2. BONE MARROW (HEMATOPOIETIC) STEM CELLS

by Jos Domen, Amy Wagers** and Irving L. Weissman****

CONTENTS

* Cellerant Therapeutics, 1531 Industrial Road, San Carlos, CA 94070. Current address: Department of Surgery, Arizona Health Sciences Center, 1501 N. Campbell Avenue, P.O. Box 245071, Tucson, AZ 85724-5071, *Email:* jdomen@surgery.arizona.edu

** Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, *Email:* amy.wagers@joslin.harvard.edu

*** Director, Institute for Cancer/Stem Cell Biology and Medicine, Professor of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, *Email:* Irv@Stanford.edu

INTRODUCTION

Blood and the system that forms it, known as the hematopoietic system, consist of many cell types with specialized functions *(see Figure 2.1).* Red blood cells (erythrocytes) carry oxygen to the tissues. Platelets (derived from megakaryocytes) help prevent bleeding. Granulocytes (neutrophils, basophils and eosinophils) and macrophages (collectively known as myeloid cells) fight infections from bacteria, fungi, and other parasites such as nematodes (ubiquitous small worms). Some of these cells are also involved in tissue and bone remodeling and removal of dead cells. B-lymphocytes produce antibodies, while T-lymphocytes can directly kill or isolate by inflammation cells recognized as foreign to the body, including many virus-infected cells and cancer cells. Many blood cells are short-lived and need to be replenished continuously; the average human requires approximately one hundred billion new hematopoietic cells each day. The continued production of these cells depends directly on the presence of Hematopoietic Stem Cells (HSCs), the ultimate, and only, source of all these cells.

HISTORICAL OVERVIEW

The search for stem cells began in the aftermath of the bombings in Hiroshima and Nagasaki in 1945. Those who died over a prolonged period from lower doses of radiation had compromised hematopoietic systems that could not regenerate either sufficient white blood cells to protect against otherwise nonpathogenic infections or enough platelets to clot their blood. Higher doses of radiation also killed the stem cells of the intestinal tract, resulting in more rapid death. Later, it was demonstrated that mice that were given doses of whole body X-irradiation developed the same radiation syndromes; at the minimal lethal dose, the mice died from hematopoietic failure approximately two weeks after radiation exposure.[1] Significantly, however, shielding a single bone or the spleen from radiation prevented this irradiation syndrome. Soon thereafter, using inbred strains of mice, scientists showed that whole-body-irradiated mice could be rescued from otherwise fatal hematopoietic failure by injection of suspensions of cells from blood-forming organs such as the bone marrow.[2] In 1956, three laboratories

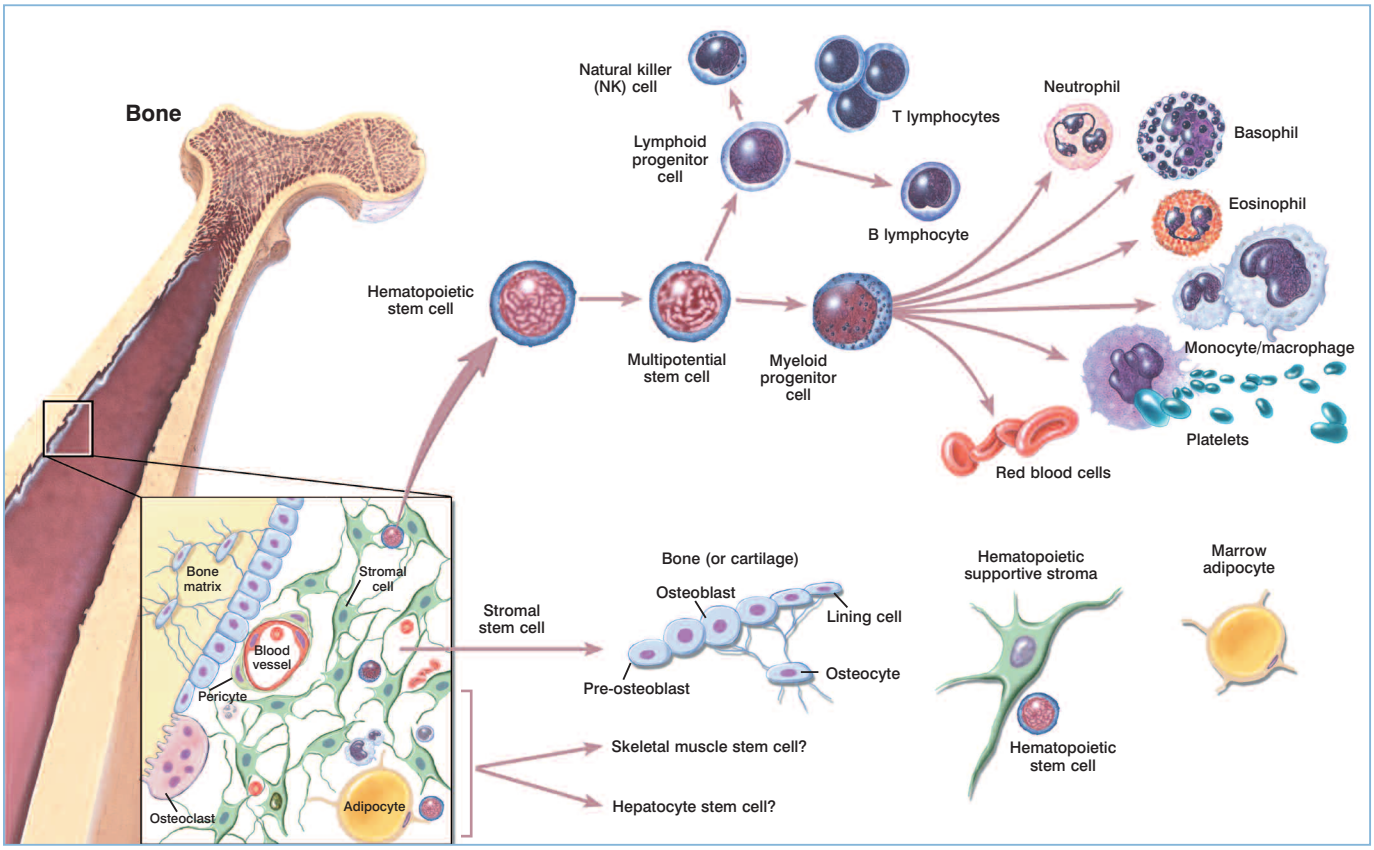

Figure 2.1. Hematopoietic and stromal cell differentiation.

demonstrated that the injected bone marrow cells directly regenerated the blood-forming system, rather than releasing factors that caused the recipients' cells to repair irradiation damage.[3–5] To date, the only known treatment for hematopoietic failure following whole body irradiation is transplantation of bone marrow cells or HSCs to regenerate the blood-forming system in the host organisms.[6,7]

The hematopoietic system is not only destroyed by the lowest doses of lethal X-irradiation (it is the most sensitive of the affected vital organs), but also by chemotherapeutic agents that kill dividing cells. By the 1960s, physicians who sought to treat cancer that had spread (metastasized) beyond the primary cancer site attempted to take advantage of the fact that a large fraction of cancer cells are undergoing cell division at any given point in time. They began using agents (*e.g.,* chemical and X-irradiation) that kill dividing cells to attempt to kill the cancer cells. This required the development of a quantitative assessment of damage to the cancer cells compared that inflicted on normal cells. Till and McCulloch began to assess quantitatively the radiation sensitivity of one normal cell type, the bone marrow cells used in transplantation, as it exists in the body. They found that, at sub-radioprotective doses of bone marrow cells, mice that died 10–15 days after irradiation developed colonies of myeloid and erythroid cells *(see Figure 2.1 for an example)* in their spleens. These colonies correlated directly in number with the number of bone marrow cells originally injected (approximately 1 colony per 7,000 bone marrow cells injected).[8] To test whether these colonies of blood cells derived from single precursor cells, they pre-irradiated the bone marrow donors with low doses of irradiation that would induce unique chromosome breaks in most hematopoietic cells but allow some cells to survive. Surviving cells displayed radiation-induced and repaired chromosomal breaks that marked each clonogenic (colony-initiating) hematopoietic cell.[9] The researchers discovered that all dividing cells within a single spleen colony, which contained different types of blood cells, contained the same unique chromosomal marker. Each colony displayed its own unique chromosomal marker, seen in its dividing cells.[9] Furthermore, when cells from a single spleen colony were re-injected into a second set of lethally-irradiated mice, donor-derived spleen colonies that contained the same unique chromosomal marker were often observed, indicating that these colonies had been regenerated from the same, single cell that had generated the first colony. Rarely, these colonies contained sufficient numbers of regenerative cells both to radioprotect secondary recipients (*e.g.,* to prevent their deaths from radiation-induced blood cell loss) and to give rise to lymphocytes and myeloerythroid cells that bore markers of the donor-injected cells.[10,11] These genetic marking experiments established the fact that cells that can both self-renew and generate most (if not all) of the cell populations in the blood must exist in bone marrow. At the time, such cells were called *pluripotent* HSCs, a term later modified to *multipotent* HSCs.[12,13] However, identifying stem cells in retrospect by analysis of randomly chromosome-marked cells is not the same as being able to isolate pure populations of HSCs for study or clinical use.

Achieving this goal requires markers that uniquely define HSCs. Interestingly, the development of these markers, discussed below, has revealed that most of the early spleen colonies visible 8 to 10 days after injection, as well as many of the later colonies, visible at least 12 days after injection, are actually derived from progenitors rather than from HSCs. Spleen colonies formed by HSCs are relatively rare and tend to be present among the later colonies.[14,15] However, these findings do not detract from Till and McCulloch's seminal experiments to identify HSCs and define these unique cells by their capacities for self-renewal and multilineage differentiation.

THE ISOLATION OF HSCS IN MOUSE AND MAN

While much of the original work was, and continues to be, performed in murine model systems, strides have been made to develop assays to study human HSCs. The development of Fluorescence Activated Cell Sorting (FACS) has been crucial for this field *(see Figure 2.2).* This technique enables the recognition and quantification of small numbers of cells in large mixed populations. More importantly, FACS-based cell sorting allows these rare cells (1 in 2000 to less than 1 in 10,000) to be purified, resulting in preparations of near 100% purity. This capability enables the testing of these cells in various assays.

