JJ, Clay D, Bourin P, Herodin F, Dupuy C, Jasmin C & Bousse-Kerdiles MC (2002) Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: Evidence for an autocrine/paracrine mechanism. *Blood* 99: 1117–1129.
- Le Bousse-Kerdiles MC, Chevillard S, Charpentier A, Romquin N, Clay D, Smadja-Joffe F, Praloran V, Dupriez B, Demory JL, Jasmin C & Martyre MC (1996) Differential expression of transforming growth factor-beta, basic fibroblast growth factor, and their receptors in CD34+ hematopoietic progenitor cells from patients with myelofibrosis and myeloid metaplasia. *Blood* 88: 4534–4546.
- Lee HW, Blasco MA, Gottlieb GJ, Horner JW, 2nd Greider CW & DePinho RA (1998) Essential role of mouse telomerase in highly proliferative organs. *Nature* 392: 569–574.
- Lemischka I (2001) Stem cell dogmas in the genomics era. *Rev Clin Exp Hematol* 5: 15–25.
- Levesque JP, Takamatsu Y, Nilsson SK, Haylock DN & Simmons PJ (2001) Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. *Blood* 98: 1289–1297.
- Liu F, Poursine-Laurent J & Link DC (1997) The granulocyte colony-stimulating factor receptor is required for the mobilization of murine hematopoietic progenitors into peripheral blood by cyclophosphamide or interleukin-8 but not flt-3 ligand. *Blood* 90: 2522–2528.
- Liu F, Poursine-Laurent J & Link DC (2000) Expression of the G-CSF receptor on hematopoietic progenitor cells is not required for their mobilization by G-CSF. *Blood* 95: 3025–3031.
- Loeuillet C, Bernard G, Remy-Martin J, Saas P, Herve P, Douay L & Chalmers D (2001) Distinct hematopoietic support by two human stromal cell lines. *Exp Hematol* 29: 736–745.
- Mackenzie TC, Shaaban AF, Radu A & Flake AW (2002) Engraftment of bone marrow and fetal liver cells after in utero transplantation in MDX mice. *J Pediatr Surg* 37: 1058–1064.
- Mahmud N, Devine SM, Weller KP, Parmar S, Sturgeon C, Nelson MC, Hewett T & Hoffman R (2001) The relative quiescence of hematopoietic stem cells in nonhuman primates. *Blood* 97: 3061–3068.
- Maris M, Woolfrey A, McSweeney PA, Sandmaier BM, Nash RA, Georges G, Maloney DG, Molina A, Chauncey T, Yu C, Zancha JM, Blume KG, Shizuru J, Niederwieser D & Storb R (2001) Nonmyeloablative hematopoietic stem cell transplantation: Transplantation for the 21st century. *Front Biosci* 6: G13–G16.
- Marshall E (2000) The business of stem cells. *Science* 287: 1419–1421.
- Marshall E (2002a) Clinical research. Gene therapy a suspect in leukemia-like disease. *Science* 298: 34–35.
- Marshall E (2002b) Gene therapy. What to do when clear success comes with an unclear risk? *Science* 298: 510–511.
- Mathioudakis G, Storb R, McSweeney PA, Torok-Storb B, Lansdorp PM, Brummendorf T, Gass MJ, Bryant EM, Storek J, Flowers ME, Gooley T & Nash RA (2000) Polyclonal hematopoiesis with variable telomere shortening in human long-term allogeneic marrow graft recipients. *Blood* 96: 3991–3994.
- Mavroudis D, Read E, Cottler-Fox M, Couriel D, Molldrem J, Carter C, Yu M, Dunbar C & Barrett J (1996) CD34+ cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood* 88: 3223–3229.
- McKay R (2002) Building animals from stem cells. *Ann N Y Acad Sci*, 961: 44.
- McKinney-Freeman SL, Jackson KA, Camargo FD, Ferrari G, Mavilio F & Goodell MA (2002) Muscle-derived hematopoietic stem cells are hematopoietic in origin. *Proc Natl Acad Sci USA* 99: 1341–1346.
- Mezey E, Chandross KJ, Harta G, Maki RA & McKercher SR (2000) Turning blood into brain: Cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290: 1779–1782.
- Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier C M & Rubinstein P (2000) Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: Graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood* 96: 2717–2722.
- Milner LA & Bigas A (1999) Notch as a mediator of cell fate determination in hematopoiesis: Evidence and speculation. *Blood* 93: 2431–2448.
