HEMATOPOIETIC STEM CELLS

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Introduction

A hematopoietic stem cell is a cell isolated from the **blood or bone marrow** that can **renew** itself, can **differentiate** to a variety of specialized cells, can **mobilize** out of the bone marrow into circulating blood, and undergoes **apoptosis**.

2 kinds of HSCs.

Long-term stem cells:

If bone marrow cells from the transplanted mouse can, in turn, be transplanted to another lethally irradiated mouse and restore its hematopoietic system over some months, they are considered to be *long-term stem cells that are* capable of self-renewal.

Short-term progenitor or precursor cells:

Other cells from bone marrow can immediately regenerate all the different types of blood cells, but under normal circumstances cannot renew themselves over the long term, and these are referred to as *short-term progenitor or precursor cells.*

They are capable of proliferating, but they have a limited capacity to differentiate into more than one cell type as HSCs do.

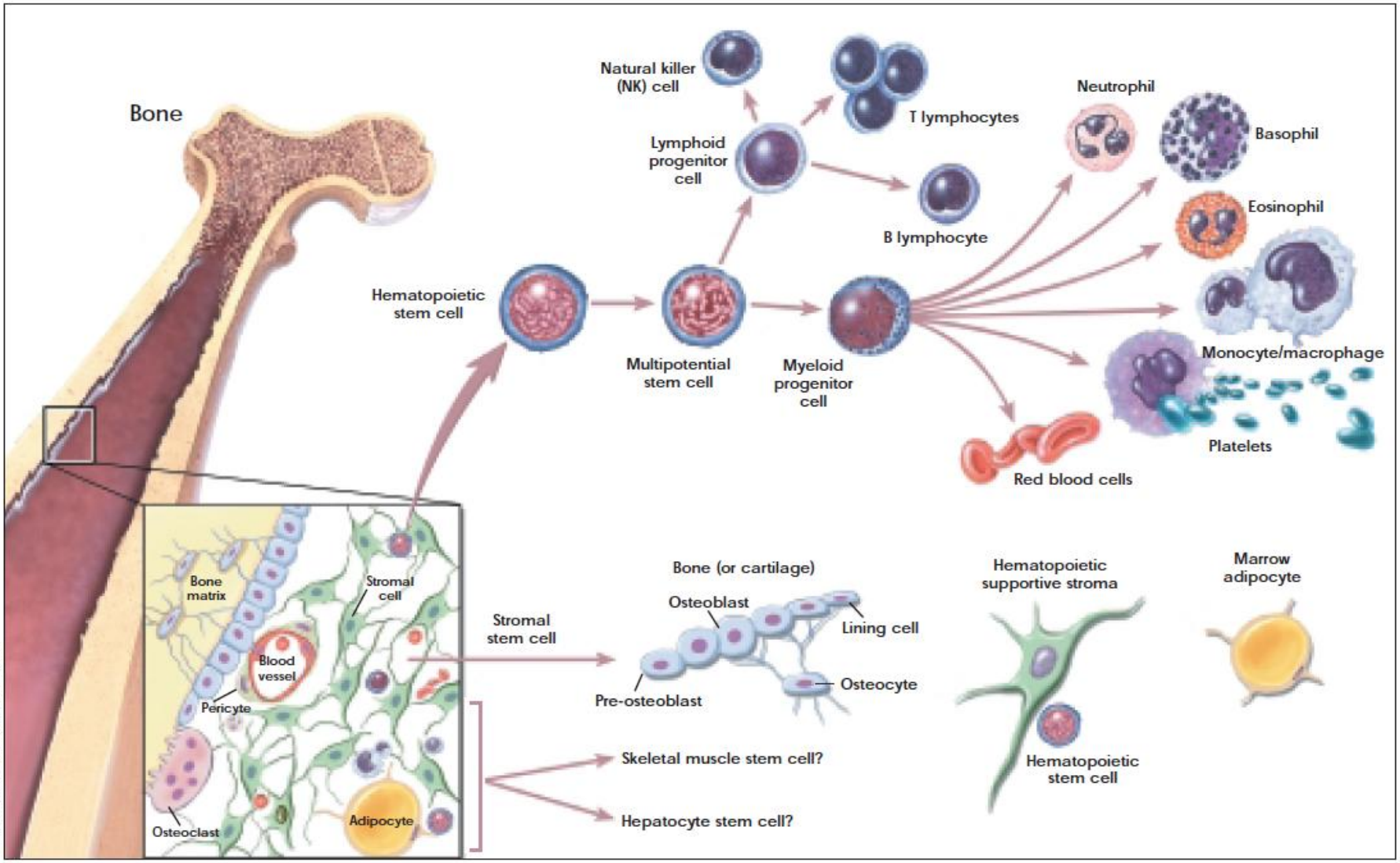

Figure 5.1. Hematopoietic and Stromal Stem Cell Differentiation.

Table 5.1. Proposed cell-surface markers of undifferentiated hematopoietic stem cells.

Listed here are cell surface markers found on mouse and human hematopoietic stem cells as they exist in their undifferentiated state *in vivo* and *in vitro*. As these cells begin to develop as distinct cell lineages the cell surface markers are no longer identified.