HSC Assays

Assays have been developed to characterize hematopoietic stem and progenitor cells *in vitro* and *in vivo (Figure 2.3).*[16,17] *In vivo* assays that are used to study

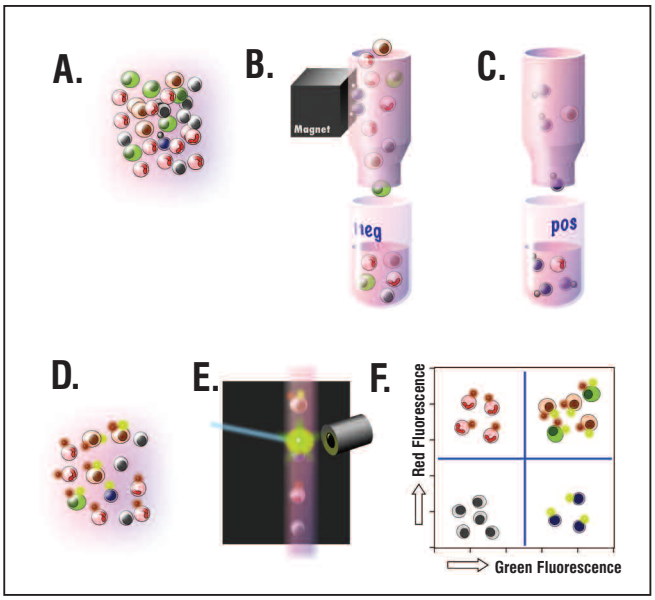

Figure 2.2. Enrichment and purification methods for hematopoietic stem cells. Upper panels illustrate column-based magnetic enrichment. In this method, the cells of interest are labeled with very small iron particles (A). These particles are bound to antibodies that only recognize specific cells. The cell suspension is then passed over a column through a strong magnetic field which retains the cells with the iron particles (B). Other cells flow through and are collected as the depleted negative fraction. The magnet is removed, and the retained cells are collected in a separate tube as the positive or enriched fraction (C). Magnetic enrichment devices exist both as small research instruments and large closed-system clinical instruments. Lower panels illustrate Fluorescence Activated Cell Sorting (FACS). In this setting, the cell mixture is labeled with fluorescent markers that emit light of different colors after being activated by light from a laser. Each of these fluorescent markers is attached to a different monoclonal antibody that recognizes specific sets of cells (D). The cells are then passed one by one in a very tight stream through a laser beam (blue in the figure) in front of detectors (E) that determine which colors fluoresce in response to the laser. The results can be displayed in a FACS-plot (F). FACS-plots (see figures 3 and 4 for examples) typically show fluorescence levels per cell as dots or probability fields. In the example, four groups can be distinguished: Unstained, red-only, green-only, and red-green double labeling. Each of these groups, *e.g.,* green fluorescence-only, can be sorted to very high purity. The actual sorting happens by breaking the stream shown in (E) into tiny droplets, each containing 1 cell, that then can be sorted using electric charges to move the drops. Modern FACS machines use three different lasers (that can activate different set of fluorochromes), to distinguish up to 8 to 12 different fluorescence colors and sort 4 separate populations, all simultaneously.

Magnetic enrichment can process very large samples (billions of cells) in one run, but the resulting cell preparation is enriched for only one parameter (*e.g.,* CD34) and is not pure. Significant levels of contaminants (such as T-cells or tumor cells) remain present. FACS results in very pure cell populations that can be selected for several parameters simultaneously (*e.g.,* Lin^{neg}, $CD34^{pos}$, $CD90^{pos}$), but it is more time consuming (10,000 to 50,000 cells can be sorted per second) and requires expensive instrumentation.

HSCs include Till and McCulloch' s classical spleen colony forming (CFU-S) assay,[8] which measures the ability of HSC (as well as blood-forming progenitor cells) to form large colonies in the spleens of lethally irradiated mice. Its' main advantage (and limitation) is the short-term nature of the assay (now typically 12 days). However, the assays that truly define HSCs are reconstitution assays.[16,18] Mice that have been "preconditioned" by lethal irradiation to accept new HSCs are injected with purified HSCs or mixed populations containing HSCs, which will repopulate the hematopoietic systems of the host mice for the life of the animal. These assays typically use different types of markers to distinguish host and donor-derived cells.

For example, allelic assays distinguish different versions of a particular gene, either by direct analysis of DNA or of the proteins expressed by these alleles. These proteins may be cell-surface proteins that are recognized by specific monoclonal antibodies that can distinguish between the variants (*e.g.,* CD45 in Figure 2.3) or cellular proteins that may be recognized through methods such as gel-based analysis. Other assays take advantage of the fact that male cells can be detected in a female host by detecting the male-cell-specific Y-chromosome by molecular assays (*e.g.,* polymerase chain reaction, or PCR).

Small numbers of HSCs (as few as one cell in mouse experiments) can be assayed using competitive reconstitutions, in which a small amount of host-type bone marrow cells (enough to radioprotect the host and thus ensure survival) is mixed in with the donor-HSC population. To establish long-term reconstitutions in mouse models, the mice are followed for at least 4 months after receiving the HSCs. Serial reconstitution, in which the bone marrow from a previously-irradiated and reconstituted mouse becomes the HSC source for

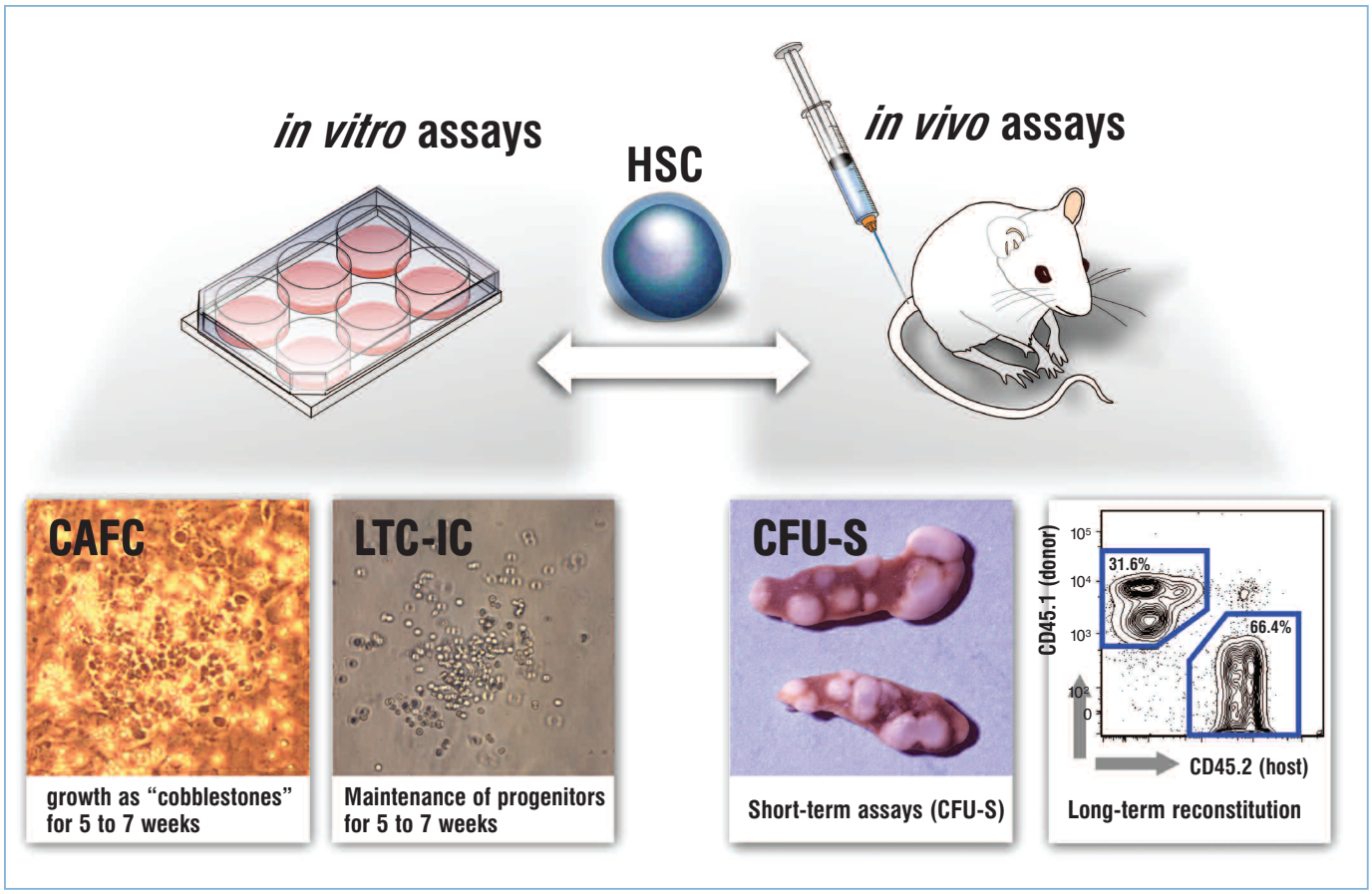

Figure 2.3. Assays used to detect hematopoietic stem cells. The tissue culture assays, which are used frequently to test human cells, include the ability of the cells to be tested to grow as "cobblestones" (the dark cells in the picture) for 5 to 7 weeks in culture. The Long Term Culture-Initiating Cell assay measures whether hematopoietic progenitor cells (capable of forming colonies in secondary assays, as shown in the picture) are still present after 5 to 7 weeks of culture.

In vivo assays in mice include the CFU-S assay, the original stem cell assay discussed in the introduction. The most stringent hematopoietic stem cell assay involves looking for the long-term presence of donor-derived cells in a reconstituted host. The example shows host-donor recognition by antibodies that recognize two different mouse alleles of CD45, a marker present on nearly all blood cells. CD45 is also a good marker for distinguishing human blood cells from mouse blood cells when testing human cells in immunocompromised mice such as NOD/SCID. Other methods such as pcr-markers, chromosomal markers, and enzyme markers can also be used to distinguish host and donor cells.

second irradiated mouse, extends the potential of this assay to test lifespan and expansion limits of HSCs. Unfortunately, the serial transfer assay measures both the lifespan and the transplantability of the stem cells. The transplantability may be altered under various conditions, so this assay is not the *sine qua non* of HSC function. Testing the *in vivo* activity of human cells is obviously more problematic.

Several experimental models have been developed that allow the testing of human cells in mice. These assays employ immunologically-incompetent mice (mutant mice that cannot mount an immune response against foreign cells) such as SCID[19–21] or NOD-SCID mice.[22,23] Reconstitution can be performed in either the presence or absence of human fetal bone or thymus implants to provide a more natural environment in which the human cells can grow in the mice. Recently NOD/SCID/cγ$^{-/-}$ mice have been used as improved recipients for human HSCs, capable of complete reconstitution with human lymphocytes, even in the absence of additional human tissues.[24] Even more promising has been the use of newborn mice with an impaired immune system (Rag-2$^{-/-}$Cγ$^{-/-}$), which results in reproducible production of human B- and T-lymphoid and myeloerythroid cells.[25] These assays

are clearly more stringent, and thus more informative, but also more difficult than the *in vitro* HSC assays discussed below. However, they can only assay a fraction of the lifespan under which the cells would usually have to function. Information on the long-term functioning of cells can only be derived from clinical HSC transplantations.

A number of assays have been developed to recognize HSCs *in vitro* (*e.g.,* in tissue culture). These are especially important when assaying human cells. Since transplantation assays for human cells are limited, cell culture assays often represent the only viable option. *In vitro* assays for HSCs include Long-Term Culture-Initializing Cell (LTC-IC) assays[26–28] and Cobble-stone Area Forming Cell (CAFC) assays.[29] LTC-IC assays are based on the ability of HSCs, but not more mature progenitor cells, to maintain progenitor cells with clonogenic potential over at least a five-week culture period. CAFC assays measure the ability of HSCs to maintain a specific and easily recognizable way of growing under stromal cells for five to seven weeks after the initial plating. Progenitor cells can only grow in culture in this manner for shorter periods of time.

