World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/1948-0210office
wjsc@wjgnet.com
doi:10.4252/wjsc.v2.i2.18

World J Stem Cells 2010 April 26; 2(2): 18-23
ISSN 1948-0210 (online)

TOPIC HIGHLIGHT

Philippe Bourin, MD, PhD, *Series Editor*

Hematopoietic stem cells in research and clinical applications: The "CD34 issue"

Zoran Ivanovic

Zoran Ivanovic, Etablissement Français du Sang Aquitaine-Limousin, Place Amélie Raba Léon, BP24, 33035 Bordeaux Cedex, France
Author contributions: Ivanovic Z solely contributed to this paper.
Correspondence to: Zoran Ivanovic, MD, PhD, Etablissement Français du Sang Aquitaine-Limousin, Place Amélie Raba Léon, BP24, 33035 Bordeaux Cedex,
France. zoran.ivanovic@efs.sante.fr
Telephone: +33-5-56908383 Fax: +33-5-56908384
Received: February 8, 2010 Revised: March 10, 2010
Accepted: March 17, 2010
Published online: April 26, 2010

Abstract

In this paper, experimental findings concerning the kinetics of hematopoietic reconstitution are compared to corresponding clinical data. Although not clearly apparent, the transplantation practice seems to confirm the basic proposals of experimental hematology concerning hematopoietic reconstitution resulting from successive waves of repopulation stemming from different subpopulations of progenitor and stem cells. One of the "first rate" parameters in clinical transplantations in hematology; i.e. the CD34+ positive cell dose, has been discussed with respect to the functional heterogeneity and variability of cell populations endowed by expression of CD34. This parameter is useful only if the relative proportion of stem and progenitor cells in the CD34+ cell population is more or less maintained in a series of patients or donors. This proportion could vary with respect to the source, pathology, treatment, processing procedure, the graft *ex vivo* treatment and so on. Therefore, a universal dose of CD34+ cells cannot be defined. In addition, to avoid further confusion, the CD34+ cells should not be named "stem cells" or "progenitor cells" since these denominations only concern functionally characterized cell entities.

Key words: Hematopoietic progenitors; Transplantation; Hematopoietic stem cells; Hematopoietic reconstitution; Granulocytopenia; CD34+; Functional stem cell definition; Immunophenotype

Peer reviewers: Shu Wang, Associate Professor, Department of Biological Sciences, National University of Singapore, Singapore; Group Leader, Institute of Bioengineering and Nanotechnology, Singapore Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore; Philippe Bourin, MD, PhD, Laboratoire de thérapie cellulaire, EFS-PM, 75 rue de Lisieux, Toulouse 31300, France; Pranela Rameshwar, PhD, Professor, Department of Medicine-Hematology/Oncology, UMDNJ-New Jersey Medical School, MSB, Room E-579, 185 South Orange Avenue, Newark, NJ 07103, United States

Ivanovic Z. Hematopoietic stem cells in research and clinical applications: The "CD34 issue". *World J Stem Cells* 2010; 2(2): 18-23 Available from: URL: http://www.wjgnet.com/1948-0210/full/v2/i2/18.htm DOI: http://dx.doi.org/10.4252/wjsc.v2.i2.18

EXPERIMENTAL HEMATOLOGY: SOURCES AND LESSONS

From the first experimental proof of the existence of hematopoietic stem cells provided by the classical experiment of Till and Mc Culloch[1] and from its consequences[2] (1961), a new discipline - experimental hematology - has developed. The first approach of experimental hematology is to characterize the functional heterogeneity of stem and progenitors cells by *in vivo* and *in vitro* functional assays; the second approach consists of searching for an immunophenotype characterizing each of the different subpopulations of stem and progenitors cells[3-6]. Although important advances have been made in terms of enrichment of stem cells by means of immuno-phenotypical properties, the initial functional characterization is still the only way

to prove the existence of the stem cell entities[7]. This functional definition could not be avoided; this can be illustrated by two major breakthroughs in stem cell biology: (1) induction of pluripotent stem cells from somatic cells[8-10]; and (2) initiating hematopoiesis from human embryonic stem cells[11].

Taken together, almost four decades of research on stem cells that exhibit a hematopoietic differentiation potential allowed an understanding of the functional heterogeneity of stem and progenitor cells, proposed a long time ago as the "generation-age hypothesis"[12]. This heterogeneity is the main factor leading to a very complex situation that does not allow simplification without losing some essential notions.