- Molineux G, Migdalska A, Szmitkowski M, Zsebo K & Dexter TM (1991) The effects on hematopoiesis of recombinant stem cell factor (ligand for c-kit) administered *in vivo* to mice either alone or in combination with granulocyte colony-stimulating factor. *Blood* 78: 961–966.
- Molineux G, Pojda Z, Hampson IN, Lord BI & Dexter TM (1990) Transplantation potential of peripheral blood stem cells induced by granulocyte colony-stimulating factor. *Blood* 76: 2153–2158.
- Monahan PE & White IG (2002) Hemophilia gene therapy: Update. *Curr Opin Hematol* 9: 430–436.
- Moore KA, Ema H & Lemischka IR (1997) *In vitro* maintenance of highly purified, transplantable hematopoietic stem cells. *Blood* 89: 4337–4347.
- Moore MA, Hattori K, Heissig B, Shieh JH, Dias S, Crystal RG & Rafii S (2001) Mobilization of endothelial and hematopoietic stem and progenitor cells by adenovector-mediated elevation of serum levels of SDF-1: VEGF, and angiopoietin-1. *Ann N Y Acad Sci* 938: 36–45; discussion 45–47.
- Morrison SJ, Prowse KR, Ho P & Weissman IL (1996a) Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity* 5: 207–216.
- Morrison SJ, Shah NM & Anderson DJ (1997) Regulatory mechanisms in stem cell biology. *Cell* 88: 287–298.

- Morrison SJ, Wandycz AM, Akashi K, Globerson A & Weissman IL (1996b) The aging of hematopoietic stem cells. *Nat Med* 2: 1011–1016.
- 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 (1997) Recombinant methionyl human stem cell factor and filgrastim for peripheral blood progenitor cell mobilization and transplantation in non-Hodgkin's lymphoma patients—results of a phase I/II trial. *Blood* 89: 3136–3147.
- Murray L, Chen B, Galy A, Chen S, Tushinski R, Uchida N, Negrin R, Tricot G, Jagannath S, Vesole D et al. (1995) Enrichment of human hematopoietic stem cell activity in the CD34 + Thy- 1 + Lin- subpopulation from mobilized peripheral blood. *Blood* 85: 368–378.
- Nichols J (2001) Introducing embryonic stem cells. *Curr Biol* 11: R503–R505.
- Nijmeijer BA, Mollevanger P, van Zelderen-Bhola SL, Kluin-Nelemans HC, Willemze R & Falkenburg JH (2001) Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. *Exp Hematol* 29: 322–329.
- Nijmeijer BA, Willemze R & Falkenburg JH (2002) An animal model for human cellular immunotherapy: specific eradication of human acute lymphoblastic leukemia by cytotoxic T lymphocytes in NOD/scid mice. *Blood* 100: 654–660.
- Odorico JS, Kaufman DS & Thomson JA (2001) Multilineage differentiation from human embryonic stem cell lines. *Stem Cells* 19: 193–204.
- Okamoto R, Yajima T, Yamazaki M, Kanai T, Mukai M, Okamoto S, Ikeda Y, Hibi T, Inazawa J & Watanabe M (2002) Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. *Nat Med* 8: 1011–1017.
- Oostendorp RA, Harvey KN, Kusadasi N, de Bruijn MF, Saris C, Ploemacher RE, Medvinsky AL & Dzierzak EA (2002) Stromal cell lines from mouse aorta-gonads-mesonephros subregions are potent supporters of hematopoietic stem cell activity. *Blood* 99: 1183–1189.
- Orkin SH & Morrison SJ (2002) Stem-cell competition. *Nature* 418: 25–27.
- Orkin SH & Zon LI (2002) Hematopoiesis and stem cells: Plasticity versus developmental heterogeneity. *Nat Immunol* 3: 323–328.
- Osawa M, Hanada K, Hamada H & Nakauchi H (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273: 242–245.
- Oscier DG (1987) Myelodysplastic syndromes. *Baillieres Clin Haematol* 1: 389–426.
- Palacios R, Golunski E & Samaridis J (1995) *In vitro* generation of hematopoietic stem cells from an embryonic stem cell line. *Proc Natl Acad Sci USA* 92: 7530–7534.
- Papayannopoulou T & Nakamoto B (1993) Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci USA* 90: 9374–9378.
- Papayannopoulou T, Priestley GV & Nakamoto B (1998) Anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the kit/mkit ligand pathway. *Blood* 91: 2231–2239.