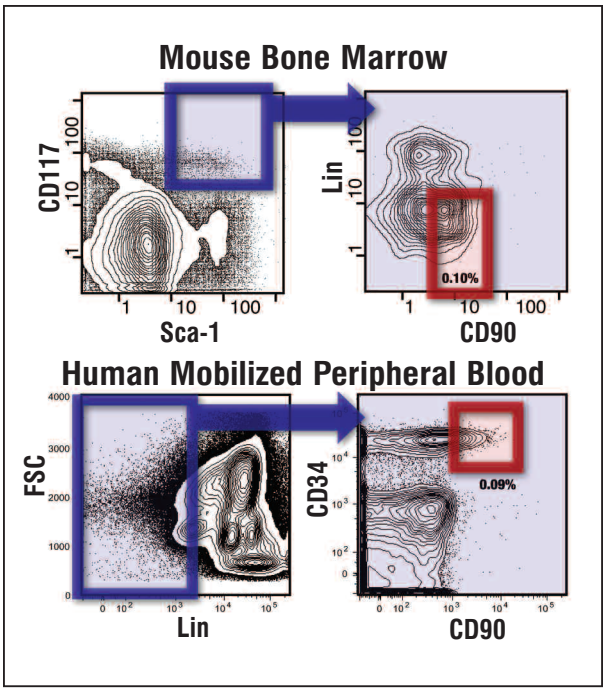

Figure 2.4. Examples of Hematopoietic Stem Cell staining patterns in mouse bone marrow (top) and human mobilized peripheral blood (bottom). The plots on the right show only the cells present in the left blue box. The cells in the right blue box represent HSCs. Stem cells form a rare fraction of the cells present in both cases.

Cell Markers Can Identify HSCs

While initial experiments studied HSC activity in mixed populations, much progress has been made in specifically describing the cells that have HSC activity. A variety of markers have been discovered to help recognize and isolate HSCs. Initial marker efforts focused on cell size, density, and recognition by lectins (carbohydrate-binding proteins derived largely from plants),[30] but more recent efforts have focused mainly on cell surface protein markers, as defined by monoclonal antibodies. For mouse HSCs, these markers include panels of 8 to 14 different monoclonal antibodies that recognize cell surface proteins present on differentiated hematopoietic lineages, such as the red blood cell and macrophage lineages (thus, these markers are collectively referred to as "Lin"),[13,31] as well as the proteins Sca-1,[13,31] CD27,[32] CD34,[33] CD38,[34] CD43,[35] CD90.1 (Thy-1.1),[13,31] CD117 (c-Kit),[36] AA4.1,[37] and MHC class I,[30] and CD150.[38] Human HSCs have been defined with respect to staining for Lin,[39] CD34,[40] CD38,[41] CD43,[35] CD45RO,[42] CD45RA,[42] CD59,[43] CD90,[39] CD109,[44] CD117,[45] CD133,[46,47] CD166,[48] and HLA DR (human).[49,50] In addition, metabolic markers/dyes such as rhodamine123 (which stains mitochondria),[51] Hoechst33342 (which identifies MDR-type drug efflux activity),[52] Pyronin-Y (which stains RNA),[53] and BAAA (indicative of aldehyde dehydrogenase enzyme activity)[54] have been described. While none of these markers recognizes functional stem cell activity, combinations (typically with 3 to 5 different markers, see examples below) allow for the purification of near-homogenous populations of HSCs. The ability to obtain pure preparations of HSCs, albeit in limited numbers, has greatly facilitated the functional and biochemical characterization of these important cells. However, to date there has been limited impact of these discoveries on clinical practice, as highly purified HSCs have only rarely been used to treat patients (discussed below). The undeniable advantages of using purified cells (*e.g.,* the absence of contaminating tumor cells in autologous transplantations) have been offset by practical difficulties and increased purification costs.

Cell Surface Marker Combinations That Define Hematopoietic Stem Cells

HSC assays, when combined with the ability to purify HSCs, have provided increasingly detailed insight into the cells and the early steps involved in the differentiation process. Several marker combinations have been developed that describe murine HSCs, including [CD117^{high}, CD90.1^{low}, Lin$^{neg/low}$, Sca-1^{pos}],[15] [CD90.1^{low}, Lin^{neg}, Sca-1^{pos} Rhodamine123^{low}],[55] [CD34$^{neg/low}$, CD117^{pos}, Sca-1^{pos}, Lin^{neg}],[33] [CD150 ^{pos}, CD48^{neg}, CD244^{neg}],[38] and "side-population" cells using Hoechst-dye.[52] Each of these combinations allows purification of HSCs to near-homogeneity. Figure 2.4 shows an example of an antibody combination that can recognize mouse HSCs. Similar strategies have been developed to purify human HSCs, employing markers such as CD34, CD38, Lin, CD90, CD133 and fluorescent substrates for the enzyme, aldehyde dehydrogenase. The use of highly purified human HSCs has been mainly experimental, and clinical use typically employs enrichment for one marker, usually CD34. CD34 enrichment yields a population of cells enriched for HSC and blood progenitor cells but still contains many other cell types. However, limited trials in which highly FACS-purified CD34^{pos} CD90^{pos} HSCs *(see Figure 2.4)* were used as a source of reconstituting cells have demonstrated that rapid reconstitution of the blood system can reliably be obtained using only HSCs.[56–58]

The purification strategies described above recognize a rare subset of cells. Exact numbers depend on the assay used as well as on the genetic background studied.[16] In mouse bone marrow, 1 in 10,000 cells is a hematopoietic stem cell with the ability to support long-term hematopoiesis following transplantation into a suitable host. When short-term stem cells, which have a limited self-renewal capacity, are included in the estimation, the frequency of stem cells in bone marrow increases to 1 in 1,000 to 1 in 2,000 cells in humans and mice. The numbers present in normal blood are at least ten-fold lower than in marrow.

None of the HSC markers currently used is directly linked to an essential HSC function, and consequently, even within a species, markers can differ depending on genetic alleles,[59] mouse strains,[60] developmental stages,[61] and cell activation stages.[62,63] Despite this, there is a clear correlation in HSC markers between divergent species such as humans and mice. However, unless the ongoing attempts at defining the complete HSC gene expression patterns will yield usable markers that are linked to essential functions for maintaining the "stemness" of the cells,[64,65] functional assays will remain necessary to identify HSCs unequivocally.[16]

Cell Surface Marker Patterns of Hematopoietic Progenitor Cells

More recently, efforts at defining hematopoietic populations by cell surface or other FACS-based markers have been extended to several of the progenitor populations that are derived from HSCs *(see Figure 2.5)*. Progenitors differ from stem cells in that they have a reduced differentiation capacity (they can generate only a subset of the possible lineages) but even more importantly, progenitors lack the ability to self-renew. Thus, they have to be constantly regenerated from the HSC population. However, progenitors do have extensive proliferative potential and can typically generate large numbers of mature cells. Among the progenitors defined in mice and humans are the Common Lymphoid Progenitor (CLP),[66,67] which in adults has the potential to generate all of the lymphoid but not myeloerythroid cells, and a Common Myeloid Progenitor (CMP), which has the potential to generate all of the mature myeloerythroid, but not lymphoid, cells.[68,69] While beyond the scope of this overview, hematopoietic progenitors have clinical potential and will likely see clinical use.[70,71]

HALLMARKS OF HSCS

HSCs have a number of unique properties, the combination of which defines them as such.[16] Among the core properties are the ability to choose between self-renewal (remain a stem cell after cell division) or differentiation (start the path towards becoming a mature hematopoietic cell). In addition, HSCs migrate in regulated fashion and are subject to regulation by apoptosis (programmed cell death). The balance between these activities determines the number of stem cells that are present in the body.

Self-Renewal

One essential feature of HSCs is the ability to self-renew, that is, to make copies with the same or very similar potential. This is an essential property because

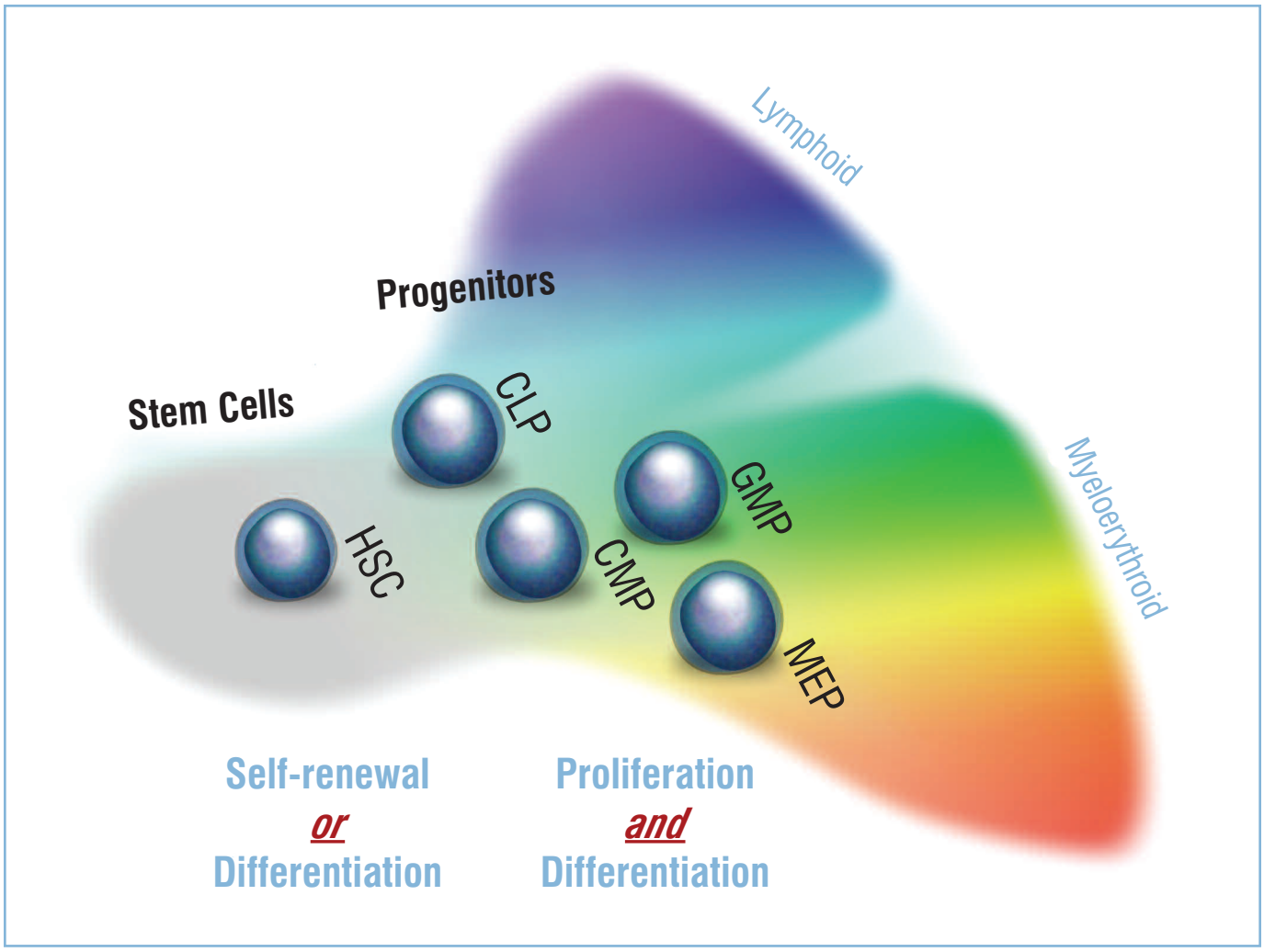

Figure 2.5. Relationship between several of the characterized hematopoietic stem cells and early progenitor cells. Differentiation is indicated by colors; the more intense the color, the more mature the cells. Surface marker distinctions are subtle between these early cell populations, yet they have clearly distinct potentials. Stem cells can choose between self-renewal and differentiation. Progenitors can expand temporarily but always continue to differentiate (other than in certain leukemias). The mature lymphoid (T-cells, B-cells, and Natural Killer cells) and myeloerythroid cells (granulocytes, macrophages, red blood cells, and platelets) that are produced by these stem and progenitor cells are shown in more detail in Figure 2.1.