The first reports revealing this heterogeneity dealt with the phenomenon of hematopoietic reconstitution after engraftment. Two phenomena; i.e. the kinetics of red blood cell repopulation (erythrocyte repopulating ability-ERA) and the kinetics of granulocyte repopulation (granulocyte repopulating ability-GRA) were reported[13-15]. It was evident that these phenomena resulted from the activity of two distinct cell populations that are more immature than morphologically recognizable precursors of these two lineages, but less immature than the multi-lineage progenitors called "colony forming unit-spleen (CFU-S)" detected by the assay of Till and McCulloch[16-21]. The development of *in vitro* assays for clonogenic progenitors showed that these two repopulating activities result from two distinct populations of committed progenitors: those of granulocyte monocyte lineage (CFU-GM) and those of erythroid lineage (CFU-E, BFU-E)[21]. But these "repopulating activities;" i.e. "committed progenitors," are different from CFU-S[22], whose population is capable, if transplanted after lethal irradiation, to protect animals from acute radiation-induced lethality ("radio-protective ability")[23]. The CFU-S population has also been shown to be heterogenous; relatively less primitive CFU-S produced colonies 8 to 9 d after injection of hematopoietic cells and the other relatively more primitive CFU-S produced colonies 12 to 14 d after the injection. In fact these sub-populations of CFU-S are overlapping[24]. Furthermore, the "late" colonies growing 12 to 14 d from more primitive multipotential progenitors contain more primitive cells, which are responsible for short-term engrafted clone maintenance, known under the generic terms "pre-CFU-S" or "marrow repopulating ability-MRA"[25-27]. Actually, this is the first population that could be considered as a real stem cell population according to current standards. Even more primitive stem cells have subsequently been found, allowing long-term maintenance of hematopoiesis after engraftment[28].

The previous paragraphs summarize 25 years of work, which enabled realization that hematopoietic stem cells and progenitors are organized as a continuum of descendant cell populations having a decreasing proliferative capacity and decreasing self renewal ability, starting from the most primitive stem cells to the last progenitors preceding precursors. In animal experimental models, the reconstitution of hematopoiesis after engraftment and consequent repopulation of peripheral blood results from successive waves of repopulation. This phenomenon stems from the heterogeneity of stem and progenitor cells since less primitive cells take less time to develop morphologically recognizable hematopoietic cells and *vice versa* for more primitive progenitors and stem cells. Some results suggest, however, that long term reconstitution could stem from short term reconstituting stem cells that are activated and exhausted in a successive manner[29-30]. This question does not interfere with the phenomenon of initial reconstitution after transplantation, for which the mechanism is well established and accepted. In summary, the works of experimental hematology imply that for a rapid and long term hematopoietic repopulation, a sufficient number of both stem cells and committed progenitors (of all categories) should be injected. With the development of *in vitro* cultures for the detection of human committed progenitors, as well as *in vivo* xenogenic transplantation models for the detection of human stem cells, the main points initially established in animal models have been confirmed for hematopoietic stem and progenitors cells issued from three main "human" sources: bone marrow, peripheral blood after mobilization and placental (cord) blood[5,31].

The concept of *ex vivo* expansion is derived directly from this knowledge. It is based on a very attractive idea to increase the number of cells and progenitors (aimed to accelerate hematopoietic reconstitution) in order to insure a secure and favorable long-term outcome of transplantation. As a matter of fact, for clinicians, the first objective of an *ex vivo* expansion is shortening the period of post transplantation agranulocytosis. The duration of this period varies between 1 and 4 wk depending on the source of transplanting cells [peripheral blood after mobilization, bone marrow, and placental (cord) blood].

On the basis of experimental hematology data from animal models[13-22], duration of this period depends mostly on the number of relatively mature progenitors present in populations of transplanted cells. On the other hand, experimental data demonstrate that, for long-term reconstitution, the presence of more primitive stem cells is required[25-28]. Accordingly, the ideal *ex vivo* expansion should allow amplification of both committed progenitors and stem cells.

IMPLEMENTATION IN CLINICAL HEMATOLOGY

From this experimental work, clinical hematology adopted the principle of hematopoietic stem cell transplantation. This practice started with bone marrow cells but other sources were preferred subsequently: hematopoietic progenitors and stem cells mobilized to peripheral blood as well as those from placental (cord) blood. The first bone marrow transplantations were allogenic, aimed to reconstitute the hematopoietic system of humans irradiated in a nuclear accident[32]. Since then, hematopoietic cell transplantation

as a clinical discipline yielded a tremendous amount of knowledge, not only related to stem cell biology, but also for immunology (e.g. discovery of the HLA system). In spite of this fact, the development of clinical transplantation sometimes neglects some fundamental points of experimental hematology. We discuss one of these points in this review.