- Papayannopoulou T, Priestley GV, Nakamoto B, Zafiroopoulos V, Scott LM & Harlan JM (2001) Synergistic mobilization of hemopoietic progenitor cells using concurrent beta1 and beta2 integrin blockade or beta2-deficient mice. *Blood* 97: 1282–1288.
- Pecora AL, Preti RA, Gleim GW, Jennis A, Zahos K, Cantwell S, Doria L, Isaacs R, Gillio AP, Michelis MA & Brochstein JA (1998) CD34+CD33- cells influence days to engraftment and transfusion requirements in autologous blood stem-cell recipients. *J Clin Oncol* 16: 2093–2104.
- Peschle C, Botta R, Muller R, Valtieri M & Ziegler BL (2001) Purification and functional assay of pluripotent hematopoietic stem cells. *Rev Clin Exp Hematol* 5: 3–14.
- Petzer AL, Zandstra PW, Piret JM & Eaves CJ (1996) Differential cytokine effects on primitive (CD34+CD38-) human hematopoietic cells: Novel responses to Flt3-ligand and thrombopoietin. *J Exp Med* 183: 2551–2558.
- Piacibello W, Sanavio F, Garetto L, Severino A, Bergandi D, Ferrario J, Fagioli F, Berger M & Aglietta M (1997) Extensive amplification and self-renewal of human primitive hematopoietic stem cells from cord blood. *Blood* 89: 2644–2653.
- Pilarski LM & Belch AR (2002) Clonotypic myeloma cells able to xenograft myeloma to nonobese diabetic severe combined immunodeficient mice copurify with CD34(+) hematopoietic progenitors. *Clin Cancer Res* 8: 3198–3204.
- Pilarski LM, Seeberger K, Coupland RW, Eshpeter A, Keats JJ, Taylor BJ & Belch AR (2002) Leukemic B cells clonally identical to myeloma plasma cells are myelomagenic in NOD/SCID mice. *Exp Hematol* 30: 221–228.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S & Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147.
- Ploemacher RE (1999) Characterisation and biology of normal human haematopoietic stem cells. *Haematologica*, 84 Suppl EHA 4: 4–7.
- Podesta M, Piaggio G, Frassoni F, Pitto A, Mordini N, Bregante S, Valeriani A & Bacigalupo A (1997) Deficient reconstitution of early progenitors after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 19: 1011–1017.
- Podesta M, Piaggio G, Frassoni F, Pitto A, Zikos P, Sessarego M, Abate M, Teresa Van Lint M, Berisso G & Bacigalupo A (1998) The assessment of the hematopoietic reservoir after immunosuppressive therapy or bone marrow transplantation in severe aplastic anemia. *Blood* 91: 1959–1965.
- Potocnik AJ, Nielsen PJ & Eichmann K (1994) *In vitro* generation of lymphoid precursors from embryonic stem cells. *Embo J* 13: 5274–5283.
- Powles R, Mehta J, Kulkarni S, Treleaven J, Millar B, Marsden J, Shepherd V, Rowland A, Sirohi B, Tait D, Horton C, Long S & Singhal S (2000) Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: A randomised trial. *Lancet* 355: 1231–1237.
- Pratt G, Rawstron AC, English AE, Johnson RJ, Jack AS, Morgan GJ & Smith GM (2001) Analysis of CD34+ cell subsets in stem cell harvests can more reliably predict rapidity and durability of engraftment than total CD34+ cell dose, but steady state levels do not correlate with bone marrow reserve. *Br J Haematol* 114: 937–943.
- Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 276: 71–74.
- Prosper F, Stroncek D & Verfaillie CM (1996) Phenotypic and functional characterization of long-term culture-initiating cells present in peripheral blood progenitor collections of normal donors treated with granulocyte colony-stimulating factor. *Blood* 88: 2033–2042.
- Punzel M, Gupta P, Roodell M, Mortari F & Verfaillie CM (1999a) Factor(s) secreted by AFT024 fetal liver cells following stimulation with human cytokines are important for human LTC-IC growth. *Leukemia* 13: 1079–1084.
- Punzel M, Moore KA, Lemischka IR & Verfaillie CM (1999b) The type of stromal feeder used in limiting dilution assays influences

- frequency and maintenance assessment of human long-term culture initiating cells. *Leukemia* 13: 92–97.
- Punzel M, Wissink SD, Miller JS, Moore KA, Lemischka IR & Verfaillie CM (1999c) The myeloid-lymphoid initiating cell (ML-IC) assay assesses the fate of multipotent human progenitors *in vitro*. *Blood* 93: 3750–3756.
- Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L & Verfaillie CM (2001) Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98: 2615–2625.