more differentiated cells, such as hematopoietic progenitors, cannot do this, even though most progenitors can expand significantly during a limited period of time after being generated. However, for continued production of the many (and often short-lived) mature blood cells, the continued presence of stem cells is essential. While it has not been established that adult HSCs can self-renew indefinitely (this would be difficult to prove experimentally), it is clear from serial transplantation experiments that they can produce enough cells to last several (at least four to five) lifetimes in mice. It is still unclear which key signals allow self-renewal. One link that has been noted is telomerase, the enzyme necessary for maintaining telomeres, the DNA regions at the end of chromosomes that protect them from accumulating damage due to DNA replication. Expression of telomerase is associated with self-renewal activity.[72] However, while absence of telomerase reduces the self-renewal capacity of mouse HSCs, forced expression is not sufficient to enable HSCs to be transplanted indefinitely; other barriers must exist.[73,74]

It has proven surprisingly difficult to grow HSCs in culture despite their ability to self-renew. Expansion in culture is routine with many other cells, including

neural stem cells and ES cells. The lack of this capacity for HSCs severely limits their application, because the number of HSCs that can be isolated from mobilized blood, umbilical cord blood, or bone marrow restricts the full application of HSC transplantation in man (whether in the treatment of nuclear radiation exposure or transplantation in the treatment of blood cell cancers or genetic diseases of the blood or blood-forming system). Engraftment periods of 50 days or more were standard when limited numbers of bone marrow or umbilical cord blood cells were used in a transplant setting, reflecting the low level of HSCs found in these native tissues. Attempts to expand HSCs in tissue culture with known stem-cell stimulators, such as the cytokines stem cell factor/steel factor (KitL), thrombopoietin (TPO), interleukins 1, 3, 6, 11, plus or minus the myeloerythroid cytokines GM-CSF, G-CSF, M-CSF, and erythropoietin have never resulted in a significant expansion of HSCs.[16,75] Rather, these compounds induce many HSCs into cell divisions that are always accompanied by cellular differentiation.[76] Yet many experiments demonstrate that the transplantation of a single or a few HSCs into an animal results in a 100,000-fold or greater expansion in the number of HSCs at the steady state while simultaneously generating daughter cells that permitted the regeneration of the full blood-forming system.[77–80] Thus, we do not know the factors necessary to regenerate HSCs by self-renewing cell divisions. By investigating genes transcribed in purified mouse LT-HSCs, investigators have found that these cells contain expressed elements of the Wnt/fzd/beta-catenin signaling pathway, which enables mouse HSCs to undergo self-renewing cell divisions.[81,82] Overexpression of several other proteins, including HoxB4[83-86] and HoxA9[87] has also been reported to achieve this. Other signaling pathways that are under investigation include Notch and Sonic hedgehog.[75] Among the intracellular proteins thought to be essential for maintaining the "stem cell" state are Polycomb group genes, including Bmi-1.[88] Other genes, such as c-*Myc* and JunB have also been shown to play a role in this process.[89,90] Much remains to be discovered, including the identity of the stimuli that govern self-renewal *in vivo,* as well as the composition of the environment (the stem cell "niche") that provides these stimuli.[91] The recent identification of osteoblasts, a cell type known to be involved in bone formation, as a critical component of this environment[92,93] will help to focus this search. For instance, signaling by Angiopoietin-1 on osteoblasts to Tie-2 receptors on HSCs has recently been suggested to regulate stem cell quiescence (the lack of cell division).[94] It is critical to discover which pathways operate in the expansion of human HSCs to take advantage of these pathways to improve hematopoietic transplantation.

Differentiation

Differentiation into progenitors and mature cells that fulfill the functions performed by the hematopoietic system is not a unique HSC property, but, together with the option to self-renew, defines the core function of HSCs. Differentiation is driven and guided by an intricate network of growth factors and cytokines. As discussed earlier, differentiation, rather than self-renewal, seems to be the default outcome for HSCs when stimulated by many of the factors to which they have been shown to respond. It appears that, once they commit to differentiation, HSCs cannot revert to a self-renewing state. Thus, specific signals, provided by specific factors, seem to be needed to maintain HSCs. This strict regulation may reflect the proliferative potential present in HSCs, deregulation of which could easily result in malignant diseases such as leukemia or lymphoma.

Migration

Migration of HSCs occurs at specific times during development (*i.e.,* seeding of fetal liver, spleen and eventually, bone marrow) and under certain conditions (*e.g.,* cytokine-induced mobilization) later in life. The latter has proven clinically useful as a strategy to enhance normal HSC proliferation and migration, and the optimal mobilization regimen for HSCs currently used in the clinic is to treat the stem cell donor with a drug such as cytoxan, which kills most of his or her dividing cells. Normally, only about 8% of LT-HSCs enter the cell cycle per day,[95,96] so HSCs are not significantly affected by a short treatment with cytoxan. However, most of the downstream blood progenitors are actively dividing,[66,68] and their numbers are therefore greatly depleted by this dose, creating a demand for a regenerated blood-forming system. Empirically, cytokines or growth factors such as G-CSF and KitL can increase the number of HSCs in the blood, especially if administered for several days following a cytoxan pulse. The optimized protocol of cytoxan plus G-CSF results in several self-renewing cell divisions for

each resident LT-HSC in mouse bone marrow, expanding the number of HSCs 12- to 15-fold within two to three days.[97] Then, up to one-half of the daughter cells of self-renewing dividing LT-HSCs (estimated to be up to 10^5 per mouse per day[98]) leave the bone marrow, enter the blood, and within minutes engraft other hematopoietic sites, including bone marrow, spleen, and liver.[98] These migrating cells can and do enter empty hematopoietic niches elsewhere in the bone marrow and provide sustained hematopoietic stem cell self-renewal and hematopoiesis.[98,99] It is assumed that this property of mobilization of HSCs is highly conserved in evolution (it has been shown in mouse, dog and humans) and presumably results from contact with natural cell-killing agents in the environment, after which regeneration of hematopoiesis requires restoring empty HSC niches. This means that functional, transplantable HSCs course through every tissue of the body in large numbers every day in normal individuals.

Apoptosis

Apoptosis, or programmed cell death, is a mechanism that results in cells actively self-destructing without causing inflammation. Apoptosis is an essential feature in multicellular organisms, necessary during development and normal maintenance of tissues. Apoptosis can be triggered by specific signals, by cells failing to receive the required signals to avoid apoptosis, and by exposure to infectious agents such as viruses. HSCs are not exempt; apoptosis is one mechanism to regulate their numbers. This was demonstrated in transgenic mouse experiments in which HSC numbers doubled when the apoptosis threshold was increased.[76] This study also showed that HSCs are particularly sensitive and require two signals to avoid undergoing apoptosis.

SOURCES OF HSCS

Bone Marrow and Mobilized Peripheral Blood

The best-known location for HSCs is bone marrow, and bone marrow transplantation has become synonymous with hematopoietic cell transplantation, even though bone marrow itself is increasingly infrequently used as a source due to an invasive harvesting procedure that requires general anesthesia. In adults, under steady-state conditions, the majority of HSCs reside in bone marrow. However, cytokine mobilization can result in the release of large numbers of HSCs into the blood. As a clinical source of HSCs, mobilized peripheral blood (MPB) is now replacing bone marrow, as harvesting peripheral blood is easier for the donors than harvesting bone marrow. As with bone marrow, mobilized peripheral blood contains a mixture of hematopoietic stem and progenitor cells. MPB is normally passed through a device that enriches cells that express CD34, a marker on both stem and progenitor cells. Consequently, the resulting cell preparation that is infused back into patients is not a pure HSC preparation, but a mixture of HSCs, hematopoietic progenitors (the major component), and various contaminants, including T cells and, in the case of autologous grafts from cancer patients, quite possibly tumor cells. It is important to distinguish these kinds of grafts, which are the grafts routinely given, from highly purified HSC preparations, which essentially lack other cell types.

Umbilical Cord Blood

In the late 1980s, umbilical cord blood (UCB) was recognized as an important clinical source of HSCs.[100,101] Blood from the placenta and umbilical cord is a rich source of hematopoietic stem cells, and these cells are typically discarded with the afterbirth. Increasingly, UCB is harvested, frozen, and stored in cord blood banks, as an individual resource (donor-specific source) or as a general resource, directly available when needed. Cord blood has been used successfully to transplant children and (far less frequently) adults. Specific limitations of UCB include the limited number of cells that can be harvested and the delayed immune reconstitution observed following UCB transplant, which leaves patients vulnerable to infections for a longer period of time. Advantages of cord blood include its availability, ease of harvest, and the reduced risk of graft-versus-host-disease (GVHD). In addition, cord blood HSCs have been noted to have a greater proliferative capacity than adult HSCs. Several approaches have been tested to overcome the cell dose issue, including, with some success, pooling of cord blood samples.[101,102] *Ex vivo* expansion in tissue culture, to which cord blood cells are more amenable than adult cells, is another approach under active investigation.[103]

The use of cord blood has opened a controversial treatment strategy — embryo selection to create a related UCB donor.[104] In this procedure, embryos are conceived by *in vitro* fertilization. The embryos are

tested by pre-implantation genetic diagnosis, and embryos with transplantation antigens matching those of the affected sibling are implanted. Cord blood from the resulting newborn is then used to treat this sibling. This approach, successfully pioneered at the University of Minnesota, can in principle be applied to a wide variety of hematopoietic disorders. However, the ethical questions involved argue for clear regulatory guidelines.[105]

Embryonic Stem Cells

Embryonic stem (ES) cells form a potential future source of HSCs. Both mouse and human ES cells have yielded hematopoietic cells in tissue culture, and they do so relatively readily.[106] However, recognizing the actual HSCs in these cultures has proven problematic, which may reflect the variability in HSC markers or the altered reconstitution behavior of these HSCs, which are expected to mimic fetal HSC. This, combined with the potential risks of including undifferentiated cells in an ES-cell-derived graft means that, based on the current science, clinical use of ES cell-derived HSCs remains only a theoretical possibility for now.

HSC PLASTICITY

An ongoing set of investigations has led to claims that HSCs, as well as other stem cells, have the capacity to differentiate into a much wider range of tissues than previously thought possible. It has been claimed that, following reconstitution, bone marrow cells can differentiate not only into blood cells but also muscle cells (both skeletal myocytes and cardiomyocytes),[107–111] brain cells,[112,113] liver cells,[114,115] skin cells, lung cells, kidney cells, intestinal cells,[116] and pancreatic cells.[117] Bone marrow is a complex mixture that contains numerous cell types. In addition to HSCs, at least one other type of stem cell, the mesenchymal stem cell (MSC), is present in bone marrow. MSCs, which have become the subject of increasingly intense investigation, seem to retain a wide range of differentiation capabilities i*n vitro* that is not restricted to mesodermal tissues, but includes tissues normally derived from other embryonic germ layers (*e.g.,* neurons).[118–120] MSCs are discussed in detail in Dr. Catherine Verfaillie's testimony to the President's Council on Bioethics at this website: http://bioethicsprint.bioethics.gov/transcripts/apr02/apr25session2.html and will not be discussed further here. However, similar claims of differentiation into multiple diverse cell types, including muscle,[111] liver,[114] and different types of epithelium[116] have been made in experiments that assayed partially- or fully-purified HSCs. These experiments have spawned the idea that HSCs may not be entirely or irreversibly committed to forming the blood, but under the proper circumstances, HSCs may also function in the regeneration or repair of non-blood tissues. This concept has in turn given rise to the hypothesis that the fate of stem cells is "plastic," or changeable, allowing these cells to adopt alternate fates if needed in response to tissue-derived regenerative signals (a phenomenon sometimes referred to as "trans-differentiation"). This in turn seems to bolster the argument that the full clinical potential of stem cells can be realized by studying only adult stem cells, foregoing research into defining the conditions necessary for the clinical use of the extensive differentiation potential of embryonic stem cells. However, as discussed below, such "transdifferentiation" claims for specialized adult stem cells are controversial, and alternative explanations for these observations remain possible, and, in several cases, have been documented directly.

