review
Haematologica 1995; 80:367-387

CD34-POSITIVE CELLS: BIOLOGY AND CLINICAL RELEVANCE

Carmelo Carlo Stella, Mario Cazzola,* Paolo De Fabritiis,° Armando De Vincentiis,# Alessandro Massimo Gianni,@ Francesco Lanza,^ Francesco Lauria,** Roberto M. Lemoli,°° Corrado Tarella,## Paola Zanon,@@ Sante Tura°°

*Cattedra di Ematologia, Università di Parma, Parma; *Dipartimento di Medicina Interna e Terapia Medica, Sezione di Medicina Interna e Oncologia Medica, Università di Pavia e IRCCS Policlinico S. Matteo, Pavia; °Dipartimento di Biopatologia Umana, Sezione di Ematologia, Università "La Sapienza", Roma; #Dompé Biotec SpA, Milano; @Centro Trapianto Midollo Osseo "Cristina Gandini", Divisione di Oncologia Medica C, Istituto Nazionale Tumori, Milano; ^Istituto di Ematologia e Fisiopatologia dell'Emostasi, Università degli Studi di Ferrara, Ferrara; **Istituto di Scienze Mediche, Università degli Studi di Milano, Milano; °°Istituto di Ematologia "Lorenzo e Ariosto Seragnoli", Università degli Studi di Bologna, Bologna; ##Cattedra di Ematologia, Università di Torino, Torino; @@Amgen S.p.A., Milano, Italy*

The CD34-positive cell: definition and morphology

Cellular expression of the CD34 antigen identifies a morphologically and immunologically heterogeneous cell population that is functionally characterized by the *in vitro* capability to generate clonal aggregates derived from early and late progenitors and the *in vivo* capacity to reconstitute the myelo-lymphopoietic system in a supralethally irradiated host.[1-3]

Immunohistochemical studies have demonstrated that the CD34 antigen is stage but not lineage specific. In fact, independently of the differentiative lineage, it is expressed only by ontogenetically early cells.[4] For years a major obstacle to the morphological identification of putative hematopoietic stem cell has been the difficulty in separating them from their direct progeny. The use of CD34 and other suitable cell surface markers (i.e. CD33, CD38, HLA-DR antigens) in fluorescence-activated cell-sorting techniques or other cell separation methods has allowed considerable progress in this field.

Positively selected, lineage committed $CD34^+$ cells and more immature, lineage negative $CD34^+$ $CD33^-$ HLA-DR- cells are shown in Figure 1 and Figure 2, respectively. On May-Grünwald-Giemsa stained preparations, $CD34^+$ cells are medium sized cells having large nuclei, eccentrically surrounded by narrow rims of deep blue cytoplasm occasionally containing cytoplasmic granules. Some normal $CD34^+$ cell nuclei show one or more pale blue nucleoli. Taken together, these findings reflect the heterogeneous proliferative status and protein synthesis of this cell population. Conversely, earlier hematopoietic progenitors, identified as $CD34^+$ $CD33^-$ $HLA\text{-}DR^-$, seem to be more homogeneous in size (small lymphocyte-like cells) and lack cytoplasmic granules and prominent nucleoli. Again, the morphology of this cellular population appears to reflect the functional characteristics of these cells (e.g. low protein synthesis, very low proliferative activity with predominantly G_0 phase).

Several monoclonal antibodies (MY10, 12.8, B1-3C5, 115.2, ICH3, TUK3, etc.) raised against the leukemic cell lines KG1 or KG1a and an anti-endothelial cell antibody (QBEND10) assigned to the CD34 cluster have been shown to identify a transmembrane glycoproteic antigen of 105-120 kD expressed on 1-3% of normal bone marrow cells, 0.01-0.1% peripheral blood cells and 0.1-0.4% cord blood cells.[5] Different antibodies recognize distinct epitopes of the same antigen. CD34 antigen expression is associated with concomitant expression of several other markers that can be classified as lineage non-specific markers (Thy1, CD38, HLA-DR, CD45RA, CD71) and lineage specific markers, including T-lymphoid (TdT, CD10, CD7, CD5, CD2), B-lymphoid (TdT, CD10, CD19), myeloid (CD33, CD13) and megakaryocytic (CD61, CD41, CD42b) markers.[5] The expression of lineage non-specific markers allows the heterogeneous $CD34^+$ population to

Correspondence: Prof. Sante Tura, Istituto di Ematologia L. e A. Seràgnoli, Policlinico S. Orsola, via Massarenti 9, 40138 Bologna, Italy.
Acknowledgments: the preparation of this manuscript was supported by grants from Dompé Biotec SpA, Milano, and Amgen S.p.A., Milano, Italy.
Received October 18, 1994; accepted May 2, 1995.

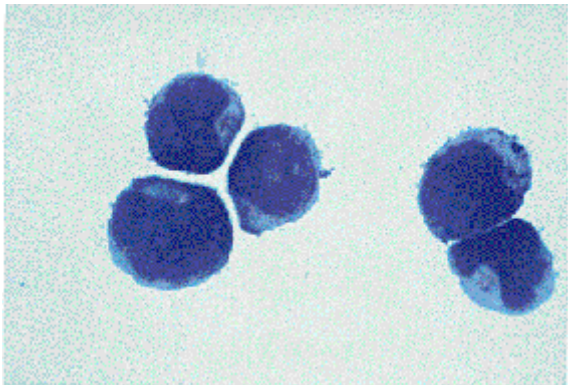

Figure 1. May-Grünwald-Giemsa (MGG) staining of cytospin preparation of enriched $CD34^+$ cells, highly purified by avidin-biotin immunoaffinity.

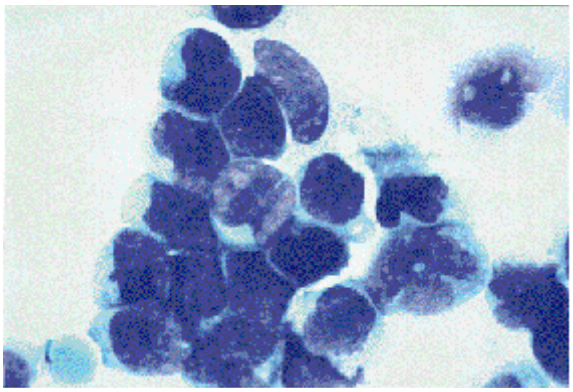

Figure 2. MGG staining of cytospin preparation of enriched $CD34^+$ $CD33^-$ $HLA\text{-}DR^-$ cells. The $CD34^+$ cell fraction was further depleted of $CD33^+$ $HLA\text{-}DR^+$ cells by immunomagnetic separation.

be divided into two distinct subpopulations characterized, respectively, by low or high expression of Thy1, CD38, HLA-DR, CD45RA, CD71. These two cell subpopulations contain early and late hematopoietic progenitor cells, respectively.[6-8]

In addition to conventional immunological markers classified on the basis of their assignation to specific clusters of differentiation, CD34 cells express receptors for a number of growth factors. Two distinct families of related receptors have been identified: (i) tyrosine kinase receptors, including the stem cell factor receptor (SCF-R, CD117) and the macrophage colony-stimulating factor receptor (M-CSF-R, CD115); (ii) hematopoietic receptors not containing a tyrosine kinase domain, such as the granulocyte-macrophage colony-stimulating factor receptor (GM-CSF-R, CDw116).[5,9]

The identification of new markers selectively expressed on primitive lymphohematopoietic cells ($CD34^+$ $CD38^-$) represents a stimulating research field. In this context, stem cell tyrosine kinase receptors (STK), such as STK-1, a human homologue of the murine Flk-2/Flt-3, are of particular relevance.[10-12] The ligands for these receptors might represent new factors able to selectively control stem cell self-renewal, proliferation and differentiation.[13-15]

Clonogenic and biologic activity

The structural and functional integrity of the hematopoietic system is maintained by a relatively small population of stem cells, located mainly in the bone marrow, that can (i) undergo self-renewal to produce more stem cells or (ii) differentiate to produce progeny which are progressively unable to self-renew, irreversibly committed to one or another of the various hematopoietic lineages, and able to generate clones of up to 10^5 lineage-restricted cells that mature into specialized cells.[16-18]

The decision of a stem cell to either self-renew or differentiate and the selection of a specific differentiation lineage by a multipotent progenitor during commitment are intrinsic properties of stem cell progenitor cells and are regulated by stochastic mechanisms.[19] Survival and amplification of hematopoietic progenitors are controlled by a number of regulatory molecules (hematopoietic growth factors) interacting according to complex modalities (synergism, recruitment, antagonism).[19] A further level of hematopoietic control is exerted by nuclear transcription factors that activate lineage-specific genes regulating growth factor responsiveness and/or the proliferative capacity of hematopoietic cells.[20]

Detection of the most primitive hematopoietic cell types is now possible due to the technique of long-term bone marrow culture. In the case of human bone marrow, a 5- to 8-week time period between initiating cultures and assessing clonogenic progenitor numbers allows quantification of a very primitive cell in the starting population, the so-called *long-term culture-initiating cell* (LTC-IC).[21] Committed prog-

enitors of the various hematopoietic cell classes can be quantitated by a number of short-term culture clonogenic assays.[19] CD34 antigen expression associated with low CD38 and CD45RA expression and variable HLA-DR expression is a typical feature of LTC-IC, CFU-Blast, CFU-T, CFU-B. In contrast, CD34 antigen expression associated with CD38 and HLA-DR expression is a typical feature of multipotent (CFU-GEMM) and lineage-restricted (CFU-GM, CFU-G, CFU-M, BFU-E, CFU-E, BFU-Meg, CFU-Meg) hematopoietic progenitor cells[5] (Figure 3). Recently reported data have shown that low or absent expression of the Thy1 or SCF receptor can be efficiently used to enrich primitive hematopoietic progenitors from the heterogeneous $CD34^+$ cell population.[7,9] Although the CD34 antigen is virtually expressed by all progenitor cells, the percentage of $CD34^+$ cells with assayable *in vitro* clonogenic activity ranges from 10 to 30%. The problem of non-clonogenic $CD34^+$ cells is still open and not adequately explained by the presence of lymphoid progenitors which are not assayable with current *in vitro* systems. Non-proliferating $CD34^+$ cells might represent a subpopulation that is not responsive to conventional myeloid hematopoietic growth factors. The non-proliferating $CD34^+$ subset might require the presence of co-factors, such as the ligand of STK-1 or the hepatocyte growth factor, able to activate stem cell-specific genes whose expression is a prerequisite for acquiring responsiveness to conventional growth factors.[14,22,23]

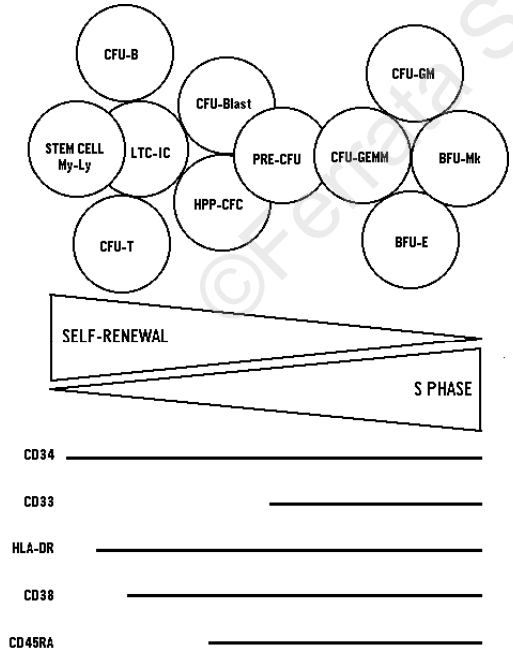

Figure 3. Cellular organization of the hematopoietic system.