- Reyes M & Verfaillie CM (2001) Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. *Ann N Y Acad Sci* 938: 231–233; discussion 233–235.
- Risau W, Sariola H, Zerwes HG, Sasse J, Ekblom P, Kemler R & Doetschman T (1988) Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. *Development* 102: 471–478.
- Robertson JA (2001) Human embryonic stem cell research: Ethical and legal issues. *Nat Rev Genet* 2: 74–78.
- Roeder I & Loeffler M (2002) A novel dynamic model of hematopoietic stem cell organization based on the concept of within-tissue plasticity. *Exp Hematol* 30: 853–861.
- Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, Berkowitz RL, Cabbad M, Dobrila NL, Taylor PE, Rosenfield RE & Stevens CE (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 339: 1565–1577.
- Rufer N, Brummendorf TH, Chapuis B, Helg C, Lansdorp PM & Roosnek E (2001) Accelerated telomere shortening in hematological lineages is limited to the first year following stem cell transplantation. *Blood* 97: 575–577.
- Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M & Lansdorp PM (1999) Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *J Exp Med* 190: 157–167.
- Sanchez-Ramos JR (2002) Neural cells derived from adult bone marrow and umbilical cord blood. *J Neurosci Res* 69: 880–893.
- Sato T, Laver JH & Ogawa M (1999) Reversible expression of CD34 by murine hematopoietic stem cells. *Blood* 94: 2548–2554.
- Schofield R (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4: 7–25.
- Shay JW (1998) Accelerated telomere shortening in bone-marrow recipients. *Lancet* 351: 153–154.
- Shay JW & Wright WE (2000) Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol* 1: 72–76.
- Shultz LD, Lang PA, Christianson SW, Gott B, Lyons B, Umeda S, Leiter E, Hesselton R, Wagar EJ, Leif JH, Kollet O, Lapidot T & Greiner DL (2000) NOD/LtSz-Rag1null mice: An immunodeficient and radioresistant model for engraftment of human hematolymphoid cells, HIV infection, and adoptive transfer of NOD mouse diabetogenic T cells. *J Immunol* 164: 2496–2507.
- Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobraaten L, Rajan TV, Greiner DL et al. (1995) Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J Immunol* 154: 180–191.
- Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, Varadi G, Kirschbaum M, Ackerstein A, Samuel S, Amar A, Brautbar C, Ben-Tal O, Eldor A & Or R (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 91: 756–763.
- Smith AG (2001) Embryo-derived stem cells: Of mice and men. *Annu Rev Cell Dev Biol* 17: 435–462.
- Storb R, Yu C, Sandmaier BM, McSweeney PA, Georges G, Nash RA & Woolfrey A (1999) Mixed hematopoietic chimerism after marrow allografts. Transplantation in the ambulatory care setting. *Ann N Y Acad Sci* 872: 372–375; discussion 375–376.
- Sutherland HJ, Eaves CJ, Eaves AC, Dragowska W & Lansdorp PM (1989) Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis *in vitro*. *Blood* 74: 1563–1570.
- Szilyvassy SJ, Meyerrose TE & Grimes B (2000) Effects of cell cycle activation on the short-term engraftment properties of *ex vivo* expanded murine hematopoietic cells. *Blood* 95: 2829–2837.
- Tabbara IA, Zimmerman K, Morgan C & Nahleh Z (2002) Allogeneic hematopoietic stem cell transplantation: complications and results. *Arch Intern Med* 162: 1558–1566.
- Takeuchi M, Sekiguchi T, Hara T, Kinoshita T & Miyajima A (2002) Cultivation of aorta-gonad-mesonephros-derived hematopoietic stem cells in the fetal liver microenvironment amplifies long-term repopulating activity and enhances engraftment to the bone marrow. *Blood* 99: 1190–1196.
- Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE & Scott EW (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 416: 542–545.
- Terstappen LW, Huang S, Safford M, Lansdorp PM & Loken MR (1991) Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+CD38– progenitor cells. *Blood* 77: 1218–1227.
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS & Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145–1147.
- Thomson JA & Marshall VS (1998) Primate embryonic stem cells. *Curr Top Dev Biol* 38: 133–165.
- Thornley I, Sutherland DR, Nayar R, Sung L, Freedman MH & Messner HA (2001) Replicative stress after allogeneic bone marrow transplantation: changes in cycling of CD34+CD90+ and CD34+CD90– hematopoietic progenitors. *Blood* 97: 1876–1878.
- Tisdale JF & Wilson DR (eds) (2002) Clinical applications of gene therapy. *Current Opinion in Molecular Therapeutics*, 4: 409–534.