Total CD34+ cell dose issue

Since the beginning of clinical transplantation practice, the total number of viable cells has been considered as a main parameter in transplantation. Though polymorphonuclear cells, monocytes, and lymphocytes do not provide hematopoietic reconstitution after transplantation, the total cell number is still considered as a first rate qualifying and prognostic factor in transplantation, especially for placental (cord) blood cells. Indeed, in most papers describing transplantation of cells issued from hematopoietic sources in the steady state, there is a correlation between engraftment, kinetics and the total cell number in the graft[33]. This correlation results from the fact that the concentration of stem and progenitor cells in hematopoietic tissues in the steady state, or after some standard therapeutic protocols, is more or less constant. Thus, the increase in total cell numbers also means an increase in stem and progenitor cell numbers. In addition, this parameter is easily and rapidly determined. Taken together, its usefulness has been confirmed. Of course, it would be wrong to consider that, due to this correlation between total cell number and transplantation outcome, the engraftment is achieved by total cells instead of stem cells.

The possibility of detecting CD34+ cells enabled researchers and technologists to approach a non-differentiated cell population containing most hematopoietic progenitors and stem cells[34], but this was not specific. Vascular endothelial cells, perivascular dendritic cells, hair follicle "stem" cells, spindle shaped cells of eccrine glands cells, for example, also express CD34[35]. This molecule, (also known as, e.g. "podocalyxin-like protein", "thrombo mucin", "gp135", *etc.*) belongs to a family of proteins ("CD34-family") that have overlapping expression patterns[36]. CD34-family proteins (CD34, podocalyxin, and endoglycan) have a serine-, threonine-, and proline rich extracellular domain that is extensively O-glycosylated and sialylated (90-170 kDa). The function of CD34 family members has not yet been definitively elucidated. However, several roles have been ascribed to these proteins; for example, the proliferation-promoting effect, differentiation-blocking effect on progenitor cells, enhancement of trafficking and migration of hematopoietic cells, and a role in cell morphogenesis[36]. Despite this expression pattern, nonspecific to hematopoietic tissue, and an elusive physiological role, the CD34 protein has become, in the minds of many in the biomedical community, the main marker endowing hematopoietic stem and progenitor cells. Furthermore, most clinicians and biologists who are not directly involved in stem cell research have a tendency to add the term "stem cells" each time they say or write "CD34+". This tendency has been a permanent source of misunderstanding and confusion and it heavily affects experimental and clinical hematology. It should therefore be repeatedly stressed that the fact that the majority of hematopoietic stem and progenitors cells express CD34+ does not mean that all CD34+ cells are stem cells or progenitors. The CD34+ cell population is very heterogeneous[34]. For example, in the CD34+ population of placental (cord) blood, 30% to 50% are progenitors (CFU-GM, BFU-E, CFU mix, and CFU-Mk) and only a small percentage are primitive stem cells. Approximately one half of the CD34+ cell population does not exhibit either progenitor or stem cell functional properties. Some stem cells do not express CD34+ in a steady state[37] and expression of this molecule could be reversible and not related to functional capacities of stem cells[38]. Here again, the CD34+ cell count in different cell populations derived from hematopoietic tissues in a steady state or mobilized in peripheral blood has been confirmed as a useful parameter of the graft concerning the kinetics of engraftment[39-44], although only a small fraction of these cells have stem cell characteristics. The dose of CD34+ cells correlates well with the dynamics of hematopoietic reconstitution compared to total cell number. This results from the fact that the proportion of progenitors in stem cells inducing "transitory" engraftment in the CD34+ population is higher than in other subpopulations. It is also relatively stable for the tissue in question. In addition, it is easy to get the count of CD34+ cells by immuno-staining and flow-cytometry. Thus, the number of CD34+ cells became a main parameter of graft quality control. Since rapidity of hematopoietic reconstitution correlates with the number of CD34+ cells per kilo of patient weight, this approximation induced a "mental shortcut" in clinical hematology; the term "CD34+ cells" is frequently equated with the term "stem cells". On the contrary, experimental hematology considers the term "stem cell" as a functional entity (or state)[7]. Even a very complex and sophisticated procedure aimed to isolate "stem cells", based on several immuno-phenotypic markers and combined with metabolic properties, only enabled a high degree of enrichment and not a completely pure stem cell population[45]. For example, Lin- CD34+ CD38- fraction from placental (cord) blood only contains 1%-2% of stem cells detectable by a functional *ex vivo* assay[46]. In addition, if steady state is disrupted, as it is in *ex vivo* expansion cultures, for instance, the relationship phenotype/function is less evident or even non-existent[47-51].