In lethally irradiated non-human primates, both autologous and allogeneic $CD34^+$ cells have been shown to have the capacity to reconstitute the myelo-lymphopoietic system, thus suggesting that the stem cell responsible for hematopoietic reconstitution is $CD34^+$.[24,25] It has also been shown that human $CD34^+$ $HLA\text{-}DR^-$ cells transplanted in utero in the fetus of sheep initiate and sustain a chimeric hematopoiesis producing human progenitor cells of all differentiative lineages.[26] Autologous $CD34^+$ cells enriched by avidin-biotin columns have been shown to be able to reconstitute myelo-lymphopoiesis in patients receiving high-dose chemoradiotherapy.[27] The results of studies using $CD34^+$ bone marrow cells in the allogeneic setting in patients receiving both related as well as unrelated allogeneic marrow transplants will soon be available.[27] In addition, trials are planned that will use allogeneic peripheral blood $CD34^+$ cells either alone or with marrow.[27]

Characterization and function of the CD34 cell surface molecule

The CD34 cell surface molecule has been biochemically characterized and both the human cDNA and gene have been cloned and sequenced in the last few years.[28,29]

CD34 is a one-pass type I transmembrane glycoprotein with a molecular weight of 105-120 kDs in either the reduced or unreduced form[28] (Figure 4). CD34 protein is not homologous to any other known protein. The minimum size of the CD34 protein is 354-amino acids and contains nine sites for N-glycosylation and a several for O-glycosylation that are essential constituents of the three epitopes of the molecule; this

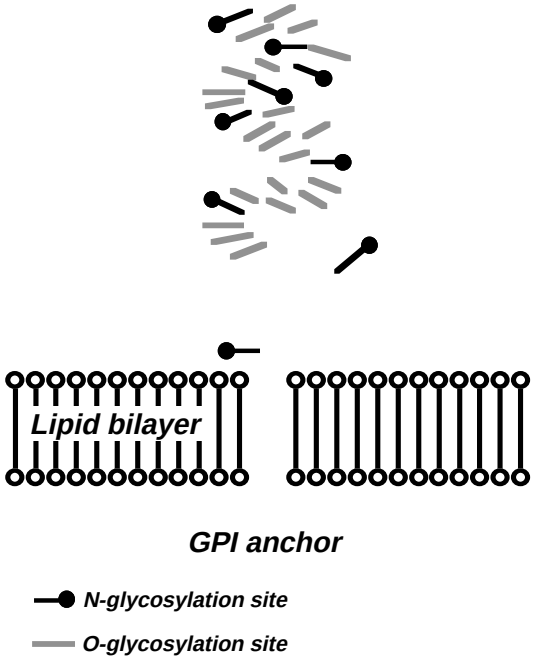

Figure 4. Schematic representation of the CD34 cell surface molecule.

molecule is also rich in sialic acid. Its biochemical composition suggests a mucin-like structure and in some respects resembles leucosialin (CD43). Sequence comparisons between human and mouse CD34 show a very low level of identity in the glycosylated region, 70% identity in the globular domain, and 92% in the transmembrane and cytoplasmic regions.

Using a KG1 cell line library, it has been shown that the human CD34 gene is located on chromosome 1, and recent studies with *in situ* hybridization have assigned its localization to band 1q32, in close proximity to other genes that encode growth factors or function molecules such as CD1, CD45, TGF2, laminin, LAM/GMP, etc.[28]

Seven CD34 monoclonal antibodies (MoAbs) were clustered at the 4th Workshop on Leukocyte Differentiation Antigens (Vienna, 1988),[30,31] and another 15 MoAbs were verified as recognizing the CD34 molecule during the 5th International Workshop on Leukocyte Differentiation Antigens (Boston, 1983), the most direct evidence being reactivity with cells transfected with CD34 cDNA and binding to CD34 protein.[32,33] The epitope specificity of the CD34 antibodies was classified into three distinct groups according to the sensitivity of the epitopes to enzymatic cleavage (which was performed using neuraminidase, chymopapain and glycoprotease from *Pasteurella haemolytica*), reactivity with fibroblasts and high endothelial venules, and cross blocking experiments (Table 1).[34,35] We know, in fact, that glycoprotease from *Pasteurella haemolytica* specifically cleaves only proteins containing sialylated O-linked glycans.[34] Based on these data, it can be further postulated that class III epitopes are more proximal to the extracellular side of the cell membrane than class I and class III epitopes.

Furthermore, for most CD34 MoAbs (with few exceptions) cross blocking experiments are

Epitope class	*Clones*	*CD34 reactivity*		
		paraffin section	frozen section	western blotting
I. Sensitive to neuraminidase (from *Vibrio cholerae*), chymopapain, glycoprotease (from *Pasteurella haemolytica*)	14G3, BI3C5, My10,* 12.8,* ICH3,* Immu-133, Immu-409	positive	negative	positive
II. Resistant to neuraminidase. Glycoprotease and chymopapain sensitive	43A1, MD34.3, MD34.1, MD34.2, QBend10, 4A1, 9044, 9049	positive	positive	positive
III. Resistant to neuraminidase, chymopapain and glycoprotease	CD34 9F2,HPCA2, 581, 553.563, Tuk 3, 115.2	negative	positive	negative

*Incomplete digestion by neuroaminidase.

Table 1. Epitope specificity of CD34 MoAbs as assessed by their differential sensitivity.

in agreement with the classification based on enzymatic cleavage of the CD34 protein. In other words, using a cocktail of CD34 MoAbs, CD34 reactivity is blocked only in the case that MoAbs belonging to the same CD34 epitope are simultaneously employed. On the contrary, the combined use of MoAbs recognizing class II and III or class I and II or class III epitopes does not affect cell reactivity. Moreover, CD34 MoAbs defining class III epitopes are unable to react with CD34 glycoprotein in Western blots because this epitope is sensitive to denaturation.

The pattern of expression of CD34 antibodies exhibited by $CD34^+$ acute leukemias is partially in accordance with that derived from epitope mapping based on the differential sensitivity of CD34 to enzymatic treatment. However, about one third of CD34 MoAbs do not seem to belong to any of these subgroups and for this peculiar pattern of expression are referred to as *atypical* CD34 reagents. The widest variation in CD34 MoAb reactivity has been demonstrated in acute myeloid leukemia (AML) samples, allowing us to postulate the occurrence of aberrant antigens or of distinct epitopes in subgroups of leukemias. Alternatively, it can be hypothesized that the expression of different antibodies could reflect the degree of maturation of leukemic cells. The presence of new, distinct non overlapping epitopes could be proposed on the basis of the data published so far in the literature. As far as the expression of CD34 in normal and leukemic cells is concerned, it has been calculated by flow cytometry that the number of molecular equivalents of soluble fluorochrome (MESF) expressed by leukemic and normal progenitors ranges from 18,200 to 322,000 and from 8,000 to 124,000, respectively.

Recent data collected by Lanza *et al.*[36] seem to indicate that class I-type MoAbs are more sensitive to freezing procedures than class II and III MoAbs, since the epitope is not identifiable following a frozen/thawed methodology.

The function of CD34 surface glycoprotein in hematopoietic stem and progenitor cells is still the object of debate. In light of recent findings, it would seem to play a relevant role in modulating cell adhesion.[36] Furthermore, it has been demonstrated that CD34 probably acts as an adhesive ligand for L-selectin. It has been further postulated that the CD34 molecule could play a protective role against proteolytic enzyme-mediated damage due to its high number of O-glycosylation sites. The cytoplasmic domain contains two sites for protein kinase C phosphorylation and one for tyrosine phosphorylation.[28]

Given the discordant reactivity of these molecules, the choice of the CD34 MoAb to employ may be important when analyzing cell positivity for the CD34 molecule in both leukemic and normal samples, and may be responsible for the differences reported by various authors in the literature concerning the prognostic role played by this antigen in acute myeloid leukemias.[37] The type of CD34 MoAb used to enumerate progenitor cells is probably relevant in the peripheral blood stem cell autograft setting as well, since both early and late engraftment following transplantation are, to some extent, related to the number of hemopoietic stem cells collected at the time of blood or bone marrow harvests, and to the degree of progenitor cell maturation related to the expression of lineage markers such as HLA-DR, CD71, CD38, CD33, and myeloperoxidase.

Techniques for $CD34^+$ cell separation

A number of different techniques have been proposed for separating $CD34^+$ cells. The common aim is to produce a cell population with optimal purity and viability by means of a low cost, rapid and simple separation technique. The first separation techniques exploited parameters such as size and cell density and were represented by Ficoll-Hypaque and Percoll density gradients. In the last two decades, the development of monoclonal antibodies has allowed a more specific and careful cell selection by identifying surface antigens used as targets for cell separation (Table 2).

FACS (Fluorescence Activated Cell Sorter)

Flow cytometry is able to physically separate different populations after incubation of cells with fluorochrome-conjugated monoclonal

antibodies. This cell sorting technique can yield a highly purified (> 98%) cell population. In addition, the use of electronic gates allows selection and recovery of several subpopulations according to antigenic expression and different characteristics such as size and cytoplasmic granularity. This technique has been very useful for studying $CD34^+$ sub-populations but cannot be employed to select large numbers of cells due to its complexity and low recovery. The recent development of *high-speed cell sorting*, however, might allow clinical utilization of this technique.

Panning

Anti-CD34 monoclonal antibodies bound to one of the surfaces of cell culture flasks were recently used to select $CD34^+$ cells. When a cell suspension is introduced into the flask, the positive population is blocked on the plastic surface, while CD34 negative cells remain in suspension and can be easily eliminated. Adherent cells should contain the $CD34^+$ population with a viability > 90%.[38]

Immunomagnetic systems

Immunomagnetic beads are uniform, superparamagnetic, polystyrene beads with affinity purified anti-mouse Ig covalently bound to the surface. They are equally suited for negative and positive cell separation; the rosetted target cell can easily be isolated by applying a magnet on the outer wall of the test tube for 1-2 minutes. Immunomagnetic beads coupled with CD34 monoclonal antibodies can be utilized for positive selection of $CD34^+$ cells to obtain a population with > 80% viable $CD34^+$ cells.[39] Similarly, immunomagnetic beads can be employed for negative depletion with monoclonal antibodies binding lineage-specific antigens.

High-affinity chromatography based on biotin-avidin interaction

This method is based on an immunoadsorption technique that relies on the high affinity interaction between the protein avidin and the vitamin biotin. Avidin-biotin immunoadsorption has been employed for both positive selection and depletion of specific cell populations.[40] The instrument includes a set of non-reusable products including biotinylated anti CD34 antibody, plastic bags, filters and a column of avidin-biotin beads. An automated version controlled by a computer which guarantees reproducibility of operation and reduces risks of operator errors has been developed for dinical use. Its capacity has recently been increased so that a single column can process more than 50×10^{10} bone marrow or peripheral blood cells in 1-2 hours and sustain bone marrow engraftment in patients submitted to autograft.[41]

Table 2. Recovery of CD34-positive cells after different separation techniques.