Mouse	Human
$CD34^{low/-}$	$CD\ 34^{+}$
$SCA\text{-}1^{+}$	$CD59^{+*}$
$Thy1^{+/low}$	$Thy1^{+}$
$CD38^{+}$	$CD38^{low/-}$
$C\text{-}kit^{+}$	$C\text{-}kit^{-/low}$
lin^{-*}	lin^{-**}

* *Only one of a family of CD59 markers has thus far been evaluated.*

** *Lin- cells lack 13 to 14 different mature blood-lineage markers.*

SOURCES OF HEMATOPOIETIC STEM CELLS

- Bone Marrow
- Peripheral Blood
- Umbilical Cord Blood
- Fetal Hematopoietic System
- Embryonic Stem Cells and Embryonic Germ Cells

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- Stem cell populations of the bone marrow

- Effectiveness of Transplants of Adult versus Umbilical Cord Blood Stem Cells

- Effectiveness in Transplants of Peripheral Versus Bone Marrow Stem Cells

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1) it can renew itself
2) it can differentiate
3) it can mobilize out of the bone marrow into circulation (or the reverse) or
4) it can undergo programmed cell death, or apoptosis.

Self-renewal of Hematopoietic Stem Cells

stem cell factor and thrombopoietin; signaling molecule gp130

Differentiation of HSCs into Components of the Blood and Immune System

in the course of producing a mature, circulating blood cell, the original hematopoietic stem cell will undergo between 17 and 19.5 divisions, “giving a net amplification of between ~170,000 and ~720,000

Migration of Hematopoietic Stem Cells Into and Out of Marrow and Tissues:

HSCs may also be found in the spleen, in peripheral blood circulation, and other tissues. Connection to the interstices of bone marrow is important to both the engraftment of transplanted cells and to the maintenance of stem cells as a self-renewing population. Connection to stroma is also important to the orderly proliferation, differentiation, and maturation of blood cells

Apoptosis and Regulation of Hematopoietic Stem Cell Populations

when they used antibodies to disrupt the adhesion of HSCs to the stroma via **VLA-4/VCAM-1**, the cells were predisposed to apoptosis

stem cells need to get two growth factor signals to continue life and avoid apoptosis: one via a protein called BCL-2, the other from steel factor, which, by itself, induces HSCs to produce progenitor cells but not to self-renew

- Leukemia and Lymphoma
- Inherited Blood Disorders
- Hematopoietic Stem Cell Rescue in Cancer Chemotherapy
- Graft-Versus-Tumor Treatment of Cancer
- Other Applications of Hematopoietic Stem Cells

Bone marrow from non-*mdx male mice was transplanted into* female *mdx mice with chronic muscle damage;* after 70 days, researchers found that nuclei from the males had taken up residence in skeletal and cardiac muscle cells.

Krause has shown in mice that a *single* selected donor hematopoietic stem cell could do more than just repopulate the marrow and hematopoietic system of the recipient. These investigators also found **epithelial cells derived from the donors in the lungs, gut, and skin of the recipient mice**. This suggests that HSCs may have grown in the other tissues in response to infection or damage from the irradiation the mice received.

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Boosting the Numbers of Hematopoietic Stem Cells