Clinical vs experimental

In general, it is more difficult to follow the specific effect of one variable in clinical rather than experimental situations. The individual variations of cellular parameters in humans are larger than in rodents. The treatment of humans should be effected within the requirements of clinical trials. In addition, the preparation of the graft is restrained to only accepted and validated procedures. After all, the interference of different human pathologies,

as well as previous treatments and therapeutic approaches, could have a big impact on the effects of transplantation. These are only some of the reasons why it is sometimes difficult to reproduce the same effect on humans that was demonstrated in animal experimentation. The apparent absence of correlations in some clinical trials, however, between two variables that correlated in animal trials, does not mean that the principle is automatically erroneous.

This should be considered in the issue of hematopoietic reconstitution after transplantation. Many papers have been published demonstrating a positive correlation between the total number of cells and the number of CD34+ cells and hematopoietic reconstitution. Determination of hematopoietic progenitors on the basis of their colony-forming capacity in culture is less practical and more time consuming than determining CD34 expression; therefore, the number of hematopoietic progenitors has not been systematically taken into consideration in analysis of hematopoietic reconstitution[33,39-44]. However, in some reports, these parameters were properly analyzed. These analyses almost always showed that the best correlation is between committed progenitors and rapidity of hematopoietic reconstitution[52-54] in comparison with total cells and CD34+ cells. Other studies have shown the absence of correlation between the total cell number, CD34+ number and clinical and hematologic outcomes[55]. This confirms a relative progenitor and stem cell source-dependent value of these parameters [unfortunately, the progenitor (CFC) number analysis was not shown]. Furthermore, short term repopulating cells, previously demonstrated in animal models, also exists in human grafts. In bone marrow grafts, for example, these short term repopulating cells have clearly demonstrated a hematopoietic reconstitution inferior to 100 d[56]. These stem cells, found in sub-populations CD34+ and CD34- and CD34+ HLA-DR-, are not correlated with a long term hematopoietic reconstitution (between 100 d and a year post transplantation). This late reconstitution, however, is correlated with CD34+ cell number, due to the presence of very primitive stem cells inside this heterogeneous population, as mentioned above[56].

These discoveries confirm that human stem cell biology is not an exception with respect to other vertebrates. This information is in favour of the "expansion concept," which postulates that *ex vivo* amplification of committed progenitors should accelerate hematopoietic reconstitution after transplantation. We could not analyze here all clinical trials that were recently reviewed dealing with the transplantation of bone marrow, peripheral blood, and cord blood hematopoietic cells after *ex vivo* expansion[57]. The initial inefficiency of this approach, however, was due to inefficient *ex vivo* protocols and/or to the study design rather than an erroneous concept. Some, however, demonstrated a positive effect on hematopoietic reconstitution after transplantation, decreasing the incidence of neutropenic fever, reduction of red blood cell transfusions, and the diminution of the duration of hospitalization[58,59]. A trial carried out with a combination of cytokines, showing a high pro-differentiation power, enabled a relatively modest expansion of total cells and progenitors. Although this trial did not provide an acceleration of hematopoietic reconstitution, it is important because transplanted cells failed to maintain short and long term reconstitution after aplasia[60]. With current knowledge, it could be proposed that the stem cells with short term and long term repopulating capacities have been exhausted in expansion cultures due to the culture conditions, especially to IL-3 and IL-1 association and the exposure of the culture to ambient oxygenation. Thus, this trial underlines the importance of the presence of primitive stem cells in a graft. Furthermore, it firmly demonstrates that the number of CD34+ cells only is not a universally appropriate parameter of the graft quality, since the primitive stem cells could be absent. Also, if a graft, as in this case an expansion product, is composed exclusively of committed progenitors without stem cells, it could only ensure a transient engraftment.