	Enrichment	*Recovery*	*Large-scale separation*
	(% $CD34^+$ in the recovered population)	(% $CD34^+$ of the original sample)	
Negative depletion by immunomagnetic beads	20-60	30-60	no
Positive selection by immunomagnetic beads	60-80	30-60	yes
Fluorescence activated cell sorter (FACS)	> 95	30 - 50	time consuming
Panning	50 - 80	30 - 60	yes
Ceprate SC®	50 - 80	40 - 70	yes

CD34-positive subpopulations: phenotypic and functional analysis

The normal $CD34^+$ cell population likely contains progenitors of all human lympho-hematopoietic lineages, including stem cells capable of hematopoietic reconstitution after bone marrow transplantation.[1] Levels of CD34 expression decline with differentiation; consequently, the earliest clonogenic cells (CFU-blast, LTC-IC, etc.) express the highest levels of CD34, while the most differentiated (CFU-G, CFU-M, CFU-E, CFU-Meg) express only low levels of CD34 (Figure 5). The CD34 antigen has been used to identify, enumerate and isolate cells from different lympho-hematopoietic lin-

eages, as well as develop *in vitro* tests for indirect evaluation of cells with different functional and clonogenic capacity.[42]

Pre-clinical studies

Several animal-human systems have been created to utilize chimeras for the study of lympho-hematopoiesis *in vivo*. The first experiments demonstrated the feasibility of transplanting human fetal stem cells to sheep fetuses; the postnatal presence of human cells in the sheep was documented at several points in time.[43] Furthermore, some early $CD34^+$ subpopulations were able to repopulate sheep bone marrow; animals were transplanted in utero with $CD34^+/DR^-$ cells and the presence of a chimeric population with the functional characteristics of hemopoietic progenitors was demonstrated in the marrow and peripheral blood in a percentage of cases.[44] Berenson *et al.*[45] also showed how hematopoietic progenitors (positive for the Ia antigen and subsequently for the CD34 antigen) could reconstitute the marrow of lethally irradiated dogs. Of the seven animals treated, all showed complete marrow engraftment after reinfusion of Ia-positive cells; only one dog died from infection.[45] Similarly, marrow cells from 5 primates (baboons) were treated *in vitro* with a biotinylated anti-CD34 antibody and then passed through a column of avidin; after autograft, all the animals showed marrow engraftment followed by hematological reconstitution comparable to that of control animals.[46] Furthermore, the demonstration that allogeneic $CD34^+$ cells can reconstitute the hematopoietic system in lethally irradiated baboons confirmed that this cell population includes pluripotent stem cells.[25]

Lymphoid precursor cells

The $CD34^+$ cell compartment contains all the cells expressing terminal deoxynucleotidyl transferase (TdT), which is an intranuclear enzyme expressed in early lymphoid cells undergoing immunoglobulin or T-cell receptor gene rearrangement. Flow cytometry has shown that the great majority of TdT^+ cells in the marrow coexpress CD34, CD19 and CD10 (B-cell pre-

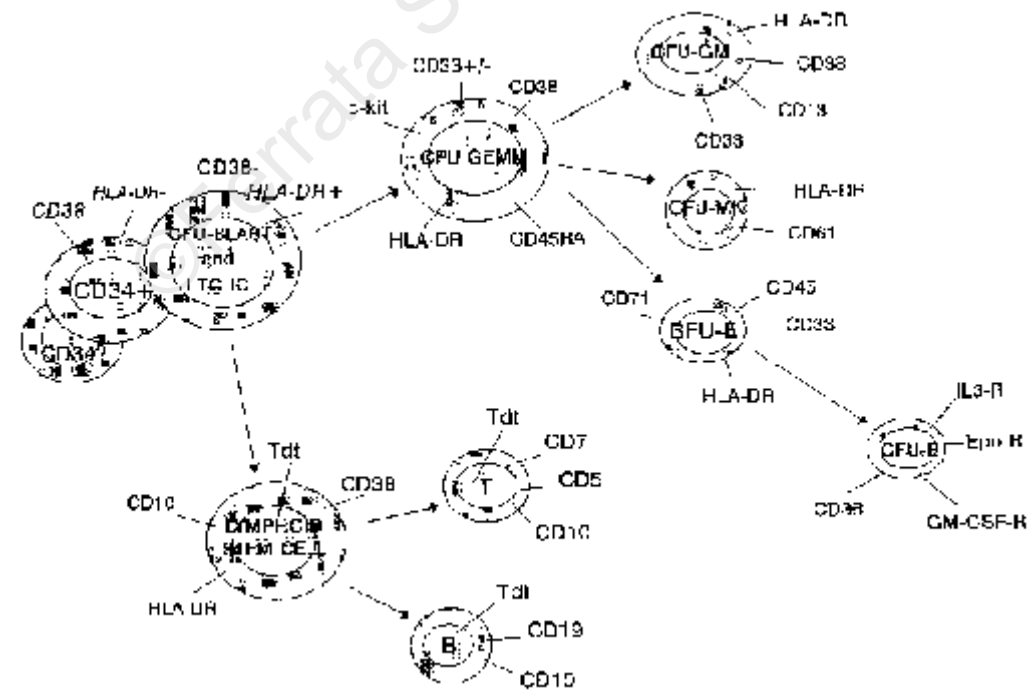

Figure 5. Functional differentiation and antigenic expression of hematopoietic cells.

cursors), as well as T-cell differentiation antigens such as CD7, CD5 and CD2. A small proportion of $CD34^+/TdT^+$ cells coexpress CD10, which might represent a common lymphoid progenitor for both B and T lineages.[47] Recently, Miller *et al.* reported that $CD34^+$ cells may also generate NK cells *in vitro*.[48]

Granulocyte-macrophage precursors

Marrow erythroid progenitors lack specific markers and therefore are difficult to identify. Glycophorin A-directed monoclonal antibodies recognize all hemoglobinized cells, but this molecule is expressed in only a small proportion of $CD34^+$ cells and is absent in clonogenic cells.[49]

High levels of CD45 are present on BFU-E, but this antigen is lost by the CFU-E stage;[50] however, the CD45RO isoform is well represented on earlier erythroid progenitors, while the CD45RA isoform is present on committed myeloid progenitors. The expression of CD71 (transferrin receptor) is currently considered to be the specific antigen for the $CD34^+$ erythroid population. CD71 is present at high levels on erythroid progenitor cells and at very low levels in all the other progenitor cells.[51] Expression of CD71 increases from stem cells to BFU-E, then declines during erythroid maturation. In addition, marrow $CD34^+$ erythroid cells might express IL-3, GM-CSF (CD116) and erythropoietin receptors, based on the action of these growth factors on CFU-erythroid cells.[52]

Myeloid precursor clonogenic cells (CFU-GM, CFU-G, CFU-M, CFU-MK) coexpress CD34, HLA-DR, CD117 (*c-kit*), CD45RA, CD33 and CD13; CD15 is present at low levels on CFU-G, while CFU-M specifically express CD115 (M-CSF receptor). CFU-MK are the only $CD34^+$ cells which express the platelet glycoproteins identified by the CD61, CD42 and CD41 monoclonal antibodies.[5] Dendritic cells also originate from bone marrow, but the conditions that direct their growth and differentiation are still poorly characterized. GM-CSF stimulates the growth of dendritic cells from mouse peripheral blood; however, it was recently reported that $CD34^+$ cells may give rise to dendritic/Langherans cells after stimulation with GM-CSF and tumor necrosis factor-α.[53]

Multilineage progenitors and stem cells

CFU-GEMM contain precursor clonogenic cells of both myeloid and erythroid lineages and express CD34, HLA-DR, CD38, CD117 and CD45RA. They also express low amounts of CD33, but not CD13. In a hypothetical differentiation scheme involving pluripotent stem cells, the lympho-hemopoietic compartment is the next cell type and can be identified *in vitro* with CFU-Blast and LTC-IC.

The lack of expression of CD38 is the most important characteristic of these early progeny, which represent 1% of CD34 and less than 0.01% of mononuclear cells. The lack of CD38 allows separation of committed progenitors ($CD34^+/CD38^+$) from earlier compartments ($CD34^+/CD38^-$) by a single marker combination.[54]

Furthermore, the earliest $CD34^+$ cells coexpress low levels of $CD45RO^6$ and are negative for staining with the fluorescent dye rhodamine 123. The role of HLA-DR in defining earlier cell types is still controversial; a series of evidence indicates that the stem cell compartment has the $CD34^+/CD38^-/HLA\text{-}DR^+$ phenotype.[8,54] Recent works, however, have not found HLA-DR in the earliest cells.[55] These data were confirmed by the possibility that HLA-DR expression may discriminate the Ph-positive leukemic compartment ($HLA\text{-}DR^+$) from normal residual cells ($HLA\text{-}DR^-$) in chronic myeloid leukemia.[56]

Adhesion molecules and cytokine receptors

A number of molecules within the integrin family have been shown to mediate interactions between early CD34-positive cells and bone marrow stromal cells. These include VLA-4/VCAM-1, VLA-5/fibronectin, LFA-1 or ICAM, and several others. Each adhesion molecule appears to mediate a specific cell interaction. Hematopoietic growth factors may be active in a soluble form or in a membrane-anchored form; adhesion molecules may be crucial for allowing anchored growth factors to bind the target cell. Table 3 lists the main adhesion molecule and growth factor receptors expressed on CD34-positive cells.

Table 3. Adhesion molecule and growth factor receptors expressed on CD34-positive cells.

Antigen name	*CD*	*Ligand*
GlyCAM-L/selectin	none	adhesion molecule
ICAM1,2,3	CD11a/CD18	adhesion molecule
H-CAM	CD44	adhesion molecule
VLA-4	CD49d/CD29	adhesion molecule
VLA-5	CD49d/CD29	adhesion molecule
FGF-R	none	growth factor
M-CSF	CD115	growth factor
GM-CSF-R	CD116	growth factor
SCF (c-kit)	CD117	growth factor
Interferon γ-R	CD119	growth factor
IL 7-R	CD127	growth factor

CD34 expression on normal and neoplastic cells

It has been known that CD34 monoclonal antibodies bind specifically to vascular endothelium ever since a 110 kd protein extracted from freshly isolated umbilical vessel endothelium was identified with CD34 antibodies in Western blots and in Northern blot analysis.[57,58] CD34 molecules have a striking ultrastructural localization on endothelial cells: they are concentrated primarily on the luminal side, in particular on membrane processes, many of which interdigitate between adjacent endothelial cells.[57,58] Since this region is an important site for leukocyte adhesion and transendothelial traffic, in contrast to previous experiences,[33] it has been hypothesized that CD34 may be antagonistic or inhibitory to the adhesive functions of vascular endothelium. This was supported by the demonstration that CD34 gene expression at the mRNA level is reciprocally down-regulated when adhesion molecules ICAM-1 and ELAM-1 are up-regulated by IL-1 during inflammatory skin lesions associated with leukocyte infiltration.[33,59] Furthermore, additional studies conducted on both paraffin embedded and cryopreserved sections demonstrated that fibroblasts also react with anti-CD34 MoAbs.[60] However, it should be noted that while CD34 MoAbs reacted with all classes of epitopes on cryopreserved sections, class III epitopes were not recognized by specific anti-CD34 MoAbs on paraffin embedded sections. Levels of CD34 expression, highest in immature hematopoietic precursor cells, decrease progressively with cell maturation.

Regarding hematologic malignancies, CD34 is expressed in a large percentage of acute leukemias.[61] The fluorescence intensity of CD34 expression is variable and higher in acute lymphoblastic (ALL) than in acute myeloblastic leukemia (AML). In these latter patients, the CD34 antigen is found on 40-60% of leukemic blasts and is most frequently associated with M0, M1 and M4 FAB cytotype, secondary leukemias, karyotypic abnormalities involving chromosome 5 or 7, P170 expression and poor prognosis.[61-64] Thus, CD34 expression may be considered the most predictive negative factor in AML patients strictly correlated with intensity of expression.[65,66] In ALL, CD34 is expressed in 70% of patients, particularly in those with a B-lineage phenotype. In these patients, unlike AML cases, the clinical relevance of CD34 expression is controversial; however, according to the findings of a Pediatric Oncology Group,[67] its expression in B-lineage cases was associated with hyperdiploidy, lower frequency of initial central nervous system (CNS) leukemia and a favorable prognosis. In T-cell ALL cases, on the other hand, CD34 expression showed a positive correlation with initial CNS leukemia and CD10 negativity, but not with any presenting favorable-risk characteristics.[67,68]

Lastly, CD34 and HLA-DR expression may be very useful in discriminating between the very few benign primitive hematopoietic progenitors and their malignant counterparts in patients with chronic myeloid leukemia (CML). In fact, it has been demonstrated that normal progenitor cells are $CD34^+$ and $HLA\text{-}DR^-$, while malignant progenitor cells, which exhibit Ph and bcr/abl rearrangement, express HLA-DR antigens.[56]