While a full discussion of this issue is beyond the scope of this overview, several investigators have formulated criteria that must be fulfilled to demonstrate stem cell plasticity.[121,122] These include *(i)* clonal analysis, which requires the transfer and analysis of single, highly-purified cells or individually marked cells and the subsequent demonstration of both "normal" and "plastic" differentiation outcomes, *(ii)* robust levels of "plastic" differentiation outcome, as extremely rare events are difficult to analyze and may be induced by artefact, and *(iii)* demonstration of tissue-specific function of the "transdifferentiated" cell type. Few of the current reports fulfill these criteria, and careful analysis of individually transplanted KTLS HSCs has failed to show significant levels of non-hematopoietic engraftment.[123,124] In addition, several reported trans-differentiation events that employed highly purified HSCs, and in some cases a very strong selection pressure for trans-differentiation, now have been shown to result from fusion of a blood cell with a non-blood cell, rather than from a change in fate of blood stem cells.[125–127] Finally, in the vast majority of cases, reported contributions of adult stem cells to cell types outside their tissue of origin are exceedingly rare, far too rare to be considered therapeutically useful. These findings have raised significant doubts about the

biological importance and immediate clinical utility of adult hematopoietic stem cell plasticity. Instead, these results suggest that normal tissue regeneration relies predominantly on the function of cell type-specific stem or progenitor cells, and that the identification, isolation, and characterization of these cells may be more useful in designing novel approaches to regenerative medicine. Nonetheless, it is possible that a rigorous and concerted effort to identify, purify, and potentially expand the appropriate cell populations responsible for apparent "plasticity" events, characterize the tissue-specific and injury-related signals that recruit, stimulate, or regulate plasticity, and determine the mechanism(s) underlying cell fusion or transdifferentiation, may eventually enhance tissue regeneration via this mechanism to clinically useful levels.

HSC SYSTEMS BIOLOGY

Recent progress in genomic sequencing and genome-wide expression analysis at the RNA and protein levels has greatly increased our ability to study cells such as HSCs as "systems," that is, as combinations of defined components with defined interactions. This goal has yet to be realized fully, as computational biology and system-wide protein biochemistry and proteomics still must catch up with the wealth of data currently generated at the genomic and transcriptional levels. Recent landmark events have included the sequencing of the human and mouse genomes and the development of techniques such as array-based analysis. Several research groups have combined cDNA cloning and sequencing with array-based analysis to begin to define the full transcriptional profile of HSCs from different species and developmental stages and compare these to other stem cells.[64,65,128–131] Many of the data are available in online databases, such as the NIH/NIDDK Stem Cell Genome Anatomy Projects (http://www.scgap.org). While transcriptional profiling is clearly a work in progress, comparisons among various types of stem cells may eventually identify sets of genes that are involved in defining the general "stemness" of a cell, as well as sets of genes that define their exit from the stem cell pool (*e.g.,* the beginning of their path toward becoming mature differentiated cells, also referred to as *commitment*). In addition, these datasets will reveal sets of genes that are associated with specific stem cell populations, such as HSCs and MSCs, and thus define their unique properties. Assembly of these datasets into pathways will greatly help to understand and to predict the responses of HSCs (and other stem cells) to various stimuli.

CLINICAL USE OF HSCS

The clinical use of stem cells holds great promise, although the application of most classes of adult stem cells is either currently untested or is in the earliest phases of clinical testing.[132,133] The only exception is HSCs, which have been used clinically since 1959 and are used increasingly routinely for transplantations, albeit almost exclusively in a non-pure form. By 1995, more than 40,000 transplants were performed annually world-wide.[134,135] Currently the main indications for bone marrow transplantation are either hematopoietic cancers (leukemias and lymphomas), or the use of high-dose chemotherapy for non-hematopoietic malignancies (cancers in other organs). Other indications include diseases that involve genetic or acquired bone marrow failure, such as aplastic anemia, thalassemia sickle cell anemia, and increasingly, autoimmune diseases.

Autologous versus Allogeneic Grafts

Transplantation of bone marrow and HSCs are carried out in two rather different settings, autologous and allogeneic. Autologous transplantations employ a patient's own bone marrow tissue and thus present no tissue incompatibility between the donor and the host. Allogeneic transplantations occur between two individuals who are not genetically identical (with the rare exceptions of transplantations between identical twins, often referred to as syngeneic transplantations). Non-identical individuals differ in their human leukocyte antigens (HLAs), proteins that are expressed by their white blood cells. The immune system uses these HLAs to distinguish between "self" and "non-self." For successful transplantation, allogeneic grafts must match most, if not all, of the six to ten major HLA antigens between host and donor. Even if they do, however, enough differences remain in mostly uncharacterized minor antigens to enable immune cells from the donor and the host to recognize the other as "non-self." This is an important issue, as virtually all HSC transplants are carried out with either non-purified, mixed cell populations (mobilized peripheral blood, cord blood, or bone marrow) or cell populations that have been enriched for HSCs (*e.g.,* by column selection

for CD34+ cells) but have not been fully purified. These mixed population grafts contain sufficient lymphoid cells to mount an immune response against host cells if they are recognized as "non-self." The clinical syndrome that results from this "non-self" response is known as graft-versus-host disease (GVHD).[136]

In contrast, autologous grafts use cells harvested from the patient and offer the advantage of not causing GVHD. The main disadvantage of an autologous graft in the treatment of cancer is the absence of a graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) response, the specific immunological recognition of host tumor cells by donor-immune effector cells present in the transplant. Moreover, the possibility exists for contamination with cancerous or pre-cancerous cells.

Allogeneic grafts also have disadvantages. They are limited by the availability of immunologically-matched donors and the possibility of developing potentially lethal GVHD. The main advantage of allogeneic grafts is the potential for a GVL response, which can be an important contribution to achieving and maintaining complete remission.[137,138]

CD34+-Enriched versus Highly Purified HSC Grafts

Today, most grafts used in the treatment of patients consist of either whole or CD34+-enriched bone marrow or, more likely, mobilized peripheral blood. The use of highly purified hematopoietic stem cells as grafts is rare.[56–58] However, the latter have the advantage of containing no detectable contaminating tumor cells in the case of autologous grafts, therefore not inducing GVHD, or presumably GVL,[139–141] in allogeneic grafts. While they do so less efficiently than lymphocyte-containing cell mixtures, HSCs alone can engraft across full allogeneic barriers (*i.e.,* when transplanted from a donor who is a complete mismatch for both major and minor transplantation antigens).[139–141] The use of donor lymphocyte infusions (DLI) in the context of HSC transplantation allows for the controlled addition of lymphocytes, if necessary, to obtain or maintain high levels of donor cells and/or to induce a potentially curative GVL-response.[142,143] The main problems associated with clinical use of highly purified HSCs are the additional labor and costs[144] involved in obtaining highly purified cells in sufficient quantities.

While the possibilities of GVL and other immune responses to malignancies remain the focus of intense interest, it is also clear that in many cases, less-directed approaches such as chemotherapy or irradiation offer promise. However, while high-dose chemotherapy combined with autologous bone marrow transplantation has been reported to improve outcome (usually measured as the increase in time to progression, or increase in survival time),[145–154] this has not been observed by other researchers and remains controversial.[155–161] The tumor cells present in autologous grafts may be an important limitation in achieving long-term disease-free survival. Only further purification/purging of the grafts, with rigorous separation of HSCs from cancer cells, can overcome this limitation. Initial small scale trials with HSCs purified by flow cytometry suggest that this is both possible and beneficial to the clinical outcome.[56] In summary, purification of HSCs from cancer/lymphoma/leukemia patients offers the only possibility of using these cells post-chemotherapy to regenerate the host with cancer-free grafts. Purification of HSCs in allotransplantation allows transplantation with cells that regenerate the blood-forming system but cannot induce GVHD.

Non-Myeloablative Conditioning

An important recent advance in the clinical use of HSCs is the development of non-myeloablative preconditioning regimens, sometimes referred to as "mini transplants."[162–164] Traditionally, bone marrow or stem cell transplantation has been preceded by a preconditioning regimen consisting of chemotherapeutic agents, often combined with irradiation, that completely destroys host blood and bone marrow tissues (a process called *myeloablation*). This creates "space" for the incoming cells by freeing stem cell niches and prevents an undesired immune response of the host cells against the graft cells, which could result in graft failure. However, myeloablation immunocompromises the patient severely and necessitates a prolonged hospital stay under sterile conditions. Many protocols have been developed that use a more limited and targeted approach to preconditioning. These non-myeloablative preconditioning protocols, which combine excellent engraftment results with the ability to perform hematopoietic cell transplantation on an outpatient basis, have greatly changed the clinical practice of bone marrow transplantation.

Additional Indications

FACS purification of HSCs in mouse and man completely eliminates contaminating T cells, and thus GVHD (which is caused by T-lymphocytes) in allogeneic transplants. Many HSC transplants have been carried out in different combinations of mouse strains. Some of these were matched at the major transplantation antigens but otherwise different (Matched Unrelated Donors or MUD); in others, no match at the major or minor transplantation antigens was expected. To achieve rapid and sustained engraftment, higher doses of HSCs were required in these mismatched allogeneic transplants than in syngeneic transplants.[139–141,165–167] In these experiments, hosts whose immune and blood-forming systems were generated from genetically distinct donors were permanently capable of accepting organ transplants (such as the heart) from either donor or host, but not from mice unrelated to the donor or host. This phenomenon is known as transplant-induced tolerance and was observed whether the organ transplants were given the same day as the HSCs or up to one year later.[139,166] Hematopoietic cell transplant-related complications have limited the clinical application of such tolerance induction for solid organ grafts, but the use of non-myeloablative regimens to prepare the host, as discussed above, should significantly reduce the risk associated with combined HSC and organ transplants. Translation of these findings to human patients should enable a switch from chronic immunosuppression to prevent rejection to protocols wherein a single conditioning dose allows permanent engraftment of both the transplanted blood system and solid organ(s) or other tissue stem cells from the same donor. This should eliminate both GVHD and chronic host transplant immunosuppression, which lead to many complications, including life-threatening opportunistic infections and the development of malignant neoplasms.

We now know that several autoimmune diseases — diseases in which immune cells attack normal body tissues — involve the inheritance of high risk-factor genes.[168] Many of these genes are expressed only in blood cells. Researchers have recently tested whether HSCs could be used in mice with autoimmune disease (*e.g.,* type 1 diabetes) to replace an autoimmune blood system with one that lacks the autoimmune risk genes. The HSC transplants cured mice that were in the process of disease development when non-myeloablative conditioning was used for transplant.[169] It has been observed that transplant-induced tolerance allows co-transplantation of pancreatic islet cells to replace destroyed islets.[170] If these results using non-myeloablative conditioning can be translated to humans, type 1 diabetes and several other auto-immune diseases may be treatable with pure HSC grafts. However, the reader should be cautioned that the translation of treatments from mice to humans is often complicated and time-consuming.