The first really successful expansion protocol[61,62] confirmed that hematopoietic reconstitution depends on the functional sub-populations of progenitor and stem cells that should be present in a graft. In addition, it presents a very interesting example of the phenomenon called "dissociation phenotype-function". During the pre-clinical development of this expansion procedure, as well as in expansion for clinical trials, we found that the expansion of progenitors with a mean value of 27 fold was accompanied with an expansion of CD34+ cells of only 3.5 fold[62,63]. In terms of absolute number, we get almost twice the number of committed progenitors than CD34 cells. This means that, in the course of *ex vivo* expansion, the culture generated the progenitors that do not express CD34 antigen (see the studies related to the transient expression of CD34)[38]. Thus, the predictable value of the CD34+ cell count could be questioned for expansion products. Indeed, the results derived from clinical trials point to the absence of correlation between the number of CD34+ cells in a graft and the duration of post-transplantation agranulocytosis[62]. On the contrary, the number of committed progenitors was well correlated with the acceleration of post transplantation hematopoietic reconstitution[62].

Concluding remarks

On the basis of experimental data, the capacity of a CD34+ cell population to reconstitute hematopoiesis quickly after engraftment, as well as in the short- and long-term perspective, depends on the presence (in sufficient number) and proportion of functionally very different CD34+ sub-populations. This proportion should vary with respect to the source (e.g. bone marrow peripheral blood[64], cord blood[5,31]), pathology, treatment, processing procedure, the graft *ex vivo* treatment[62] and so on.

For all these reasons, the same number of CD34+ cells could give completely different results related to the rapidity of hematopoietic reconstitution and the short and long term maintenance of hematopoiesis. Considering this, it would not be expected that the number of CD34+

cells would become the universal "first rate" parameter for clinical transplantation, and that a universal CD34+ cell dose could be defined.

Also, to avoid further confusion in research and clinical practice, the heterogenous population of cells endowed by CD34+ antigen expression should not be named as "stem cells" or "progenitor cells". These denominations only concern functionally characterized cell entities.