Positive and negative regulators of hematopoietic progenitor cells

The hematopoietic stem cell is defined by its

extensive self-renewal capacity, multilineage differentiation potential and capacity for of long-term reconstitution of normal marrow function in lethally irradiated animals.[68] Transplantation of retrovirally marked murine stem cells has shown that only a few multilineage progenitor cells induce the repopulation of engrafted hematopoietic tissue,[69] suggesting that hematopoiesis may be supported by a succession of short-lived clones. Moreover, experimental evidence indicates that the processes of self-renewal, differentiation and selection of lineage potentials are intrinsic properties of the stem cells and occur according to a stochastic (random) model.[70] In the steady state, most of the stem cells are quiescent (G_0 phase) and begin active cycling randomly.[71] Conversely, survival and proliferation of hematopoietic progenitors are regulated by cytokines, which also act in preventing apoptosis.[72] According to this model, the induction of differentiation by a cytokine may be considered as the consequence of the proliferation of a specific population stimulated by that factor. The broad term *cytokines* includes *growth factors* such as fibroblast growth factor, *colony stimulating factors* (CSFs) (e.g. granulocyte-CSF, granulocyte-macrophage-CSF), and interleukins (ILs). Depending on their target cells and different proliferative potential, cytokines can be divided into three categories:[71] 1) late-acting, lineage-specific factors; 2) intermediate-acting, lineage-nonspecific factors; 3) early-acting growth factors inducing the recruitment of dormant early progenitors in the cell cycle (Figure 6).[71]

The majority of late-acting factors support the proliferation and maturation of lineage-committed progenitors and the functional properties of terminally differentiated cells. Erythropoietin (Epo) is the physiologic regulator of erythropoiesis[73] and thrombopoietin has the same role in thrombopoiesis,[74] while M-CSF and IL-5 are considered specific for macrophages and eosinophils, respectively. G-CSF exerts its activity on committed neutrophil precursors, although it has been shown to be a synergistic factor for primitive hematopoietic cells.[75]

Intermediate-acting, lineage-nonspecific factors include IL-3, IL-4 and GM-CSF. Their activity is mainly directed toward progenitor cells in the intermediate stages of hematopoietic development. However, they interact with later-

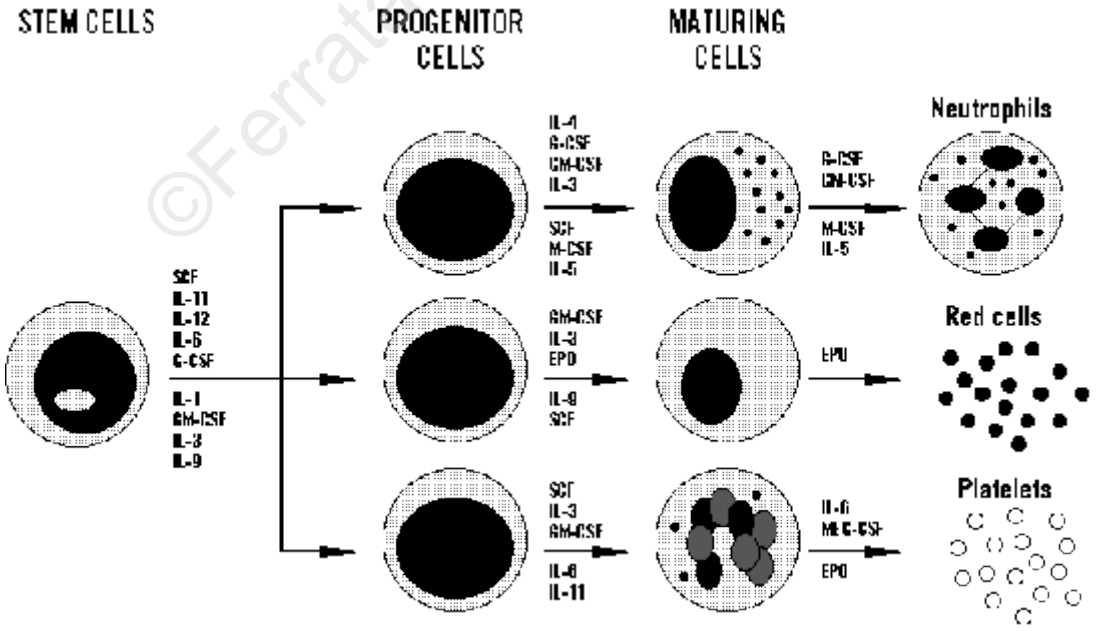

Figure 6. Cytokines exert their activity at different levels of the hematopoietic differentiation pathway. Each progenitor cell is concurrently affected by multiple regulators.

acting growth factors for the production of more mature cells,[76] as well as with the cytokines capable of triggering stem cell cycling. On their own, they act as survival factors for stem cells and appear to stimulate early hematopoietic precursors only after their exit from G_0.[77]

Several cytokines have recently been identified for their capacity to stimulate the proliferation of the earliest hematopoietic cells. Mapping studies of normal blast cell colony formation from single progenitors have shown that IL-6, G-CSF, IL-11, *stem cell factor* (SCF), IL-12 and *leukemia inhibitory factor* (LIF) recruit dormant stem cells into the cell cycle that are then able to respond to additional growth factors.[77] Whereas the permissive factors retain limited proliferative potential by themselves, they strongly enhance the stimulatory activity of IL-3, GM-CSF, G-CSF and EPO on $CD34^+$ and more immature $CD34^+$ lineage-progenitors.[78] In addition to positive interaction with intermediate-and late-acting growth factors, SCF synergistically or additively augments the colony-forming ability of other early-acting growth factors, such as IL-11, IL-12 and IL-6, on myeloid and bilineage (i.e. lymphomyeloid) primitive cells.[79] Recently, the ligands for the STK-1 or FLT3 receptor, and the hepatocyte growth factor were shown to be able to stimulate very primitive hematopoietic progenitor cells. However, their biological activity is still under investigation.

The main sources of both positive and negative regulatory proteins are accessory myeloid cells and the stromal component of the bone marrow. In general, microenvironment cells do not constitutively produce cytokines, rather transcription and/or translation processes are rapidly induced by cytokines such as IL-1, TNF. The extracellular matrix also participates in the regulation of hematopoiesis by binding growth factors and presenting them in a biologically active form to bone marrow progenitor cells.[80]

Most data suggest that stem cells express low levels of growth factor receptors and require multiple proliferative stimuli to enter into the cell cycle, while committed progenitor cells can be effectively stimulated by individual cytokines.[23] Combinations of two or more growth factors[81] can stimulate hematopoietic cells either by amplifying the progeny cell production of single precursors (synergy) or by inducing additional clonogenic cells to proliferate (recruitment). Examples of these two types of enhancement are given in Figures 7 and 8, where $CD34^+$ $CD33^-$ DR^- cells are simultaneously stimulated by two or three growth factors. The molecular basis regulating the complex interplay between cytokines is still largely unknown. However, one proposed mechanism for growth factor synergism is the induction of CSF receptors on early hematopoietic progenitor cells.[82] This appears to be a coordinate cascade transactivation via up-modulation of growth factor receptors that leads to proliferation and differentiation of human marrow cells.[83] Conversely, the structural homology between some growth factors,[84] the presence of shared receptor subunits on the cell membrane[85] and common signal-transduction

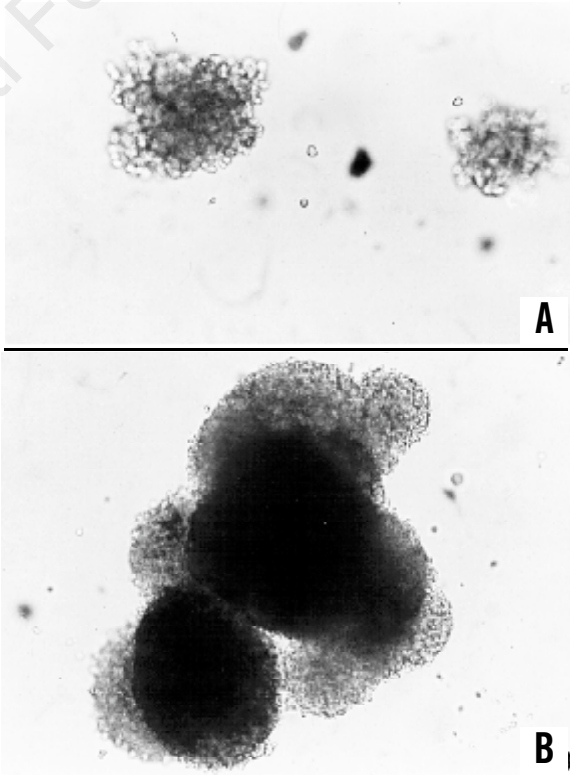

Figure 7. Human $CD34^+$ CD33- DR- cells were stimulated by IL-9 (A) or IL-9 and SCF (B) in the presence of EPO. The addition of SCF induced the growth of large multicentered BFU-E colonies containing > 10,000 cells. The different size of the colonies indicates amplification of stem cell progeny (synergy).

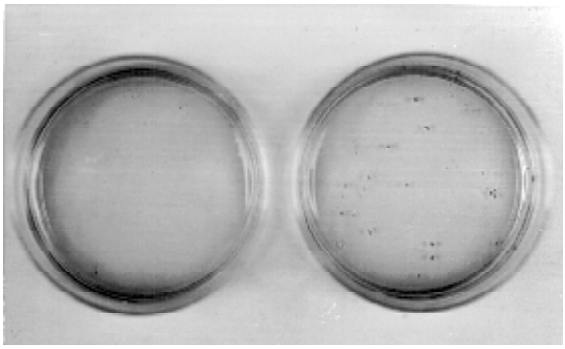

Figure 8. The addition of SCF to IL-11, in the presence of EPO, results in a much higher number of colony-forming cells derived from $CD34^+$ $CD33^-$ DR^- progenitors (right), as compared to the IL-11 and EPO combination (left) (recruitment).

proteins[86] provide a potential explanation for their functional similarities and the apparent redundancy of their activity.

The growth of hematopoietic progenitor cells is also regulated by soluble negative regulators such as macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor-α (TNF-α), interferons (IFNs), prostaglandins and transforming growth factor-β (TGF-β). The TGF-β family of proteins includes at least five isoforms (TGF-β1-5) which are encoded by different genes[87] and produced by stromal cells, platelets and bone cells. Moreover, a subset of very primitive murine hematopoietic cells has been shown to secrete active TGF-β1 by an autocrine mechanism.[88] TGF-β1 and 2 isoforms are bimodal regulators of murine and human hematopoietic progenitor cells, and their activity is based upon the differentiation state of the target cells and the presence of growth factors.[89] In the human system, for instance, CFU-GEMM derived from purified $CD34^+$ $CD33^-$ cells are inhibited by TGF-β1, whereas more committed progenitors such as CFU-G or CFU-GM are not affected. In addition, high proliferative potential-CFC (HPP-CFC) responsive to a combination of CSFs are markedly inhibited by TGF-β1, while more mature CFU-GM are actually enhanced when GM-CSF is used as colony forming factor.[78] TGF-β1 and 2-induced myelosuppression is partially counteracted by early-acting growth factors such as G-CSF, IL-6 and fibroblast growth factor.[90] On the other hand, TGF-β3 has been shown to be a more potent suppressor of human BM precursors and its activity on hematopoiesis is only inhibitory,[89] although the synergistic growth factors IL-11 and IL-9 seem to be able to oppose the negative regulation of TGF-β3 on human $CD34^+$ cells.[91] Several potential modes of action of the TGF-β family have been suggested, including down-modulation of cytokine receptors,[92] interaction with the underphosphorylated form of the retinoblastoma gene product in late G_1 phase,[93] and alteration of gene expression.[94] Early studies have shown that TGF-β1 and 3 exert their activity on normal and leukemic cells by lengthening or arresting the G_1 phase of the cell cycle,[95] and this effect is functional to protect normal CD34 positive cells from the toxicity of alkylating agents *in vitro*.[96] More recent investigations have demonstrated that TGF-β regulates the responsiveness of mice hematopoietic cells to SCF, which is known to be the main synergistic factor for both murine and human stem cells, through a decrease in *c-kit* mRNA stability that leads to decreased cell-surface expression.[97]

Similarly to TGF-β, TNF-α has been reported to have both inhibitory and stimulatory effects on hematopoietic progenitor cells. TNF-α potentiated the IL-3 and GM-CSF-mediated growth of human $CD34^+$ cells in short-term liquid culture assay. However, it inhibited the growth promoting activity of G-CSF.[98] Two TNF receptors with molecular weights of 55 and 75 kd, respectively, have recently been identified.[99] Whereas the p55 receptor mediates TNF-α effects on committed progenitor cells, the p75 receptor is involved in signaling the inhibition of murine primitive cells.