Hematopoietic Stem Cell Banking

Banking is currently a routine procedure for UCB samples. If expansion of fully functional HSCs in tissue culture becomes a reality, HSC transplants may be possible by starting with small collections of HSCs rather than massive numbers acquired through mobilization and apheresis. With such a capability, collections of HSCs from volunteer donors or umbilical cords could be theoretically converted into storable, expandable stem cell banks useful on demand for clinical transplantation and/or for protection against radiation accidents. In mice, successful HSC transplants that regenerate fully normal immune and blood-forming systems can be accomplished when there is only a partial transplantation antigen match. Thus, the establishment of useful human HSC banks may require a match between as few as three out of six transplantation antigens (HLA). This might be accomplished with stem cell banks of as few as 4,000–10,000 independent samples.

LEUKEMIA (AND CANCER) STEM CELLS

Leukemias are proliferative diseases of the hematopoietic system that fail to obey normal regulatory signals. They derive from stem cells or progenitors of the hematopoietic system and almost certainly include several stages of progression. During this progression, genetic and/or epigenetic changes occur, either in the DNA sequence itself (genetic) or other heritable modifications that affect the genome (epigenetic). These (epi)genetic changes alter cells from the normal hematopoietic system into cells capable of robust leukemic growth. There are a variety of leukemias, usually classified by the predominant pathologic cell types and/or the clinical course of the disease. It has been proposed that these are diseases in which self-

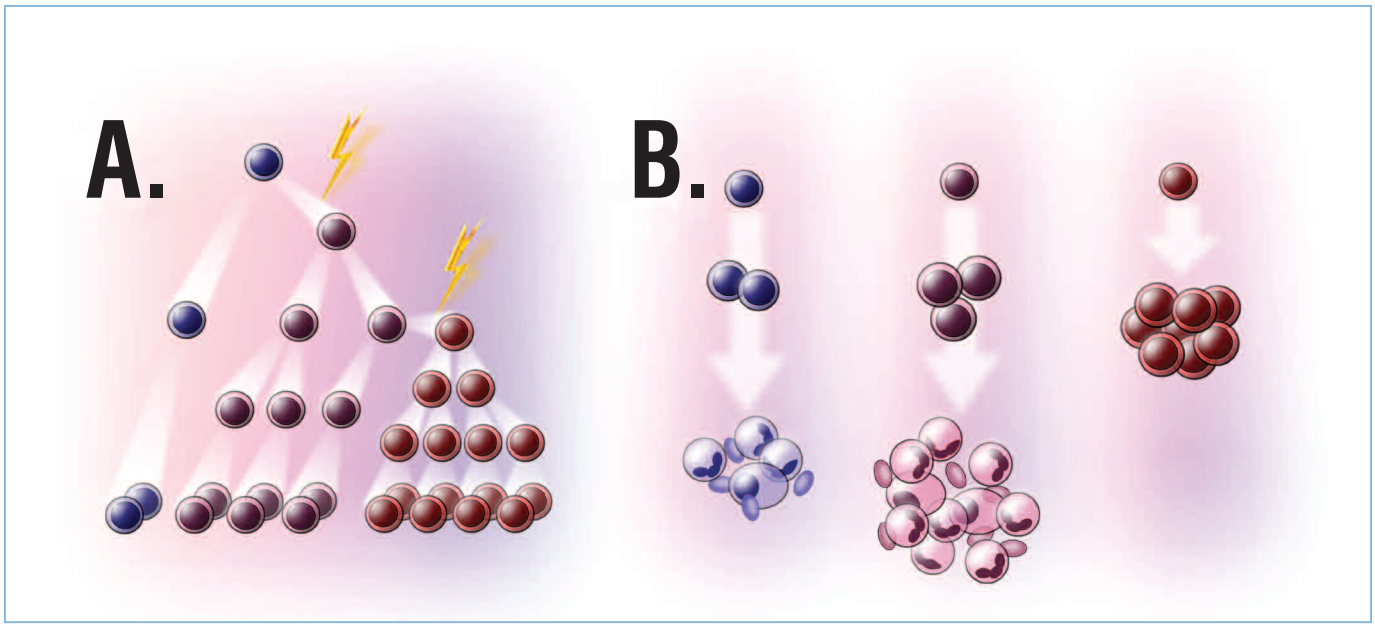

Figure 2.6. Leukemic progression at the hematopoietic stem cell level. Self-renewing HSCs are the cells present long enough to accumulate the many activating events necessary for full transformation into tumorigenic cells. Under normal conditions, half of the offspring of HSC cell divisions would be expected to undergo differentiation, leaving the HSC pool stable in size. (A) (Pre) leukemic progression results in cohorts of HSCs with increasing malignant potential. The cells with the additional event (two events are illustrated, although more would be expected to occur) can outcompete less-transformed cells in the HSC pool if they divide faster (as suggested in the figure) or are more resistant to differentiation or apoptosis (cell death), two major exit routes from the HSC pool. (B) Normal HSCs differentiate into progenitors and mature cells; this is linked with limited proliferation (left). Partially transformed HSCs can still differentiate into progenitors and mature cells, but more cells are produced. Also, the types of mature cells that are produced may be skewed from the normal ratio. Fully transformed cells may be completely blocked in terminal differentiation, and large numbers of primitive blast cells, representing either HSCs or self-renewing, transformed progenitor cells, can be produced. While this sequence of events is true for some leukemias (*e.g.,* AML), not all of the events occur in every leukemia. As with non-transformed cells, most leukemia cells (other than the leukemia stem cells) can retain the potential for (limited) differentiation.

renewing but poorly regulated cells, so-called "leukemia stem cells" (LSCs), are the populations that harbor all the genetic and epigenetic changes that allow leukemic progression.[171–176] While their progeny may be the characteristic cells observed with the leukemia, these progeny cells are not the self-renewing "malignant" cells of the disease. In this view, the events contributing to tumorigenic transformation, such as interrupted or decreased expression of "tumor suppressor" genes, loss of programmed death pathways, evasion of immune cells and macrophage surveillance mechanisms, retention of telomeres, and activation or amplification of self-renewal pathways, occur as single, rare events in the clonal progression to blast-crisis leukemia. As LT HSCs are the only self-renewing cells in the myeloid pathway, it has been proposed that most, if not all, progression events occur at this level of differentiation, creating clonal cohorts of HSCs with increasing malignancy *(see Figure 2.6).* In this disease model, the final event, explosive self-renewal, could occur at the level of HSC or at any of the known progenitors *(see Figures 2.5 and 2.6).* Activation of the β-catenin/lef-tcf signal transduction and transcription pathway has been implicated in leukemic stem cell self-renewal in mouse AML and human CML.[177] In both cases, the granulocyte-macrophage progenitors, not the HSCs or progeny blast cells, are the malignant self-renewing entities. In other models, such as the JunB-deficient tumors in mice and in chronic-phase CML in humans, the leukemic stem cell is the HSC itself.[90,177] However, these HSCs still respond to regulatory signals, thus representing steps in the clonal progression toward blast crisis *(see Figure 2.6).*

Many methods have revealed contributing proto-oncogenes and lost tumor suppressors in myeloid leukemias. Now that LSCs can be isolated, researchers should eventually be able to assess the full sequence of events in HSC clones undergoing leukemic

transformation. For example, early events, such as the AML/ETO translocation in AML or the BCR/ABL translocation in CML can remain present in normal HSCs in patients who are in remission (*e.g.,* without detectable cancer).[177,178] The isolation of LSCs should enable a much more focused attack on these cells, drawing on their known gene expression patterns, the mutant genes they possess, and the proteomic analysis of the pathways altered by the proto-oncogenic events.[173,176,179] Thus, immune therapies for leukemia would become more realistic, and approaches to classify and isolate LSCs in blood could be applied to search for cancer stem cells in other tissues.[180]

SUMMARY

After more than 50 years of research and clinical use, hematopoietic stem cells have become the best-studied stem cells and, more importantly, hematopoietic stem cells have seen widespread clinical use. Yet the study of HSCs remains active and continues to advance very rapidly. Fueled by new basic research and clinical discoveries, HSCs hold promise for such indications as treating autoimmunity, generating tolerance for solid organ transplants, and directing cancer therapy. However, many challenges remain. The availability of (matched) HSCs for all of the potential applications continues to be a major hurdle. Efficient expansion of HSCs in culture remains one of the major research goals. Future developments in genomics and proteomics, as well as in gene therapy, have the potential to widen the horizon for clinical application of hematopoietic stem cells even further.

REFERENCES

1. Jacobsen LO, Marks EK, Gaston EO, Zirkle RE. Effect of spleen protection on mortality following X-irradiation. *J Lab Clin Med.* Vol 34; 1949:1538-1543.
2. Lorenz E, Uphoff ED, Reid TR, Shelton E. Modification of acute irradiation injury in mice and guinea pigs by bone marrow injection. *Radiology.* Vol 58; 1951:863-877.
3. Gengozian N, Urso IS, Congdon CC, Conger AD, Makinodan T. Thymus specificity in lethally irradiated mice treated with rat bone marrow. *Proc Soc Exp Biol Med.* Vol 96; 1957:714-720.
4. Ford CE, Hamerton JL, Barnes DWH, Loutit JF. Cytological identification of radiation-chimaeras. *Nature.* Vol 177; 1956:452-454.
5. Nowell PC, Cole LJ, Habermyer JG, Roan PL. Growth and continued function of rat marrow cells in x-irradiated mice. *Cancer Res.* Vol 16; 1956:258-261.
6. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science.* Vol 287; 2000:1442-1446.
7. Manz MG, Akashi K, Weissman IL. Biology of Hematopoietic Stem and Progenitor Cells. In: Appelbaum FR, ed. *Thomas' Hematopoietic Cell Transplantation.* Third ed. Malden, MA: Blackwell Publishing; 2004.
8. Till J, McCulloch E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res.* Vol 14; 1961:213-224.
9. Becker A, McCulloch E, Till J. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature.* Vol 197; 1963:452-454.
10. Siminovitch L, McCulloch E, Till J. The distribution of colony-forming cells among spleen colonies. *J Cell Comp Physiol.* Vol 62; 1963:327-336.
11. Wu AM, Till JE, Siminovitch L, McCulloch EA. Cytological evidence for a relationship between normal hematopoietic colony-forming cells and cell of the lymphoid system. *J Exp Med.* Vol 127; 1963:455-467.
12. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell.* Vol 100; 2000:157-168.
13. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science.* Vol 241; 1988:58-62.
14. Na Nakorn T, Traver D, Weissman IL, Akashi K. Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J Clin Invest.* 2002;109:1579-1585.
15. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1994;1:661-673.
16. Domen J, Weissman IL. Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate. *Mol Med Today.* Vol 5; 1999:201-208.
17. Ploemacher RE. Stem cells: characterization and measurement. *Baillieres Clin Haematol.* Vol 10; 1997:429-444.
18. Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol.* Vol 11; 1995:35-71.
19. McCune JM, Namikawa H, Kaneshima H, Schultz LD, al e. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science.* Vol 241; 1988:1632-1639.
20. Kamel-Reid S, Dick JE. Engraftment of immune deficient mice with human hematopoietic stem cells. *Science.* Vol 242; 1988:1706-1709.
21. Peault B, Weissman IL, Baum C, McCune JM, Tsukamoto A. Lymphoid reconstitution of the human fetal thymus in SCID mice with CD34+ precursor cells. *J Exp Med.* Vol 174; 1991:1283-1286.