REFERENCES

1 **Till JE**, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961; **14**: 213-222
2 **McCulloch EA**, Till JE. Perspectives on the properties of stem cells. *Nat Med* 2005; **11**: 1026-1028
3 **Roeder I**, Horn K, Sieburg HB, Cho R, Muller-Sieburg C, Loeffler M. Characterization and quantification of clonal heterogeneity among hematopoietic stem cells: a model-based approach. *Blood* 2008; **112**: 4874-4883
4 **Chao MP**, Seita J, Weissman IL. Establishment of a normal hematopoietic and leukemia stem cell hierarchy. *Cold Spring Harb Symp Quant Biol* 2008; **73**: 439-449
5 **Weissman IL**, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood* 2008; **112**: 3543-3553
6 **Weissman IL**. The road ended up at stem cells. *Immunol Rev* 2002; **185**: 159-174
7 **Zipori D**. The nature of stem cells: state rather than entity. *Nat Rev Genet* 2004; **5**: 873-878
8 **Vodyanik MA**, Bork JA, Thomson JA, Slukvin II. Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood* 2005; **105**: 617-626
9 **Bhatia M**. Hematopoiesis from human embryonic stem cells. *Ann N Y Acad Sci* 2007; **1106**: 219-222
10 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147
11 **Yu J**, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917-1920
12 **Rosendaal M**, Hodgson GS, Bradley TR. Organization of haemopoietic stem cells: the generation-age hypothesis. *Cell Tissue Kinet* 1979; **12**: 17-29
13 **Constable TB**, Blackett NM. Comparison of effect of four cytotoxic agents on granulocytic and erythroid repopulating ability of rat bone marrow. *J Natl Cancer Inst* 1972; **48**: 941-948
14 **Standen GR**, Blackett NM. Effect of daily administration of cytotoxic drugs on the erythroid and granulocytic repopulating ability of rat bone marrow. *Acta Haematol* 1980; **63**: 252-256
15 **Marsh JC**, Blackett NM. A direct assay of granulocytic repopulating ability. *Exp Hematol* 1978; **6**: 135-140
16 **Milenković P**, Pavlović-Kentera V. Erythroid repopulating ability of bone marrow cells in polycythaemic mice. *Acta Haematol* 1979; **61**: 258-263
17 **Milenković P**. [The effect of cyclophosphamide on the erythropoietic stem cell compartment] *Bilt Hematol Transfuz* 1980; **8**: 91-97
18 **Dunn CD**. The effect of certain nitrogen mustard derivatives on bone marrow colony-forming units and erythroid repopulating ability. I. Rats. *Eur J Cancer* 1972; **8**: 509-516
19 **Dunn CD**. The effect of certain nitrogen mustard derivatives on bone marrow colony-forming units and erythroid repopulating ability. II. Mice. *Eur J Cancer* 1972; **8**: 517-522
20 **Milenković P**, Biljanović-Paunović L, Lukic M, Pavlović-Kentera V. Erythroid-committed progenitors and spleen colony-forming cells in adult thymus-deprived mice. *Cell Tissue Kinet* 1983; **16**: 429-440
21 **Constable TB**, Blackett NM. The relationship between granulocytic and erythroid repopulating ability. *Exp Hematol* 1974; **2**: 131-137
22 **Milenković P**, Pavlović-Kentera V. Regeneration of erythroid committed precursor cells in polycythaemic mice treated with cyclophosphamide. *Exp Hematol* 1980; **8**: 44-51
23 **Ploemacher RE**, Brons NH. Isolation of hemopoietic stem cell subsets from murine bone marrow: I. Radioprotective ability of purified cell suspensions differing in the proportion of day-7 and day-12 CFU-S. *Exp Hematol* 1988; **16**: 21-26
24 **Ploemacher RE**, Brons NH. Cells with marrow and spleen repopulating ability and forming spleen colonies on day 16, 12, and 8 are sequentially ordered on the basis of increasing rhodamine 123 retention. *J Cell Physiol* 1988; **136**: 531-536
25 **Ploemacher RE**, Brons RH. Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell. *Exp Hematol* 1989; **17**: 263-266
26 **Duke-Cohan JS**, Davies AJ, Wallis VJ. Heterogeneity within the hematopoietic stem cell compartment: evidence for a marrow-seeding stem cell distinct from CFU-s. *Int J Cell Cloning* 1985; **3**: 44-56
27 **Hodgson GS**, Bradley TR. Properties of haematopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell? *Nature* 1979; **281**: 381-382
28 **Jones RJ**, Celano P, Sharkis SJ, Sensenbrenner LL. Two phases of engraftment established by serial bone marrow transplantation in mice. *Blood* 1989; **73**: 397-401
29 **Drize NJ**, Keller JR, Chertkov JL. Local clonal analysis of the hematopoietic system shows that multiple small short-living clones maintain life-long hematopoiesis in reconstituted mice. *Blood* 1996; **88**: 2927-2938
30 **Drize NJ**, Olshanskaya YV, Gerasimova LP, Manakova TE, Samoylina NL, Todria TV, Chertkov JL. Lifelong hematopoiesis in both reconstituted and sublethally irradiated mice is provided by multiple sequentially recruited stem cells. *Exp Hematol* 2001; **29**: 786-794
31 **Guenechea G**, Gan OI, Dorrell C, Dick JE. Distinct classes of human stem cells that differ in proliferative and self-renewal potential. *Nat Immunol* 2001; **2**: 75-82
32 **Mathe G**, Jammet H, Pendic B, Schwarzenberg L, Duplan JF, Maupin B, Latarjet R, Larrieu MJ, Kalic d, Djukic Z. [Transfusions and grafts of homologous bone marrow in humans after accidental high dosage irradiation.] *Rev Fr Etud Clin Biol* 1959; **4**: 226-238
33 **Smith RJ**, Sweetenham JW. A mononuclear cell dose of 3 x 10(8)/kg predicts early multilineage recovery in patients with malignant lymphoma treated with carmustine, etoposide, Ara-C and melphalan (BEAM) and peripheral blood progenitor cell transplantation. *Exp Hematol* 1995; **23**: 1581-1588
34 **Silvestri F**, Banavali S, Baccarani M, Preisler HD. The CD34 hemopoietic progenitor cell associated antigen: biology and clinical applications. *Haematologica* 1992; **77**: 265-273
35 **Nickoloff BJ**. The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin-fixed normal skin, and on proliferating endothelial cells and stromal spindle-shaped cells in Kaposi's sarcoma. *Arch Dermatol* 1991; **127**: 523-529
36 **Nielsen JS**, McNagny KM. Novel functions of the CD34 family. *J Cell Sci* 2008; **121**: 3683-3692
37 **Engelhardt M**, Lübbert M, Guo Y. CD34(+) or CD34(-): which is the more primitive? *Leukemia* 2002; **16**: 1603-1608
38 **Dao MA**, Arevalo J, Nolta JA. Reversibility of CD34 expression on human hematopoietic stem cells that retain the capacity for secondary reconstitution. *Blood* 2003; **101**: 112-118
39 **Sawada H**, Wake A, Yamasaki Y, Izumi Y. [CD34+ cell dose and hematologic recovery in allogeneic peripheral blood