Furthermore, it was recently shown that TNF-α is capable of inhibiting the multi-growth factor (GM-CSF, IL-3, G-CSF, SCF, IL-1)-dependent growth of human HPP-CFC derived from $CD34^+$ cells through interaction with both p55 and p75 receptors, while the p55 receptor exclusively mediates the bifunctional activity of TNF-α on more mature BM precursors responsive to single cytokines.[100]

MIP-1α is a peptide of 69-amino acids with a molecular weight of 7.8 kd produced by activated macrophages, T-cells and fibroblasts.[101] It belongs to a large family of putative cytokines

that includes MIP-1β, MIP-2 and IL-8 (chemokines). Biologic characterization has shown that MIP-1α enhances the M-CSF- and GM-CSF-dependent growth of CFU-GM, while it inhibits the colony-forming ability of hematopoietic precursors present in a cell population enriched for $CD34^{++}$ DR^{+} cells stimulated with erythropoietin, IL-3 and GM-CSF.[102]

Taken together, these results indicate that a complex interplay between positive and negative regulatory proteins determines the proliferation or inhibition of early hematopoietic progenitor cells (Figure 9). In general, the activity of inhibitors of hemopoiesis appears to be reversible, lineage-nonspecific and directed at the early stages of differentiation. In addition, TGF-β has shown some degree of differential activity between normal and neoplastic lymphoid cells.[96] Thus, negative regulators may be clinically relevant to the protection of the hematopoietic stem cell compartment from the dose-limiting toxicity of neoplastic disease therapy.[96,103,104]

Collection of $CD34^{+}$ cells

Bone marrow and peripheral blood are the only sources of immature hemopoietic precursors identified as $CD34^{+}$ cells. A diagnostic marrow sample contains only a few $CD34^{+}$ cells, while even fewer of them are present in peripheral blood samples taken under steady state conditions. Large quantities of $CD34^{+}$ cells can be collected with massive marrow harvests, such as for transplantation purposes. Nevertheless, marrow $CD34^{+}$ cells are somewhat elusive due to their scattering among the predominant $CD34^{+}$ hematopoietic population.[105] Recently developed cell separation procedures allow collection of highly enriched $CD34^{+}$ cell populations. However, this is generally accomplished through aspecific and often unacceptable cell loss.[106] So far the limited number of immature precursors commonly obtained from both bone marrow and peripheral blood has been the major obstade to a simple identification and analysis of marrow $CD34^{+}$ cells. Indeed, the growing interest in $CD34^{+}$ cells is primarily the result of the development of new therapeutic modalities that allow easy access to large quantities of hemopoietic precursors through the peripheral blood. The key role in these innovative approaches is represented by the introduction of hemopoietic growth factors for clinical use.[107]

At present GM-CSF and G-CSF are the most extensively employed and studied hemopoietic growth factors in the clinical setting. From the very beginning, it was observed that GM-CSF or G-CSF administration was associated with an increase in circulating hemopoietic progenitors.[108,109]

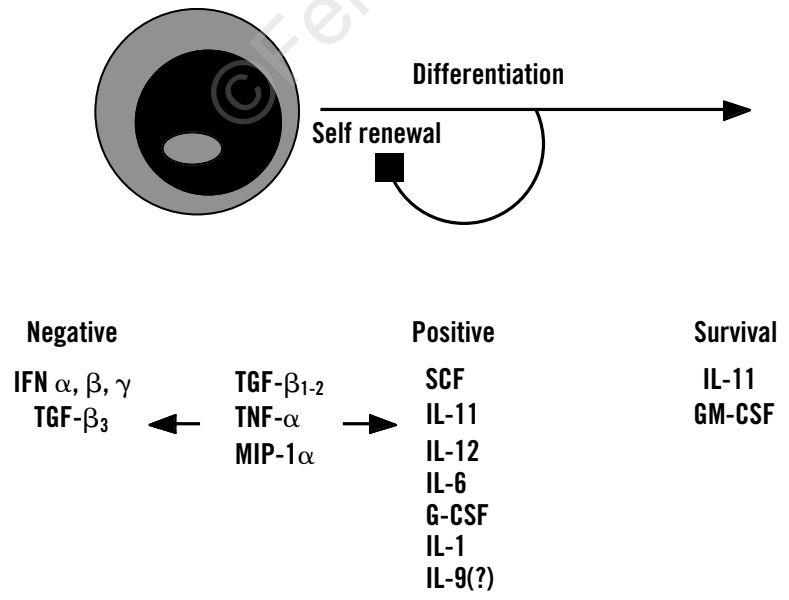

Figure 9. Interplay between positive and negative regulatory proteins acting on hematopoietic stem cells.

Later on, it was demonstrated that this phenomenon could be extensively and reproducibly amplified by combining growth factor administration with high-dose chemotherapy.[110-112]

Indeed hemopoietic progenitors are massively, though transiently, mobilized into peripheral blood during hemopoietic recovery following high-dose chemotherapy given with growth factor support. Such an abundance of immature hemopoietic cells makes them easily recognizable by cell sorting techniques that employ anti-CD34 MoAbs.[113] Under optimal conditions, the proportion of $CD34^+$ cells may reach values as high as 20-30% of the total leukocyte count. In steady state conditions $CD34^+$ cells do not exceed 4% of the total marrow population, while they are undetectable by cell sorting techniques in the peripheral blood. Chemotherapy and growth factors do not merely induce a relative increase of immature hemopoietic cells; their absolute number is amplified several times over basal conditions. This allows collection of sufficient amounts of hemopoietic progenitors for autografting purposes by means of a few leukapheresis procedures.[110,113,114]

Values of $10\text{-}20\times10^4$ CFU-GM/kg represent the minimal required dose for marrow engraftment with peripheral blood progenitors.[115-117] In fact, much higher quantities of circulating progenitors can be collected using appropriate mobilization procedures. Under optimal conditions, circulating $CD34^+$ cell peak values may range between 150 and 700/μL on days of maximal mobilization. As a rule, at least 8×10^6 $CD34^+$ cells/kg or more can thus be collected with 1 to 3 leukapheresis procedures.[114] These huge quantities, approximately corresponding to 50×10^4 CFU-GM/kg, must be considered the ideal threshold dose of peripheral blood progenitors for autografting purposes. Indeed values of 8×10^6 $CD34^+$ cells/kg or more guarantee a rapid and durable hemopoietic recovery when circulating progenitors are used as the sole source for marrow reconstitution following submyeloablative treatment.[118-121]

Thus far, massive $CD34^+$ cell mobilization has been most commonly observed when growth factor is administered following high-dose cyclophosphamide, given at 7 g/sqm. Indeed chemotherapy that induces profound leukocytopenia seems to be crucial for optimal mobilization; for instance, cyclophosphamide at doses lower than 7 g/sqm produces a reduced mobilizing stimulus.[111,122] Several other chemotherapy schedules have also been successfully employed for mobilization purposes. The principal chemotherapy protocols reported to be highly effective in inducing $CD34^+$ cell mobilization are summarized in Table 4.[110, 111, 115, 117, 122-127]

As stressed earlier, hemopoietic growth factors play a key role in mobilization. This has been clearly documented with cyclophosphamide. A median peak value of 75 $CD34^+$ cells/μL has been recorded following high-dose cyclophosphamide alone, whereas 420 and 500/μL are the median values recorded when GM-CSF or G-CSF, respectively, are added to cyclophosphamide.[112,113,128,129] Extensive growth factor-induced mobilization is further substantiated by the possibility of collecting sufficient $CD34^+$ cells using growth factor alone.[130,131] In this setting the most promising experiences have been

Table 4. Main chemotherapy protocols employed in hematopoietic progenitor mobilization.

Authors	*Protocol characteristics*	*Drug(s) employed*	*Dosage*
Gianni *et al.*[110]	single agent	cyclophosphamide	high
Tarella *et al.*[123] Gianni *et al.*[124]	single agent	etoposide	high
Kotasek *et al.*[121] Tarella *et al.*[122]	single agent	cyclophosphamide	intermediate
Dreyfus *et al.*[125]	single agent	cytarabine	high
Schimazaki *et al.*[126]	multiple drugs	etoposide cytarabine	high
Kawano *et al.*[115]	multiple drugs	daunorubicin cyclophosphamide etoposide	intermediate
Pettengell *et al.*[127]	multiple drugs	doxorubicin cyclophosphamide etoposide	intermediate
Hass *et al.*[117]	multiple drugs	cytarabine mitoxantrone	high

produced with G-CSF. The results reported indicate new opportunities for the utilization of mobilized progenitors in allogeneic transplantation procedures.[132,133] Combined chemotherapy and growth factors remain the most efficient mobilization procedure at this time. However, ongoing studies are directed at improving mobilization efficiency with growth factors alone. For example, G-CSF at doses higher than 5 μg/kg/day up to 20 μg/kg/day may have higher mobilization capacity.[134] Further improvement might derive from the clinical availability of new cytokines, such as IL-3, SCF and others, to be employed alone or in combination with G- or GM-CSF.[135-137] However, the potentially high mobilizing activity of such new cytokine combinations must be accompanied by no or very few side effects in order to be considered for a wide clinical applicability.

Mobilizing protocols generally include daily delivery of growth factors, starting 1 to 3 days after chemotherapy administration and continuing until harvesting procedures are completed. Growth factor is usually administered for a total of 7-12 consecutive days. The most convenient route of delivery is subcutaneous, with 1 to 2 doses per day. Progenitor cell harvests are performed during hemopoietic recovery, provided that progenitor cell mobilization is documented. Indeed various parameters have been considered as an indirect indication of progenitor mobilization, including an increase in WBC or, alternatively, in monocytes, basophils or platelets.[138-140] However, $CD34^+$ cell evaluation remains mandatory for an accurate definition of the extent of progenitor mobilization.[141,142]

$CD34^+$ cells should be monitored daily from the early stages of hemopoietic recovery. Detection of circulating $CD34^+$ cells is not sufficient reason for starting leukapheresis procedures; an adequate number of $CD34^+$ cells (>20-50/μL) and WBC > 1.0×10^9/L and platelet count > 30×10^9/L are required for safe and effective progenitor cell harvesting.[114,142] Leukapheresis are performed using continuous-flow blood cell separators. A complete leukapheresis procedure generally takes 2-3 hours and the total blood volume processed ranges from 6 to 10 liters.[114,140,143,144] The harvested cells are resuspended in freezing medium and then cryopreserved for subsequent transplantation. One to 3 leukapheresis repeated on consecutive days usually provide large amounts of progenitor cells capable of rapid engraftment after submyeloablative treatments.

Factors and procedures favoring $CD34^+$ cell mobilization have been well established. However, other conditions adversely influencing the mobilization phenomenon should be considered. A major limitation is represented by impaired marrow function, as can occur in previously treated patients.[111] In fact, patients at first relapse following a single treatment protocol maintain an adequate mobilization capacity;[145] however, few if any mobilized $CD34^+$ cells can be obtained from heavily treated patients previously exposed to multiple chemotherapy courses. Mobilization is also profoundly reduced and often totally abolished in patients previously exposed to radiotherapy, especially if it was delivered to the pelvis or to extended vertebral areas.[117,123] Lastly, marrow invasion by tumor cells may negatively affect mobilization capacity. This is typically reported in myeloma patients in whom the extent of $CD34^+$ cells often correlates with the degree of marrow involvement by tumoral plasma cells.[122] In conclusion, several new findings have dramatically improved the procedures for collection of large amounts of $CD34^+$ cells. However, optimal marrow function is a prerequisite for exploiting fully the activity of all those factors known for their mobilization-inducing capacity.

$CD34^+$ positive cells in umbilical cord blood

Significant numbers of human hematopoietic stem cells can be found in umbilical cord blood and can be used for allogeneic bone marrow transplantation. Mayani *et al.*[146] found that 1-2% of the total number of cord blood-derived low-density cells express high levels of the CD34 antigen and low or undetectable levels of the antigens CD45RA and CD71. These populations were highly enriched in clonogenic cells (34%), in particular in multipotent progenitors (12%). By culturing these cells at low concen-

tration for 8-10 days in highly defined serum-free liquid cultures supplemented with various hematopoietic cytokines, it was possible to achieve a significant expansion (about 100-fold) of the $CD34^+$ cell population.