22. Larochelle A, Vormoor J, Hanenberg H, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. *Nat Med.* Vol 2; 1996:1329-1337.

23. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci USA.* Vol 94; 1997:5320-5325.

24. Hiramatsu H, Nishikomori R, Heike T, et al. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. *Blood.* Vol 102; 2003:873-880.

25. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science.* Vol 304; 2004:104-107.

26. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J Cell Physiol.* Vol 91; 1977:335-344.

27. Whitlock CA, Witte ON. Long-term culture of B lymphocytes and their precursors from murine bone marrow. *Proc Natl Acad Sci USA.* Vol 79; 1982:3608-3612.

28. Sutherland HJ, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc Natl Acad Sci USA.* Vol 87; 1990:3584-3588.

29. Ploemacher R, van der Sluijs J, Voerman JSA, Bron NHC. An in vitro limiting-dilution assay of long-term repopulating hematopoietic stem cell in the mouse. *Blood.* Vol 74; 1989:2755-2763.

30. Bauman JG, de Vries P, Pronk B, Visser JW. Purification of murine hemopoietic stem cells and committed progenitors by fluorescence activated cell sorting using wheat germ agglutinin and monoclonal antibodies. *Acta Histochem Suppl.* Vol 36; 1988:241-253.

31. Uchida N, Weissman IL. Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. *J Exp Med.* Vol 175; 1992:175-184.

32. Wiesmann A, Phillips RL, Mojica M, et al. Expression of CD27 on murine hematopoietic stem and progenitor cells. *Immunity.* Vol 12; 2000:193-199.

33. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. Vol 273; 1996:242-245.

34. Randall TD, Lund FE, Howard MC, Weissman IL. Expression of murine CD38 defines a population of long-term reconstituting hematopoietic stem cells. *Blood.* Vol 87; 1996:4057-4067.

35. Moore T, Huang S, Terstappen LW, Bennett M, Kumar V. Expression of CD43 on murine and human pluripotent hematopoietic stem cells. *J Immunol.* Vol 153; 1994:4978-4987.

36. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci USA.* Vol 89; 1992:1502-1506.

37. Jordan CT, McKearn JP, Lemischka IR. Cellular and developmental properties of fetal hematopoietic stem cells. *Cell.* Vol 61; 1990:953-963.

38. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopioetic stem and progenitor cells and reveal enothelial niches for stem cells. *Cell.* 2005;121:1109-1121.

39. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci USA.* Vol 89; 1992:2804-2808.

40. 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.* Vol 133; 1984:157-165.

41. Terstappen LW, Huang S, Safford M, Lansdorp PM, Loken MR. Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+CD38– progenitor cells. *Blood.* Vol 77; 1991:1218-1227.

42. Lansdorp PM, Sutherland HJ, Eaves CJ. Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. *J Exp Med.* Vol 172; 1990:363-366.

43. Hill B, Rozler E, Travis M, et al. High-level expression of a novel epitope of CD59 identifies a subset of CD34+ bone marrow cells highly enriched for pluripotent stem cells. *Exp Hematol.* Vol 24; 1996:936-943.

44. Sutherland DR, Yeo EL, Stewart AK, et al. Identification of CD34+ subsets after glycoprotease selection: engraftment of CD34+Thy-1+Lin– stem cells in fetal sheep. *Exp Hematol.* Vol 24; 1996:795-806.

45. Gunji Y, Nakamura M, Osawa H, et al. Human primitive hematopoietic progenitor cells are more enriched in KITlow cells than in KIThigh cells. *Blood.* Vol 82; 1993:3283-3289.

46. Miraglia S, Godfrey W, Yin AH, et al. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood.* Vol 90; 1997:5013-5021.

47. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood.* Vol 90; 1997:5002-5012.

48. Uchida N, Yang Z, Combs J, et al. The characterization, molecular cloning, and expression of a novel hematopoietic cell antigen from CD34+ human bone marrow cells. *Blood.* Vol 89; 1997:2706-2716.

49. Srour EF, Brandt JE, Leemhuis T, Ballas CB, Hoffman R. Relationship between cytokine-dependent cell cycle progression and MHC class II antigen expression by human CD34+ HLA-DR- bone marrow cells. *J Immunol.* Vol 148; 1992:815-820.

50. Tsukamoto A, Weissman I, Chen B, et al. Phenotypic and functional analysis of hematopoietic stem cells in mouse and man. In: Mertelsman La, ed. *Hematopoietic Stem Cells, Biology and Therapeutic Applications:* Phenotypic and functional analysis of hematopoietic stem cells in mouse and man.; 1995.

51. Spangrude GJ, Johnson GR. Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 87; 1990:7433-7437.

52. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med.* Vol 183; 1996:1797-1806.

53. Gothot A, Pyatt R, McMahel J, Rice S, Srour EF. Functional heterogeneity of human CD34(+) cells isolated in subcompartments of the G0 /G1 phase of the cell cycle. *Blood.* Vol 90; 1997:4384-4393.

54. Storms RW, Trujillo AP, Springer JB, et al. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proc Natl Acad Sci USA.* Vol 96; 1999:9118-9123.

55. Kim M, Cooper DD, Hayes SF, Spangrude GJ. Rhodamine-123 staining in hematopoietic stem cells of young mice indicates mitochondrial activation rather than dye efflux. *Blood.* Vol 91; 1998:4106-4117.

56. Negrin RS, Atkinson K, Leemhuis T, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. *Biol Blood Marrow Transplant.* Vol 6; 2000:262-271.

57. Michallet M, Philip T, Philip I, et al. Transplantation with selected autologous peripheral blood CD34+Thy-1+ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. *Exp Hematol.* Vol 28; 2000:858-870.

58. Vose JM, Bierman PJ, Lynch JC, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin's lymphoma. *Biol Blood Marrow Transplant.* Vol 7; 2001:680-687.

59. Spangrude GJ, Brooks DM. Phenotypic analysis of mouse hematopoietic stem cells shows a Thy-1- negative subset. *Blood.* Vol 80; 1992:1957-1964.

60. Spangrude GJ, Brooks DM. Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. *Blood.* Vol 82; 1993:3327-3332.

61. Morrison SJ, Hemmati HD, Wandycz AM, Weissman IL. The purification and characterization of fetal liver hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 92; 1995:10302-10306.

62. Randall TD, Weissman IL. Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. *Blood.* Vol 89; 1997:3596-3606.

63. Sato T, Laver JH, Ogawa M. Reversible Expression of CD34 by Murine Hematopoietic Stem Cells. *Blood.* Vol 94; 1999:2548-2554.

64. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science.* Vol 298; 2002:597-600.

65. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science.* Vol 298; 2002:601-604.

66. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell.* Vol 91; 1997:661-672.

67. Galy A, Travis M, Cen D, Chen B. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity.* Vol 3; 1995:459-473.

68. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature.* Vol 404; 2000:193-197.

69. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc Natl Acad Sci USA.* Vol 99; 2002:11872-11877.

70. Arber C, BitMansour A, Sparer TE, et al. Common lymphoid progenitors rapidly engraft and protect against lethal murine cytomegalovirus infection after hematopoietic stem cell transplantation. *Blood.* Vol 102; 2003:421-428.

71. BitMansour A, Burns SM, Traver D, et al. Myeloid progenitors protect against invasive aspergillosis and Pseudomonas aeruginosa infection following hematopoietic stem cell transplantation. *Blood.* Vol 100; 2002:4660-4667.

72. Greenwood MJ, Lansdorp PM. Telomeres, telomerase, and hematopoietic stem cell biology. *Arch Med Res.* Vol 34; 2003:489-495.

73. Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat Med.* Vol 9; 2003:369-371.

74. Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL. Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood.* Vol 102; 2003:517-520.

75. Reya T. Regulation of hematopoietic stem cell self-renewal. *Recent Prog Horm Res.* Vol 58; 2003:283-295.

76. Domen J, Weissman IL. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J Exp Med.* Vol 192; 2000:1707-1718.

77. Smith LG, Weissman IL, Heimfeld S. Clonal analysis of hematopoietic stem-cell differentiation in vivo. *Proc Natl Acad Sci USA.* Vol 88; 1991:2788-2792.

78. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science.* Vol 297; 2002:2256-2259.

79. Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. *Development.* Vol 124; 1997:1929-1939.

80. Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. *Curr Biol.* Vol 7; 1997:805-808.

81. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* Vol 423; 2003:409-414.

82. Willert K, Brown JD, Danenberg E, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature.* Vol 423; 2003:448-452.

83. Sauvageau G, Thorsteinsdottir U, Eaves CJ, et al. Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo. *Genes Dev.* Vol 9; 1995:1753-1765.

84. Antonchuk J, Sauvageau G, Humphries RK. HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. *Cell.* Vol 109; 2002:39-45.

85. Krosl J, Austin P, Beslu N, Kroon E, Humphries RK, Sauvageau G. In vitro expansion of hematopoietic stem cells by recombinant TAT-HOXB4 protein. *Nat Med.* Vol 9; 2003:1428-1432.

86. Amsellem S, Pflumio F, Bardinet D, et al. Ex vivo expansion of human hematopoietic stem cells by direct delivery of the HOXB4 homeoprotein. *Nat Med.* Vol 9; 2003:1423-1427.

87. Thorsteinsdottir U, Mamo A, Kroon E, et al. Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. *Blood.* Vol 99; 2002:121-129.

88. Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M. Stem cells and cancer; the polycomb connection. *Cell.* Vol 118; 2004:409-418.

89. Wilson A, Murphy MJ, Oskarsson T, et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* Vol 18; 2004:2747-2763.

90. Passegue E, Wagner EF, Weissman IL. JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. *Cell.* Vol 119; 2004:431-443.

91. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. *Science.* Vol 287; 2000:1427-1430.

92. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature.* Vol 425; 2003:841-846.

93. Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature.* Vol 425; 2003:836-841.

94. Arai F, Hirao A, Ohmura M, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell.* Vol 118; 2004:149-161.

95. Cheshier SH, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self- renewing hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 96; 1999:3120-3125.

96. Bradford GB, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol.* Vol 25; 1997:445-453.

97. Morrison SJ, Wright DE, Weissman IL. Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. *Proc Natl Acad Sci USA.* Vol 94; 1997:1908-1913.

98. Wright DE, Wagers AJ, Gulati AP, Johnson FL, Weissman IL. Physiological migration of hematopoietic stem and progenitor cells. *Science.* Vol 294; 2001:1933-1936.

99. Abkowitz JL, Robinson AE, Kale S, Long MW, Chen J. Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. *Blood.* Vol 102; 2003:1249-1253.

100. Barker JN, Wagner JE. Umbilical cord blood transplantation: current practice and future innovations. *Crit Rev Oncol Hematol.* Vol 48; 2003:35-43.

101. Koh LP, Chao NJ. Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol Blood Marrow Transplant.* Vol 10; 2004:1-22.

102. Wagner Je J, Barker J. Umbilical cord blood transplantation: Novel approaches toward improving engraftment. *Biol Blood Marrow Transplant.* Vol 10; 2004:733.

103. Jaroscak J, Goltry K, Smith A, et al. Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell System. *Blood.* Vol 101; 2003:5061-5067.