stem cell transplantation] *Rinsho Ketsueki* 2000; **41**: 500-506
40 **Lee SH**, Lee MH, Lee JH, Min YH, Lee KH, Cheong JW, Lee J, Park KW, Kang JH, Kim K, Kim WS, Jung CW, Choi SJ, Lee JH, Park K. Infused CD34+ cell dose predicts long-term survival in acute myelogenous leukemia patients who received allogeneic bone marrow transplantation from matched sibling donors in first complete remission. *Biol Blood Marrow Transplant* 2005; **11**: 122-128
41 **Jansen EM**, Hanks SG, Terry C, Akard LP, Thompson JM, Dugan MJ, Jansen J. Prediction of engraftment after autologous peripheral blood progenitor cell transplantation: CD34, colony-forming unit-granulocyte-macrophage, or both? *Transfusion* 2007; **47**: 817-823
42 **Chang YJ**, Xu LP, Liu DH, Liu KY, Han W, Chen YH, Yu-Wang, Chen H, Wang JZ, Zhang XH, Zhao XY, Huang XJ. Platelet engraftment in patients with hematologic malignancies following unmanipulated haploidentical blood and marrow transplantation: effects of CD34+ cell dose and disease status. *Biol Blood Marrow Transplant* 2009; **15**: 632-638
43 **Mehta J**, Mehta J, Frankfurt O, Altman J, Evens A, Tallman M, Gordon L, Williams S, Winter J, Krishnamurthy J, Duffey S, Singh V, Meagher R, Grinblatt D, Kaminer L, Singhal S. Optimizing the CD34 + cell dose for reduced-intensity allogeneic hematopoietic stem cell transplantation. *Leuk Lymphoma* 2009; **50**: 1434-1441
44 **Chang YJ**, Xu LP, Liu DH, Liu KY, Han W, Chen YH, Wang Y, Chen H, Wang JZ, Zhang XH, Zhao XY, Huang XJ. The impact of CD34+ cell dose on platelet engraftment in pediatric patients following unmanipulated haploidentical blood and marrow transplantation. *Pediatr Blood Cancer* 2009; **53**: 1100-1106
45 **McKenzie JL**, Takenaka K, Gan OI, Doedens M, Dick JE. Low rhodamine 123 retention identifies long-term human hematopoietic stem cells within the Lin-CD34+CD38- population. *Blood* 2007; **109**: 543-545
46 **Yahata T**, Ando K, Sato T, Miyatake H, Nakamura Y, Muguruma Y, Kato S, Hotta T. A highly sensitive strategy for SCID-repopulating cell assay by direct injection of primitive human hematopoietic cells into NOD/SCID mice bone marrow. *Blood* 2003; **101**: 2905-2913
47 **Dorrell C**, Gan OI, Pereira DS, Hawley RG, Dick JE. Expansion of human cord blood CD34(+)CD38(-) cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. *Blood* 2000; **95**: 102-110
48 **Xu R**, Reems JA. Umbilical cord blood progeny cells that retain a CD34+ phenotype after ex vivo expansion have less engraftment potential than unexpanded CD34+ cells. *Transfusion* 2001; **41**: 213-218
49 **Donaldson C**, Denning-Kendall P, Bradley B, Hows J. The CD34(+)CD38(neg) population is significantly increased in haemopoietic cell expansion cultures in serum-free compared to serum-replete conditions: dissociation of phenotype and function. *Bone Marrow Transplant* 2001; **27**: 365-371
50 **Danet GH**, Lee HW, Luongo JL, Simon MC, Bonnet DA. Dissociation between stem cell phenotype and NOD/SCID repopulating activity in human peripheral blood CD34(+) cells after ex vivo expansion. *Exp Hematol* 2001; **29**: 1465-1473
51 **Ivanovic Z**, Hermitte F, Brunet de la Grange P, Dazey B, Belloc F, Lacombe F, Vezon G, Praloran V. Simultaneous maintenance of human cord blood SCID-repopulating cells and expansion of committed progenitors at low O2 concentration (3%). *Stem Cells* 2004; **22**: 716-724
52 **al-Fiar F**, Prince HM, Imrie K, Stewart AK, Crump M, Keating A. Bone marrow mononuclear cell count does not predict neutrophil and platelet recovery following autologous bone marrow transplant: value of the colony-forming unit granulocyte-macrophage (CFU-GM) assay. *Cell Transplant* 1997; **6**: 491-495