These last results were recently confirmed by Traycott *et al.*[147] in an elegant study showing that umbilical cord blood $CD34^+$ cells rapidly exit G_0-G_1 phases and start to cycle in response to stem cell factor. This feature would make cord blood $CD34^+$ cells more suitable candidates than bone marrow cells for *ex vivo* expansion.

It is clear that *in vitro* expansion and maturation of hematopoietic progenitor cells might be of particular relevance for transplantation of cord blood hematopoietic cells; however, information about the effects of such an expansion on the cells required for long-term hematopoietic reconstitution is highly desirable.

The dual role of peripheral blood hematopoietic progenitor cells in oncohematology

$CD34^+$ progenitor cells circulating in the peripheral blood represent an enriched and easily accessible source of two distinct cell populations: committed progenitor cells and hematopoietic stem cells.

Although circulating progenitors are commonly called *stem cells*, the presence of circulating stem cells has been formally proved only in mice.[148] In humans the presence of hematopoietic stem cells in the peripheral blood is almost certain (see below), but to call reinfusion of circulating progenitors '*transplantation*' of peripheral blood stem cells (PBSC or similar acronyms) is hardly appropriate. This terminology overlooks the fact that the tremendous interest in peripheral blood autografting is not (at least so far) a consequence of its being a simple surrogate for autologous bone marrow transplantation (as the term PBSC would suggest), but rather derives from the unique property of this procedure to reduce the duration of the severe pancytopenia that follows submyeloablative treatments from two-three weeks (when bone marrow cells are used) to a few days only.[110,149] The reason for the rapid recovery which occurs after circulating progenitor autotransfusion (CPAT) has not been formally proved, but it is most likely a consequence of the much larger (10- to 100-fold higher) amount of committed progenitors reinfused when a patient is autografted with blood-derived (as opposed to marrow-derived) cells. The most likely hypothesis is that these late progenitors (post-progenitors) of granulocytes and platelets are capable of giving rise to mature progeny within a few days, thus allowing submyeloablated patients to survive the initial aplastic phase.

The fundamental role of committed progenitor cells was elegantly proved in mice by Jones *et al.*[150] None of the lethally irradiated animals transplanted with a pure population of stem cells free of more mature progenitors (CFU-GM, CFU-S) survived the initial aplasia. A more recent paper[151] challenged this '*conventional wisdom*' model, and maintained that peripheral blood stem cells *per se* are capable of rapidly maturing *in vivo*.

Other authors, using a very similar approach, reached an opposing conclusion.[152] In humans the most convincing, albeit indirect, evidence in favor of the role of committed progenitors in accelerating post-transplant recovery was provided by Robertson *et al.*,[153] who documented a significantly prolonged hematopoietic recovery for patients undergoing autologous bone marrow transplantation purged *ex vivo* with anti-CD33 monoclonal antibodies. This result, which occurred after a treatment that selectively kills the most mature progenitor cells (expressing the CD33 surface antigen), represents convincing indirect proof of the role of committed progenitors in early engraftment.

The clinical role of committed progenitors in reducing the morbidity and mortality of high-dose therapy has expanded since hemopoietic growth factors have become clinically available. In fact, infusion of rhGM-CSF or rhG-CSF, in particular after administration of myelotoxic doses of certain stemcell-sparing agents, allows easy collection of an amount of CFU-GM/kg body weight 10 to 100 times higher than that contained in a bone marrow harvest.[110]

As already documented by a large number of

papers, the use of circulating progenitors has brought about a dramatic change in the perspectives of high-dose submyeloablative regimens. In fact, these once specialized, expensive and highly toxic treatments are now well tolerated, easy to administer, and clinically useful (cost effective). As an example, it is worth mentioning the initial Milan Cancer Institute experience in a group of over 50 poor-risk breast cancer patients who received high-dose melphalan plus an optimal amount of circulating progenitors (5×10^4 CFU-GM/kg body weight). These patients required a median of 10.5, 11 and 12.5 days to score >0.5, 1.0 and 2.0×10^9/L neutrophils/μL, respectively. Moreover, more than half of them did not require platelet transfusions, while the remaining ones needed only one or two transfusions during the first week after autografting. Such mild to moderate toxicity was never described before the clinical availability of committed progenitor cells.

In conclusion, born as a *'compassionate'* surrogate of bone marrow autografting, today CPAT is rapidly replacing ABMT. In fact, today it is the latter that should be considered a *'compassionate need'* procedure, useful in those few patients unable to mobilize a sufficient number of circulating progenitor cells.

The second, distinct role of circulating progenitors is related to the presence of stem cells, i.e. totipotent precursors responsible for durable reconstitution of all lympho-hematopoietic lineages following marrow ablative therapy. Since virtually no high-dose treatment that can be safely administered to humans is genuinely myeloablative, formal proof of the existence of circulating stem cells must await either stable transduction of DNA markers into autografted cells or the use of this cell population for allografting.

These experiments are presently underway in several laboratories,[154,155] and preliminary data do confirm the presence of stem cells in the peripheral blood of humans. Their utilization is expected to revolutionize fields like allogeneic bone marrow transplantation and somatic gene therapy, whenever the target of genetic manipulations is the hematopoietic cell.[156]

References

1. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984; 133:157-65.
2. Sutherland RD, Keating A. The CD34 antigen: structure, biology and potential clinical applications. J Hematother 1992; 1:115-29.
3. Andrews RG, Singer JW, Bernstein ID. Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of the CD33 and CD34 antigens and light scatter properties. J Exp Med 1989; 169:1721-31.
4. Andrews RG, Singer JW, Bernstein ID. Monoclonal antibody 12.8 recognizes a 115-kd molecule present on both unipotent and multipotent hematopoietic colony-forming cells and their precursors. Blood 1986; 67:842-52.
5. Civin CI, Gore SD. Antigenic analysis of hematopoiesis: a review. J Hematother 1993; 2:137-44.
6. Lansdorp PM, Sutherland HJ, Eaves CJ. Selective expression of CD45 isoforms on functional subpopulations of $CD34^+$ hematopoietic cells from human bone marrow. J Exp Med 1990; 172:363-6.
7. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM. Isolation of a candidate human hematopoietic stem-cell population. Proc Natl Acad Sci USA 1992; 89:2804-8.
8. Huang S, Terstappen LWMM. Lymphoid and myeloid differentiation of single human $CD34^+$, $HLA\text{-}DR^-$, $CD38^-$ hematopoietic stem cells. Blood 1994; 83:1515-26.
9. Gunji Y, Nakamura M, Osawa H, et al. Human primitive hematopoietic progenitor cells are more enriched in KITlow cells than in KIThigh cells. Blood 1993; 82:3283-9.
10. Rosnet O, Marchetto S, de Lapeyriere O, Birnbaum D. Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. Oncogene 1991; 6:1641-50.
11. Matthews W, Jordan CT, Wiegand GW, Pardoll D, Lemischka IR. A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. Cell 1991; 65: 1143-52.
12. Small D, Levenstein M, Kim E, et al. STK-1, the human homologue of Flk-2/Flt-3, is selectively expressed in $CD34^+$ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. Proc Natl Acad Sci USA 1994; 91:459-63.
13. Lyman SD, James L, van den Bos T, et al. Molecular cloning of a ligand for the flt 3 / flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell 1993; 75: 1157-67.
14. Lyman SD, James L, Johnson L, et al. Cloning of the human homologue of the murine flt3 ligand: a growth factor for early hematopoietic progenitor cells. Blood 1994; 83:2795-801.
15. Hannum C, Culpepper J, Campbell D, et al. Ligand for FLT3/FLK2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant RNAs. Nature 1994; 368:643-8.
16. McCulloch EA. Stem cells in normal and leukemic hemopoiesis (Henry Stratton lecture, 1982). Blood 1983; 62:1-13.
17. Metcalf D. The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature 1989; 339:27-30.
18. Orlic D, Bodine DM. What defines a pluripotent hematopoietic stem cell (PHSC): will the real PHSC stand up! Blood 1994; 84:3919-94.
19. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81:2844-53.

20. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 1994; 371:221-6.
21. Sutherland HJ, Eaves CJ, Eaves AJ, Dragowskas W, Lansdorp PM. Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 1989; 74:1563-70.
22. Kmiecik TE, Keller JE, Rosen E, van de Woude GF. Hepatocyte growth factor is a synergistic factor for the growth of hematopoietic progenitor cells. Blood 1992; 80:2454-7.
23. Metcalf D. Hematopoietic regulators: redundancy or subtlety? Blood 1993; 82:3515-23.
24. Berenson RJ, Andrews RG, Bensinger WI, et al. CD34$^+$ marrow cells engraft lethally irradiated baboons. J Clin Invest 1988; 81:951-9.
25. Andrews RG, Bryant EM, Bartelmez SH, et al. CD34 marrow cells, devoid of T and B lymphocytes, reconstitute stable lymphopoiesis and myelopoiesis in lethally irradiated allogeneic baboons. Blood 1992; 80:1693-9.
26. Srour EF, Zanjani ED, Cornetta K, et al. Persistence of human multilineage, self-renewing lymphohematopoietic stem cells in chimeric sheep. Blood 1993; 82:3333-42.
27. Berenson R. Transplantation of hematopoietic stem cells. J Hematother 1993; 2:347-9.
28. Greaves MF, Brown J, Molgaard HV, et al. Molecular features of CD34: a hemopoietic progenitor cell-associated molecule. Leukemia 1992; 6:31-6.
29. Silvestri F, Banavali S, Baccarani M, Preisler HD. Progenitor cell associated antigen CD34: biology and clinical applications. Haematologica 1992; 77:265-72.
30. Peschle CH, Köller U. Cluster report: CD34. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGKr, eds. Leukocyte typing IV: white cell differentiation antigens. Oxford:Oxford University Press, 1989: 817-8.
31. Civin CI, Trishmann TM, Fackler MJ, et al. Report on the CD34 cluster workshop. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, von dem Borne AEGKr, eds. Leukocyte typing IV: white cell differentiation antigens. Oxford:Oxford University Press, 1989: 818-25.
32. Lanza F, Moretti S, Papa S, Malavasi F, Castoldi G. Report on the fifth international workshop on human leukocyte differentiation antigens, Boston, November 3-7, 1993. Haematologica 1994; 79:374-86.
33. Greaves MF, Colman SM, Bühring HJ, et al. Report on the CD34 cluster workshop. In: Schlossman SF , Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein RL, Springer TA, Tedder TF, Tood RF, eds. Leukocyte typing V: White cell differentiation antigens. Oxford:Oxford University Press, 1995, in press.
34. Sutherland DR, Marsh JCW, Davidson J, Baker MA, Keating A, Mellors A. Differential sensitivity of CD34 epitopes to cleavage by *Pasteurella haemolytica* glycoprotease: implications for purification of CD34-positive progenitor cells. Exp Hematol 1992; 20:590-9.
35. Lanza F, Castoldi GL. Large scale enrichment of CD34$^+$ cells by Percoll density gradients: a CML-based study design. In: Wunder E, Serke S, Solovat H, Henon P, eds. Hematopoietic stem cells. Dayton:AlphaMed Press, 1993:255-70.
36. Lanza F, Bi S, Castoldi GL, Goldman JM. Abnormal expression of N-CAM (CD56) adhesion molecule on myeloid and progenitor cells from chronic myeloid leukemia. Leukemia 1993; 7:1570-5.
37. Lanza F, Rigolin GM, Moretti S, Latorraca A, Castoldi GL. Prognostic value of immunophenotypic characteristic of blasts cells in acute myeloid leukemia. Leuk Lymphoma 1994; 13(Suppl 1):81-5.
38. Morecki S, Topalian SL, Myers WW, Okrongly D, Okarma TB, Rosenberg SA. Separation and growth of human CD4$^+$ and CD8$^+$ tumor infiltrating lymphocytes and peripheral blood mononuclear cells by direct positive panning on covalently attached monoclonal antibody coated-flasks. J Biol Resp Modifiers 1990; 9:463-74.
39. Civin CI, Strauss LC, Fackler MJ, Trismann TM, Wiley JM, Loken RL. Positive stem cell selection. Basic science. In: S Gross, A Gee, DA Worthington White, eds. Bone Marrow Purging and Processing. New York: Wiley-Liss, 1990:387-402.
40. Berenson RJ, Bensinger WI, Kalamasz D. Elimination of Daudi lymphoblasts from human bone marrow using avidin-biotin immunoadsorption. Blood 1986; 67:509-15.
41. Berenson RJ, Bensinger WI, Hill RS, et al. Engraftment after infusion of CD34$^+$ marrow cells in patients with breast cancer or neuroblastoma. Blood 1991; 77:1717-22.
42. Carlo-Stella C, Mangoni L, Piovani G, Garau D, Almici C, Rizzoli V. Identification of Philadelphia-negative granulocyte-macrophage colony-forming units generated by stroma-adherent cells from chronic myelogenous leukemia patients. Blood 1994; 83:1373-80.
43. Zanjani ED, Pallavicini MG, Ascensao JL, et al. Engraftment and long-term expression of human fetal hemopoietic stem cells in sheep following transplantation in utero. J Clin Invest 1992; 89:1178-88.
44. Srour EF, Zanjani ED, Brandt JE, et al. Sustained human hematopoiesis in sheep transplanted in utero during early gestation with fractionated adult human bone marrow cells. Blood 1992; 79:1404-12.
45. Berenson RJ, Bensinger WI, Kalamasz D, et al. Engraftment of dogs with Ia-positive marrow cells isolated by Avidin-Biotin immunoadsorption. Blood 1987; 69:1363-7.
46. Berenson RJ, Andrews RG, Bensinger WI, et al. Antigen CD34$^+$ marrow cells engraft lethally irradiated baboons. J Clin Invest 1988; 81:951-5.
47. Gore SD, Kastan MB, Civin CI. Normal human bone marrow precursors that express terminal deoxynucleotidyl transferase include T-cell precursors and possible lymphoid stem cells. Blood 1991; 77:1681-90.
48. Miller JS, Verfaillie C, McGlave P. The generation of human natural killer cells from CD34$^+$/DR$^-$ primitive progenitors in long term bone marrow culture. Blood 1992; 80:2182-7.
49. Loken MR, Civin CI, Bighee WL, Langlois RG, Jensen RH. Coordinate glycosylation and cell surface expression of glycophorin A during normal human erythropoiesis. Blood 1987; 70:1959-61.
50. Shaw VO, Civin CI, Loken MR. Flow cytometric analysis of human bone marrow. IV. Differential quantitative expression of T-200 common leukocyte antigen during normal hematopoiesis. J Immunol 1988; 140:1861-7.
51. Loken MR, Shaw VO, Dattilio K, Civin CI. Flow cytometric analysis of human bone marrow. I. Normal erythroid development. Blood 1987; 69:255-63.
52. Sawada K, Krantz SB, Kans JS, et al. Purification of human colony-forming units-erythroid and demonstration of specific binding of erythropoietin. J Clin Invest 1987; 80:357-66.
53. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-α cooperate in the generation of dendritic Langherans cells. Nature 1992; 360:258-61.
54. Terstappen LWMM, Huang S, Safford M, Lansdorp P, Loken MR. Sequential generation of hematopoietic colonies derived from single nonlineage-committed CD34$^+$/CD38$^-$ progenitor cells. Blood 1991; 77:1218-27.
55. De Fabritiis P, Dowding C, Bungey J, et al. Phenotypic characterization of normal and CML CD34-positive cells: only the most primitive CML progenitors include Ph-neg cells. Leuk Lymphoma 1993; 11:51-61.
56. Verfaillie C, Miller WJ, Boylan K, McGlave PB. Selection of