104. Grewal SS, Kahn JP, MacMillan ML, Ramsay NK, Wagner JE. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood.* Vol 103; 2004:1147-1151.

105. Wolf SM, Kahn JP, Wagner JE. Using preimplantation genetic diagnosis to create a stem cell donor: issues, guidelines & limits. *J Law Med Ethics.* Vol 31; 2003:327-339.

106. Vodyanik MA, Bork JA, Thomson JA, Slukvin, II. Human embryonic stem cell-derived CD34+ cells: efficient production in the co-culture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood;* 2004.

107. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science.* Vol 279; 1998:1528-1530.

108. Gussoni E, Soneoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature.* Vol 401; 1999:390-394.

109. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* Vol 410; 2001:701-705.

110. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA.* Vol 98; 2001:10344-10349.

111. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* Vol 107; 2001:1395-1402.

112. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science.* Vol 290; 2000:1775-1779.

113. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science.* Vol 290; 2000:1779-1782.

114. Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med.* Vol 6; 2000:1229-1234.

115. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. *Science.* Vol 284; 1999:1168-1170.

116. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell.* Vol 105; 2001:369-377.

117. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest.* Vol 111; 2003:843-850.

118. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* Vol 276; 1997:71-74.

119. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* Vol 284; 1999:143-147.

120. Verfaillie CM, Schwartz R, Reyes M, Jiang Y. Unexpected potential of adult stem cells. *Ann NY Acad Sci.* Vol 996; 2003:231-234.

121. Lemischka I. A few thoughts about the plasticity of stem cells. *Exp Hematol.* Vol 30; 2002:848-852.

122. Verfaillie CM. Adult stem cells: assessing the case for pluripotency. *Trends Cell Biol.* Vol 12; 2002:502-508.

123. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell.* Vol 116; 2004:639-648.

124. Sherwood RI, Christensen JL, Conboy IM, et al. Isolation of Adult Mouse Myogenic Progenitors; Functional Heterogeneity of Cells within and Engrafting Skeletal Muscle. *Cell.* Vol 119; 2004:543-554.

125. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature.* Apr 24 2003;422(6934):897-901.

126. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature.* Vol 425; 2003:968-973.

127. Camargo FD, Finegold M, Goodell MA. Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. *J Clin Invest.* Vol 113; 2004:1266-1270.

128. Phillips RL, Ernst RE, Brunk B, et al. The genetic program of hematopoietic stem cells. *Science.* Vol 288; 2000:1635-1640.

129. Terskikh AV, Easterday MC, Li L, et al. From hematopoiesis to neuropoiesis: evidence of overlapping genetic programs. *Proc Natl Acad Sci USA.* Vol 98; 2001:7934-7939.

130. Park IK, He Y, Lin F, et al. Differential gene expression profiling of adult murine hematopoietic stem cells. *Blood.* Vol 99; 2002:488-498.

131. Terskikh AV, Miyamoto T, Chang C, Diatchenko L, Weissman IL. Gene expression analysis of purified hematopoietic stem cells and committed progenitors. *Blood.* Vol 102; 2003:94-101.

132. Koc ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol.* Vol 18; 2000:307-316.

133. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage.* Vol 10; 2002:199-206.

134. Santos GW. Historical background to hematopoietic stem cell transplantation. In: Atkinson K, ed. *Clinical bone marrow and blood stem cell transplantation.* Cambridge, UK: Cambridge University Press; 2000:1-12.

135. Horowitz MM. Uses and growth of hematopoietic cell transplantationj. In: Forman SJ, ed. *Hematopoietic cell transplantation.* Second ed. Malden, MA: Blackwell Science Inc; 1999:12-18.

136. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs.-host disease. *Biol Blood Marrow Transplant.* Vol 5; 1999:347-356.

137. Thomas ED, Blume KG, Forman SJ. Hematopoietic cell transplantation. 2nd ed. Malden, MA: Blackwell Science, Inc.; 1999.

138. Atkinson K. Clinical bone marrow and blood stem cell transplantation. Cambridge, UK: Cambridge University Press; 2000.

139. Gandy KL, Weissman IL. Tolerance of allogeneic heart grafts in mice simultaneously reconstituted with purified allogeneic hematopoietic stem cells. *Transplantation.* Vol 65; 1998:295-304.

140. Shizuru JA, Jerabek L, Edwards CT, Weissman IL. Transplantation of purified hematopoietic stem cells: requirements for overcoming the barriers of allogeneic engraftment. *Biol Blood Marrow Transplant.* Vol 2; 1996:3-14.

141. Wang B, El-Badri NS, Cherry, Good RA. Purified hematopoietic stem cells without facilitating cells can repopulate fully allogeneic recipients across entire major histocompatibility complex transplantation barrier in mice. *Proc Natl Acad Sci USA.* Vol 94; 1997:14632-14636.

142. Slavin S, Morecki S, Weiss L, Or R. Donor lymphocyte infusion: the use of alloreactive and tumor-reactive lymphocytes for immunotherapy of malignant and non-malignant diseases in conjunction with allogeneic stem cell transplantation. *J Hematother Stem Cell Res.* Vol 11; 2002:265-276.

143. Feinstein L, Sandmaier B, Maloney D, et al. Nonmyeloablative hematopoietic cell transplantation. Replacing high-dose cytotoxic therapy by the graft-versus-tumor effect. *Ann NY Acad Sci.* Vol 938; 2001:328-337; discussion 337-329.

144. Goldman JM. Autologous hematopoietic stem cell transplantation for chronic myeloid leukemia. In: Atkinson K, ed. *Clinical bone marrow and blood stem cell transplantation.* Cambridge, UK: Cambridge University Press; 2000:279-286.

145. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. *N Engl J Med.* Vol 335; 1996:91-97.

146. Haioun C, Lepage E, Gisselbrecht C, et al. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: updated results of the prospective study LNH87-2. Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol.* Vol 15; 1997:1131-1137.

147. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. *N Engl J Med.* Vol 336; 1997:1290-1297.

148. Matthay KK, Villablanca JG, Seeger RC, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis- retinoic acid. Children's Cancer Group. *N Engl J Med.* Vol 341; 1999:1165-1173.

149. Damon LE, Hu WW, Stockerl-Goldstein KE, et al. High-dose chemotherapy and hematopoietic stem cell rescue for breast cancer: experience in California. *Biol Blood Marrow Transplant.* Vol 6; 2000:496-505.

150. Nieto Y, Champlin RE, Wingard JR, et al. Status of high-dose chemotherapy for breast cancer: a review. *Biol Blood Marrow Transplant.* Vol 6; 2000:476-495.

151. Somlo G, Chow W, Hamasaki V, et al. Tandem-cycle high-dose melphalan and cisplatin with peripheral blood progenitor cell support in patients with breast cancer and other malignancies. *Biol Blood Marrow Transplant.* Vol 7; 2001:284-293.

152. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med.* Vol 348; 2003:1875-1883.

153. Rodenhuis S, Bontenbal M, Beex LV, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer. *N Engl J Med.* Vol 349; 2003:7-16.

154. Monitoring treatment and survival in chronic myeloid leukemia. Italian Cooperative Study Group on Chronic Myeloid Leukemia and Italian Group for Bone Marrow Transplantation. *J Clin Oncol.* Vol 17; 1999:1858-1868.

155. Harousseau JL, Cahn JY, Pignon B, et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM). *Blood.* Vol 90; 1997:2978-2986.

156. Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. *N Engl J Med.* Vol 339; 1998:1649-1656.

157. Stevens RF, Hann IM, Wheatley K, Gray RG. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. *Br J Haematol.* Vol 101; 1998:130-140.

158. Hortobagyi GN, Buzdar AU, Theriault RL, et al. Randomized trial of high-dose chemotherapy and blood cell autografts for high-risk primary breast carcinoma. *J Natl Cancer Inst.* Vol 92; 2000:225-233.

159. Bashey A, Corringham S, Garrett J, et al. A phase II study of two cycles of high-dose chemotherapy with autologous stem cell support in patients with metastatic breast cancer who meet eligibility criteria for a single cycle. *Bone Marrow Transplant.* Vol 25; 2000:519-524.

160. Stadtmauer EA, O'Neill A, Goldstein LJ, et al. Conventional-dose chemotherapy compared with high-dose chemotherapy plus autologous hematopoietic stem-cell transplantation for metastatic breast cancer. Philadelphia Bone Marrow Transplant Group. *N Engl J Med.* Vol 342; 2000:1069-1076.

161. Tallman MS, Gray R, Robert NJ, et al. Conventional adjuvant chemotherapy with or without high-dose chemotherapy and autologous stem-cell transplantation in high-risk breast cancer. *N Engl J Med.* Vol 349; 2003:17-26.

162. Slavin S. Non-myeloablative stem cell transplantation for induction of host-versus-graft tolerance for adoptive immunotherapy of malignant and nonmalignant diseases and towards transplantation of organ allografts. *Transplant Proc.* Vol 34; 2002:3371-3373.

163. Spitzer TR. The expanding applications of non-myeloablative stem cell transplantation. *Pediatr Transplant.* Vol 7 Suppl 3; 2003:95-100.

164. Maris M, Sandmaier BM, Maloney DG, et al. Non-myeloablative hematopoietic stem cell transplantation. *Transfus Clin Biol.* Vol 8; 2001:231-234.

165. Uchida N, Tsukamoto A, He D, Friera AM, Scollay R, Weissman IL. High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. *J Clin Invest.* Vol 101; 1998:961-966.

166. Shizuru JA, Weissman IL, Kernoff R, Masek M, Scheffold YC. Purified hematopoietic stem cell grafts induce tolerance to alloantigens and can mediate positive and negative T cell selection. *Proc Natl Acad Sci USA.* Vol 97; 2000:9555-9560.

167. Gandy KL, Domen J, Aguila H, Weissman IL. *CD8+ TCR+ and CD8+ TCR+ cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers:* Immunity; 1999.

168. McDevitt HO. The role of MHC class II molecules in susceptibility and resistance to autoimmunity. *Curr Opin Immunol.* 1998;10:677-681.

169. Beilhack GF, Landa RR, Masek MA, Shizuru JA. Prevention of type 1 diabetes with major histocompatibility complex-compatible and nonmarrow ablative hematopoietic stem cell transplants. *Diabetes.* 2005;54:1770-1779.

170. Beilhack GF, Scheffold YC, Weissman IL, et al. Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes.* 2003;52:59-68.

171. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* Vol 414; 2001:105-111.

172. Hope KJ, Jin L, Dick JE. Human acute myeloid leukemia stem cells. *Arch Med Res.* Vol 34; 2003:507-514.

173. Guzman ML, Jordan CT. Considerations for targeting malignant stem cells in leukemia. *Cancer Control.* Vol 11; 2004:97-104.

174. Scadden DT. Cancer stem cells refined. *Nat Immunol.* Vol 5; 2004:701-703.

175. Passegue E, Jamieson CH, Ailles LE, Weissman IL. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc Natl Acad Sci USA.* Vol 100 Suppl 1; 2003:11842-11849.

176. Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF. Therapeutic implications of cancer stem cells. *Curr Opin Genet Dev.* Vol 14; 2004:43-47.

177. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med.* Vol 351; 2004:657-667.

178. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. *Proc Natl Acad Sci USA.* Vol 97; 2000:7521-7526.

179. Jones RJ, Matsui WH, Smith BD. Cancer stem cells: are we missing the target? *J Natl Cancer Inst.* Vol 96; 2004:583-585.

180. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene.* Vol 23; 2004:7274-7282.