53 **Miyamoto T**, Shinozuka T, Maeda H, Hirasawa T, Muramatsu T, Murakami M, Makino T, Itagaki H, Nakamura Y. Effect of peripheral blood progenitor cell dose on hematopoietic recovery: identification of minimal progenitor cell requirements for rapid engraftment. *Bone Marrow Transplant* 2004; **33**: 589-595
54 **Kozlowska-Skrzypczak M**, Gil L, Komarnicki M. Factors affecting neutrophil recovery after autologous bone marrow-derived stem cell transplantation in patients with acute myeloid leukemia. *Transplant Proc* 2009; **41**: 3868-3872
55 **Islam MS**, Anoop P, Datta-Nemdharry P, Sage D, Gordon-Smith EC, Turner D, Wiltshire S, O'Regan L, Marsh JC. Implications of CD34+ cell dose on clinical and haematological outcome of allo-SCT for acquired aplastic anaemia. *Bone Marrow Transplant* 2009; Epub ahead of print
56 **Zubair AC**, Kao G, Daley H, Schott D, Freedman A, Ritz J. CD34(+) CD38(-) and CD34(+) HLA-DR(-) cells in BM stem cell grafts correlate with short-term engraftment but have no influence on long-term hematopoietic reconstitution after autologous transplantation. *Cytotherapy* 2006; **8**: 399-407
57 **Ivanovic Z**, Boiron JM. [Ex vivo expansion of hematopoietic stem cells: concept and clinical benefit] *Transfus Clin Biol* 2009; **16**: 489-500
58 **Paquette RL**, Dergham ST, Karpf E, Wang HJ, Slamon DJ, Souza L, Glaspy JA. Ex vivo expanded unselected peripheral blood: progenitor cells reduce posttransplantation neutropenia, thrombocytopenia, and anemia in patients with breast cancer. *Blood* 2000; **96**: 2385-2390
59 **Paquette RL**, Dergham ST, Karpf E, Wang HJ, Slamon DJ, Souza L, Glaspy JA. Culture conditions affect the ability of ex vivo expanded peripheral blood progenitor cells to accelerate hematopoietic recovery. *Exp Hematol* 2002; **30**: 374-380
60 **Holyoake TL**, Alcorn MJ, Richmond L, Farrell E, Pearson C, Green R, Dunlop DJ, Fitzsimons E, Pragnell IB, Franklin IM. CD34 positive PBPC expanded ex vivo may not provide durable engraftment following myeloablative chemoradiotherapy regimens. *Bone Marrow Transplant* 1997; **19**: 1095-1101
61 **Reiffers J**, Cailliot C, Dazey B, Attal M, Caraux J, Boiron JM. Abrogation of post-myeloablative chemotherapy neutropenia by ex-vivo expanded autologous CD34-positive cells. *Lancet* 1999; **354**: 1092-1093
62 **Boiron JM**, Dazey B, Cailliot C, Launay B, Attal M, Mazurier F, McNiece IK, Ivanovic Z, Caraux J, Marit G, Reiffers J. Large-scale expansion and transplantation of CD34(+) hematopoietic cells: in vitro and in vivo confirmation of neutropenia abrogation related to the expansion process without impairment of the long-term engraftment capacity. *Transfusion* 2006; **46**: 1934-1942
63 **Ivanovic Z**, Duchez P, Dazey B, Hermitte F, Lamrissi-Garcia I, Mazurier F, Praloran V, Reiffers J, Vezon G, Boiron JM. A clinical-scale expansion of mobilized CD 34+ hematopoietic stem and progenitor cells by use of a new serum-free medium. *Transfusion* 2006; **46**: 126-131
64 **Ivanovic Z**, Kovacevic-Filipovic M, Jeanne M, Ardilouze L, Bertot A, Szyporta M, Hermitte F, Lafarge X, Duchez P, Vlaski M, Milpied N, Pavlovic M, Praloran V, Boiron JM. CD34+ cells obtained from "good mobilizers" are more activated and exhibit lower ex vivo expansion efficiency than their counterparts from "poor mobilizers". *Transfusion* 2010; **50**: 120-127

S- Editor Li LF **L- Editor** Lutze M **E- Editor** Yang C