benign primitive hematopoietic progenitors in chronic myelogenous leukemia on the basis of HLA-DR antigen expression. Blood 1992; 79:1003-10.
57. Beschoner WE, Civin CI, Strauss LC. Localization of hemopoietic progenitor cells in tissue with the anti-My-10 monoclonal antibody. Am J Pathol 1985; 119:1-6.
58. Fina L, Molgaard HV, Robertson D, et al. Expression of the CD34 gene in vascular endothelial cells. Blood 1990; 75: 2417-26.
59. Delia D, Lampugnani MG, Resnati M, et al. CD34 expression is regulated reciprocally with adhesion molecules in vascular endothelial cells *in vitro*. Blood 1993; 81:1001-8.
60. Majdic O, Johannes Stockl, Pickl WF, et al. Signaling and induction of enhanced cytoadhesiveness via the hematopoietic progenitor cell surface molecule CD34. Blood 1994; 83: 1226-34.
61. Borowitz MJ, Gockerman JP, Moore JO, et al. Clinicopathologic and cytogenetic features of CD34 (MY10)-positive acute nonlymphocytic leukemia. Am J Clin Pathol 1989; 91:265-70.
62. Campos L, Guyotat D, Archimbaud E, et al. Surface marker expression in adults with acute myelocytic leukemia: correlations with initial characteristics, morphology and response to therapy. Br J Haematol 1989; 72:161-6.
63. Geller RB, Zahurak M, Hurwitz CA, et al. Prognostic importance of immunophenotyping in adults with acute myelocytic leukaemia: the significance of the stem-cell glycoprotein CD34 (My10). Br J Haematol 1990; 76:340-7.
64. Myint H, Lucie NP. The prognostic significance of the CD34 antigen in acute myeloid leukaemia. Leuk Lymphoma 1992; 7:425-9.
65. Lauria F, Raspadori D, Ventura MA, et al. CD7 expression does not affect the prognosis in acute myeloid leukemia. Blood 1994; 83:3097-8.
66. Borowitz Mj, Shuster JJ, Civin CI, et al. Prognostic significance of CD34 expression in childhood B-precursor acute lymphocytic leukemia: a Pediatric Oncology Group Study. J Clin Oncol 1990; 8:1389-96.
67. Pui CH, Hancock ML, Head DR, et al. Clinical significance of CD34 expression in childhood acute lymphoblastic leukemia. Blood 1993; 82:889-98.
68. Till JE, McCulloch EA. Hemopoietic stem cell differentiation. Biochim Biophys Acta 1980; 605:431-59.
69. Van Zant G, Chen JJ, Scott-Micus K. Developmental potential of hematopoietic stem cells determined using retrovirally marked allogenic marrow. Blood 1991; 77:756-63.
70. Till JE, McCulloch EA, Siminovitc L. A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. Proc Natl Acad Sci USA 1964; 51:29-34.
71. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81:2844-53.
72. Bergamaschi G, Rosti V, Danova M, Lucotti C, Cazzola M. Apoptosis: biological and clinical aspects. Haematologica 1994; 79:86-93.
73. Barosi G. Control of erythropoietin production in man. Haematologica 1993; 78:77-9.
74. Cazzola M. The end of a long search: at last thrombopoietin. Haematologica 1994; 79:397-9.
75. Ikebuchi K, Clark SC, Ihle JN, Souza LM, Ogawa M. Granulocyte colony-stimulating factor enhances interleukin-3-dependent proliferation of multipotential hemopoietic progenitors. Proc Natl Acad Sci USA 1988; 85:3445-9.
76. Sonoda Y, Yang YC, Wong GG, Clark SC, Ogawa M. Analysis in serum-free culture of the targets of recombinant human hemopoietic factors: interleukin-3 and granulocyte/macrophage colony-stimulating factor are specific for early developmental stages. Proc Natl Acad Sci USA 1988; 85: 4360-6.
77. Leary AG, Zeng HQ, Clark SC, Ogawa M. Growth factor requirements for survival in G0 and entry into the cell cycle of primitive human hematopoietic progenitors. Proc Natl Acad Sci USA 1992; 89:4013-7.
78. Moore MAS. Clinical implications of positive and negative hematopoietic stem cell regulators. Blood 1991; 78:1-19.
79. Hyrayama F, Shih JP, Awgulewitsch A, Warr GW, Clark SC, Ogawa M. Clonal proliferation of murine lymphohematopoietic progenitors in culture. Proc Natl Acad Sci USA 1992; 89: 5907-11.
80. Gordon MY, Riley GP, Watt SM, Greaves MF. Compartimentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. Nature 1987; 326:403-5.
81. Montagna C, Massaro P, Morali F, Foa P, Maiolo AT, Eridani S. In vitro sensitivity of human erythroid progenitors to hemopoietic growth factors: studies on primary and secondary polycythemia. Haematologica 1994; 79:311-8.
82. Jacobsen SEW, Ruscetti FW, Dubois CM, Wine J, Keller JR. Induction of colony-stimulating factor receptor expression on hematopoietic progenitor cells: Proposed mechanism for growth factor synergism. Blood 1992; 80:678-87.
83. Testa U, Pelosi E, Gabbianelli M, et al. Cascade of transactivation of growth factor receptors in early human hematopoiesis. Blood 1993; 81:1442-56.
84. Hirano T, Yasukawa K, Harada H, et al. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 1986; 324: 73-6.
85. Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared b subunit for the human IL-3 and GM-CSF receptors. Cell 1991; 66: 1165-9.
86. Yin T, Taga T, Lik-Shing Tsang M, Yasukawa K, Kishimoto T, Yang YC. Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. J Immunol 1993; 151: 2555-61.
87. Massague J. The transforming growth factor-family. Ann Rev Cell Biol 1990; 6:597-641.
88. Ploemacher RE, van Soest PL, Boudewijn A. Autocrine transforming growth factor 1 blocks colony formation and progenitor cell generation by hemopoietic stem cells stimulated with steel factor. Stem Cells 1993; 11:336-47.
89. Jacobsen SEW, Keller JR, Ruscetti FW, Kondaiah P, Roberts AB, Falk LA. Bidirectional effects of transforming growth factor β (TGF-β) on colony-stimulating factor-induced human myelopoiesis *in vitro*: differential effects of distinct TGF- isoforms. Blood 1991; 78:2239-47.
90. Keller JR, Jacobsen SEW, Dubois CM, Hestdal K, Ruscetti FW. Transforming growth factor-α: a bidirectional regulator of hematopoietic cell growth. Int J Cell Cloning 1992; 10:2-11.
91. Lemoli RM, Fogli M, Fortuna A, Tura S. Interleukin-11 (IL-11) and IL-9 counteract the inhibitory activity of transforming growth factor β3 (TGF β3) on human primitive hemopoietic progenitor cells. Haematologica 1995; 80:5-12.
92. Jacobsen SEW, Ruscetti FW, Dubois CM, Lee J, Boone TC, Keller JR. Transforming growth factor-β transmodulates the expression of colony stimulating factor receptors on murine hematopoietic progenitor cell lines. Blood 1991; 77:1706-16.
93. Laiho M, Di Caprio JA, Ludlow JW, Livingstone DM, Massague J. Growth inhibition by TGF-beta linked to suppression of retinoblastoma protein phosphorylation. Cell 1990; 62: 175-85.
94. Takehara K, Le Roy EC, Grotendorst GR. TGF-β inhibition of endothelial cell proliferation: alteration of EGF binding and EGF-induced growth-regulatory (competence) gene expression. Cell 1987; 49:415-22.
95. Strife A, Lambek C, Perez A, et al. The effects of TGF-β3 on