- Traycoff CM, Hoffman R, Zanjani ED, Cornetta K, Law P, Gianni AM, Bregni M, Siena S, Abboud MR, Laver J et al. (1994) Measurement of marrow repopulating potential of human hematopoietic progenitor and stem cells using a fetal sheep model. *Prog Clin Biol Res* 389: 281–291.
- Tsai RY, Kittappa R & McKay RD (2002) Plasticity, niches, and the use of stem cells. *Dev Cell* 2: 707–712.
- Turner CW, Yeager AM, Waller EK, Wingard JR & Fleming WH (1996) Engraftment potential of different sources of human hematopoietic progenitor cells in BXH Mice. *Blood* 87: 3237–3244.
- Ueda T, Yoshino H, Kobayashi K, Kawahata M, Ebihara Y, Ito M, Asano S, Nakahata T & Tsuji K (2000) Hematopoietic repopulating ability of cord blood CD34(+) cells in NOD/Shi-scid mice. *Stem Cells* 18: 204–213.
- Van Den Berg DJ, Sharma AK, Bruno E & Hoffman R (1998) Role of members of the Wnt gene family in human hematopoiesis. *Blood* 92: 3189–3202.
- Vassiliou G, Amrolia P & Roberts IA (2001) Allogeneic transplantation for haemoglobinopathies. *Best Pract Res Clin Haematol* 14: 807–822.
- Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB & Lansdorp PM (1994) Evidence for a mitotic clock in human hem-

- atopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci USA* 91: 9857–9860.
- Verfaillie CM, Almeida-Porada G, Wissink S & Zanjani ED (2000) Kinetics of engraftment of CD34(–) and CD34(+) cells from mobilized blood differs from that of CD34(–) and CD34(+) cells from bone marrow. *Exp Hematol* 28: 1071–1079.
- Wagers AJ, Sherwood RI, Christensen JL & Weissman IL (2002) Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297: 2256–2259.
- Wang JC, Lapidot T, Cashman JD, Doedens M, Addy L, Sutherland DR, Nayar R, Laraya P, Minden M, Keating A, Eaves AC, Eaves CJ & Dick JE (1998) High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood* 91: 2406–2414.
- Weaver A, Ryder D, Crowther D, Dexter TM & Testa NG (1996) Increased numbers of long-term culture-initiating cells in the apheresis product of patients randomized to receive increasing doses of stem cell factor administered in combination with chemotherapy and a standard dose of granulocyte colony-stimulating factor. *Blood* 88: 3323–3328.
- Wiles MV & Keller G (1991) Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development* 111: 259–267.
- Woods NB, Ooka A & Karlsson S (2002) Development of gene therapy for hematopoietic stem cells using lentiviral vectors. *Leukemia* 16: 563–569.
- Wynn R, Thornley I, Freedman M & Saunders EF (1999) Telomere shortening in leucocyte subsets of long-term survivors of allogeneic bone marrow transplantation. *Br J Haematol* 105: 997–1001.
- Wynn RF, Cross MA, Hatton C, Will AM, Lashford LS, Dexter TM & Testa NG (1998) Accelerated telomere shortening in young recipients of allogeneic bone marrow transplants. *Lancet* 351: 178–181.
- Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, Naito M & Nakao K (2000) Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature* 408: 92–96.
- Yan XQ, Hartley C, McElroy P, Chang A, McCrea C & McNiece I (1995) Peripheral blood progenitor cells mobilized by recombinant human granulocyte colony-stimulating factor plus recombinant rat stem cell factor contain long-term engrafting cells capable of cellular proliferation for more than two years as shown by serial transplantation in mice. *Blood* 85: 2303–2307.
- Ying QL, Nichols J, Evans EP & Smith AG (2002) Changing potency by spontaneous fusion. *Nature* 416: 545–548.
- Zanjani ED, Ascensao JL, Flake AW, Harrison MR & Tavasoli M (1992) The fetus as an optimal donor and recipient of hemopoietic stem cells. *Bone Marrow Transplant* 10: 107–114.
- Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H & Sorrentino BP (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 7: 1028–1034.
- Ziegler BL, Valtieri M, Porada GA, De Maria R, Muller R, Masella B, Gabbianelli M, Casella I, Pelosi E, Bock T, Zanjani ED & Peschle C (1999) KDR receptor: A key marker defining hematopoietic stem cells. *Science* 285: 1553–1558.
- Zon LI (1995) Developmental biology of hematopoiesis. *Blood* 86: 2876–2891.