the growth of highly enriched hematopoietic progenitor cells derived from normal human bone marrow and peripheral blood. Cancer Res 1991; 15:4828-36.
96. Lemoli RM, Strife A, Clarkson BD, Haley JD, Gulati SC. TGF-β3 protects normal human hematopoietic progenitor cells treated with 4-hydroperoxycyclophosphamide *in vitro*. Exp Hematol 1992; 20:1252-6.
97. Dubois CM, Ruscetti FW, Stankowa J, Keller JR. Transforming growth factor-β regulates c-kit message stability and cell-surface protein expression in hematopoietic progenitors. Blood 1994; 83:3138-45.
98. Caux C, Saeland S, Favre C, Duvert V, Mannoni P, Bancherau J. Tumor necrosis factor-α strongly potentiates interleukin-3 and granulocyte-macrophage colony-stimulating factor-induced proliferation of human $CD34^+$ hematopoietic progenitor cells. Blood 1990; 75:2292-8.
99. Schall TJ, Lewis M, Koller KJ, et al. Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 1990; 61:361-4.
100. Rusten LS, Jacobsen FW, Lesslauer W, Loetscher H, Smeland EB, Jacobsen SEW. Bifunctional effects of tumor necrosis factor-α (TNF-α) on the growth of mature and primitive human hematopoietic progenitor cells: involvement of p55 and p75 TNF receptors. Blood 1994; 83:3152-9.
101. Graham GJ, Wright EG, Hewick R, et al. Identification and characterization of an inhibitor of hematopoietic stem cell proliferation. Nature 1990; 344:442-4.
102. Broxmeyer HE, Sherry B, Lu L, et al. Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation in vitro by bone marrow myeloid progenitor cells. Blood 1992; 76:1110-6.
103. Dunlop DJ, Wright EG, Lorimore S, et al. Demonstration of stem cell inhibition and myeloprotective effects of SCI/MIP 1a *in vivo*. Blood 1992; 79:2221-5.
104. Moreb J, Zucali JR, Rueth S. The effects of tumor necrosis factor-α on early human hematopoietic progenitor cells treated with 4-hydroperoxycyclophosphamide. Blood 1990; 76:681-9.
105. Andrews RG, Singer JW, Bernstein ID. Precursors of colony forming cells in human can be distinguished from colony forming cells by expression of the CD33 and CD34 antigens and light scatter properties. J Exp Med 1989; 169:1721-31.
106. Gabbianelli M, Sargiacomo M, Pelosi E, Testa U, Isacchi G, Peschle C. *Pure* human hematopoietic progenitors: permissive action of basic fibroblast growth factor. Science 1990; 249:1561-4.
107. Peters W, Rosner G, Ross M, et al. Comparative effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on priming peripheral blood progenitor cells for use with autologous bone marrow transplantation after high-dose chemotherapy. Blood 1993; 81:1709-19.
108. Duhrsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G, Metcalf D. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. Blood 1988; 72:2074-81.
109. Socinski MA, Elias A, Schnipper L, Cannistra SA, Antman KH, Griffin JD. Granulocyte-macrophage colony-stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. Lancet 1988; i:1194-8.
110. Gianni AM, Siena S, Bregni M, et al. Granulocyte-macrophage colony-stimulating factor to harvest circulating haematopoietic stem cells for autotransplantation. Lancet 1989; ii:580-5.
111. Kotasek D, Shepherd KM, Sage RE, et al. Factors affecting blood stem cell collections following high-dose cyclophosphamide mobilization in lymphoma, myeloma and solid tumors. Bone Marrow Transplant 1992; 9:11-7.
112. Teshima T, Harada M, Takamatsu Y, et al. Cytotoxic drug and cytotoxic drug/G-CSF mobilization of peripheral blood stem cells and their use for autografting. Bone Marrow Transpl 1992; 10:215-20.
113. Siena S, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM. Circulation of CD34-positive hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide treated patients: enhancement by intravenous recombinant human GM-CSF. Blood 1989; 74:1905-14.
114. Ravagnani F, Siena S, Bregni M, Sciorelli G, Gianni AM, Pellegris G. Large scale collection of circulating haematopoietic progenitors in cancer patients treated with high-dose cyclophosphamide and recombinant human GM-CSF. Eur J Cancer 1990; 26:562-4.
115. Kawano Y, Takaue Y, Watanabe T, et al. Effects of progenitor cell dose and preleukapheresis use of human recombinant granulocyte colony-stimulating factor on the recovery of hematopoiesis after blood stem cell autografting in children. Exp Hematol 1993; 21:103-8.
116. Bensinger W, Singer J, Appelbaum F, et al. Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 1993; 81:3158-63.
117. Haas R, Mohle R, Fruhauf S, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 1994; 83:3787-94.
118. To LB, Dyson PG, Juttner CA. Cell-dose effect in circulating stem-cell autografting. Lancet 1986; ii:404-5.
119. Gianni AM, Tarella C, Siena S, et al. Durable and complete hematopoietic reconstitution after autografting of rhGM-CSF exposed peripheral blood progenitor cells. Bone Marrow Transplant 1991; 6:143-5.
120. Hohaus S, Goldschmidt H, Ehrhardt R, Haas R. Successful autografting following myeloablative conditioning therapy with blood stem cells mobilized by chemotherapy plus rhG-CSF. Exp Hematol 1993; 21:508-14.
121. Siena S, Bregni M, Di Nicola M, et al. Durability of hematopoiesis following myeloablative cancer tharapy and autografting with peripheral blood hematopoietic progenitors. Ann Oncol 1994; 5:935-41.
122. Tarella C, Boccadoro M, Omedè P, et al. Role of chemotherapy and GM-CSF on hemopoietic progenitor cell mobilization in multiple myeloma. Bone Marrow Transplant 1993; 11: 271-7.
123. Tarella C, Ferrero D, Siena S, et al. Conditions influencing the expansion of the circulating hemopoietic progenitor cell compartment. Haematologica 1990; 75:11-4.
124. Gianni AM, Bregni M, Siena S, et al. Granulocyte-macrophage colony stimulating factor or granulocyte-colony stimulating factor infusion makes high-dose etoposide a safe outpatient regimen that is effective in lymphoma and myeloma patients. J Clin Oncol 1992; 10:1955-62.
125. Dreyfus F, Leblond V, Belanger C, et al. Peripheral blood stem cell collection and autografting in high risk lymphoma. Bone Marrow Transpl 1992; 10:409-13.
126. Shimazaki C, Oku N, Ashihara E, et al. Collection of peripheral blood stem cells mobilized by high-dose Ara-C plus VP-16 or aclarubicin followed by recombinant human granulocyte-colony stimulating factor. Bone Marrow Transpl 1992; 10:341-6.
127. Pettengell R, Testa NG, Swindell R, Crowther D, Dexter TM. Transplantation potential of hematopoietic cell released into the circulation during routine chemotherapy for non-Hodgkin's lymphoma. Blood 1993; 82:2239-48.
128. Tarella C, Ferrero D, Bregni M, et al. Peripheral blood expansion of early progenitor cells after high-dose cyclophosphamide and rhGM-CSF. Eur J Cancer 1991; 27:22-7.

129. Bender JG, Lum L, Unverzagt KL, et al. Correlation of colony-forming cells, long-term culture initiating cells and CD34+ cells in apheresis products from patients mobilized for peripharal blood progenitors with different regimens. Bone Marrow Transpl 1994; 13:479-85.
130. Haas R, Ho AD, Bredthauer U, et al. Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. Exp Hematol 1990; 18:94-8.
131. Sheridan WP, Begley CG, Juttner C, et al. Effect of peripheral blood progenitor cells mobilized by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. Lancet 1992; i:640-4.
132. Weaver CH, Buckner CD, Longin K, et al. Syngeneic transplantation with peripheral blood mononuclear cells collected after the administration of recombinant human granulocyte colony-stimulating factor. Blood 1993; 82:1981-4.
133. Matsunaga T, Sakamaki S, Ohi S, Hirayama Y, Niitsu Y. Recombinant human granulocyte colony-stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation. Bone Marrow Transpl 1993; 11:103-8.
134. Schwinger W, Mache Ch, Urban Ch, Beaufort F, Toglhofer W. Single dose of filgrastim (rhG-CSF) increases the number of hematopoietic progenitors in the peripheral blood of adult volunteers. Bone Marrow Transpl 1993; 11:489-92.
135. Brugger W, Bross K, Frisch J, et al. Mobilization of peripheral blood progenitor cells by sequential administration of Interleukin-3 and granulocyte-macrophage colony-stimulating factor following polychemotherapy with etoposide, ifosfamide and cisplatin. Blood 1992; 79:1193-200.
136. D'Hondt V, Guillaume T, Humblet Y, et al. Tolerance of sequential or simultaneous administration of IL-3 and G-CSF in improving peripheral blood stem cell harvesting following multi-agent chemotherapy: a pilot study. Bone Marrow Transpl 1994; 13:261-4.
137. de Revel T, Appelbaum FR, Storb R, et al. Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the mobilization of peripheral blood cells that engraft lethally irradiated dogs. Blood 1994; 83:3795-9.
138. Liu KY, Akashi K, Harada M, Takamatsu Y, Niho Y. Kinetics of circulating haematopoietic progenitors during chemotherapy-induced mobilization with or without granulocyte colony-stimulating factor. Br J Haematol 1993; 84:31-8.
139. Bishop MR, Anderson JR, Jackson JD, et al. High-dose therapy and peripheral blood progenitor cell transplantation: effects of recombinant human granulocyte-macrophage colony-stimulating factor on the autograft. Blood 1994; 83: 610-6.
140. Jansen WE. Peripheral blood and bone marrow hematopoietic stem cells: are they the same? Semin Oncol 1993; 20:19-27.
141. Kessinger A, Armitage JO. The evolving role of autologous peripheral stem cell transplantation following high-dose therapy for malignancies. Blood 1991; 77:211-3.
142. Siena S, Bregni M, Brando B, et al. Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. Blood 1992; 77:400-9.
143. Menichella G, Pierelli L, Foddai ML, et al. Autologous blood stem cell harvesting and transplantation in patients with advanced ovarian cancer. Br J Haematol 1991; 79:444-50.
144. Pettengell R, Morgenstern GR, Woll PJ, et al. Peripheral blood progenitor cell transplantation in lymphoma and leukemia using a single apheresis. Blood 1993; 82:3770-7.
145. Caracciolo D, Gavarotti P, Aglietta M, et al. High-dose sequential (HDS) chemotherapy with blood and marrow cell autograft as salvage treatment in very poor prognosis, relapsed non-Hodgkin lymphoma. Bone Marrow Transplant 1993; 12:621-5.
146. Mayani H, Dragowska W, Lansdorp PM. Cytokine-induced selective expansion and maturation of erythroid versus myeloid progenitors from purified cord blood precursor cells. Blood 1993; 81:3252-8.
147. Traycott CM, Abboud MR, Laver J, Clapp DW, Srour EF. Rapid exit from G0-G1 phases of cell cycle in response to stem cell factor confers on umbilical cord blood CD34+ cells an enhanced ex vivo expansion potential. Exp Hematol 1994; 22:1264-72.
148. Molineux G, Pojda Z, Hampson IN, Lord BI, Dexter TM. Transplantation potential of peripheral blood stem cells induced by granulocyte colony-stimulating factor. Blood 1990; 76:2153-8.
149. Gianni AM, Bregni M, Siena S, et al. Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy? Hematol Oncol 1989; 7:139-48.
150. Jones RJ, Wagner JE, Celano P, Zicha MS, Sharkis SJ. Separation of pluripotent hematopoietic stem cells from spleen colony forming cells. Nature 1990; 347:188-9.
151. Uchida N, Aguila HL, Fleming WH, Jerabek L, Weissman IL. Rapid and sustained hematopoietic recovery in lethally irradiated mice transplanted with purified Thy-1.1loLin-Sca-1+ hematopoietic stem cells. Blood 1994; 83:3758-79.
152. Bradford G, Williams N, Barber L, Bertoncello I. Temporal thrombocytopenia after engraftment with defined stem cells with long-term marrow reconstitution activity. Exp Hematol 1993; 21:1615-20.
153. Robertson MJ, Soiffer RJ, Freedman AS, et al. Human bone marrow depleted of CD33-positive cells mediates delayed but durable reconstitution of hematopoiesis: clinical trial of MY9 monoclonal antibody-purged autografts for the treatment of acute myeloid leukemia. Blood 1992; 79:2229-36.
154. Dreger P, Suttorp M, Haferlach T, et al. Allogeneic granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for treatment of engraftment failure after bone marrow transplantation. Blood 1993; 81:401-9.
155. Brenner MK, Rill DR, Moen RC, et al. Gene marking to trace origin of relapse after autologous bone-marrow transplantation. Lancet 1993; i: 85-6.
156. Bregni M, Magni M, Siena S, Di Nicola M, Bonadonna G, Gianni AM. Human peripheral blood hematopoietic progenitors are optimal targets of retroviral mediated gene transfer. Blood 1992; 80:1418-22.