Outfoxing the Immune System in Host, Graft, and Pathogen Attacks

Understanding the Differentiating Environment and Developmental Plasticity

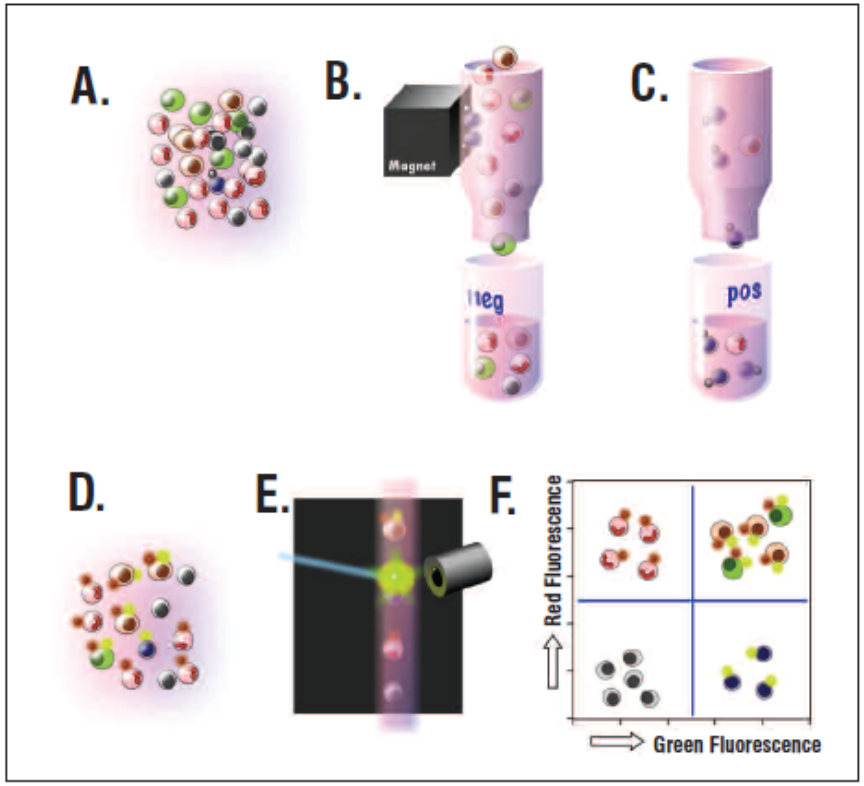

Figure 2.2. Enrichment and purification methods for hematopoietic stem cells. Upper panels illustrate column-based magnetic enrichment. In this method, the cells of interest are labeled with very small iron particles (A). These particles are bound to antibodies that only recognize specific cells. The 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.

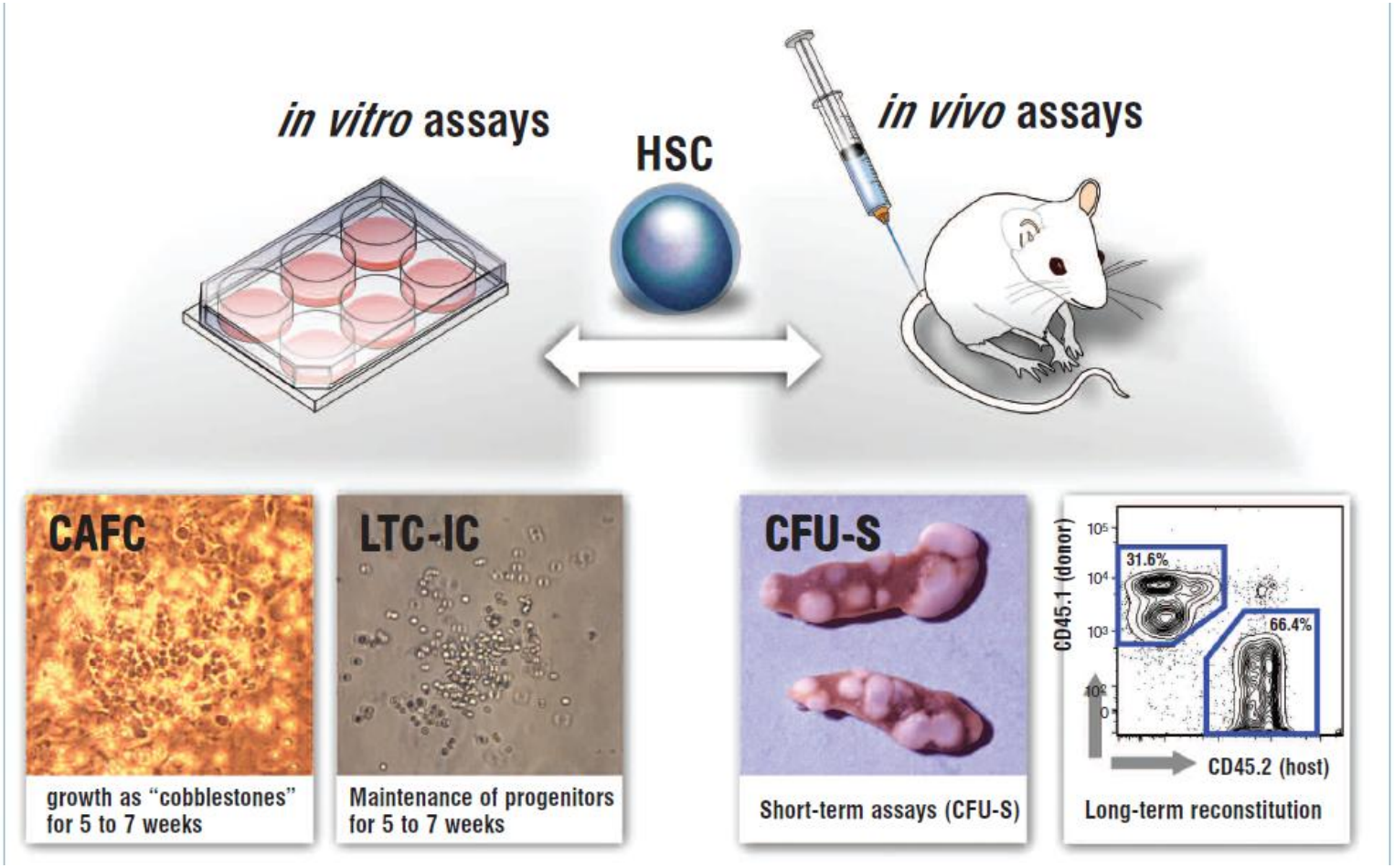

Figure 2.3. Assays used to detect hematopoietic stem cells. The tissue culture assays, which are used frequently to test human cells, include the ability of the cells to be tested to grow as "cobblestones" (the dark cells in the picture) for 5 to 7 weeks in culture. The Long Term Culture-Initiating Cell 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.

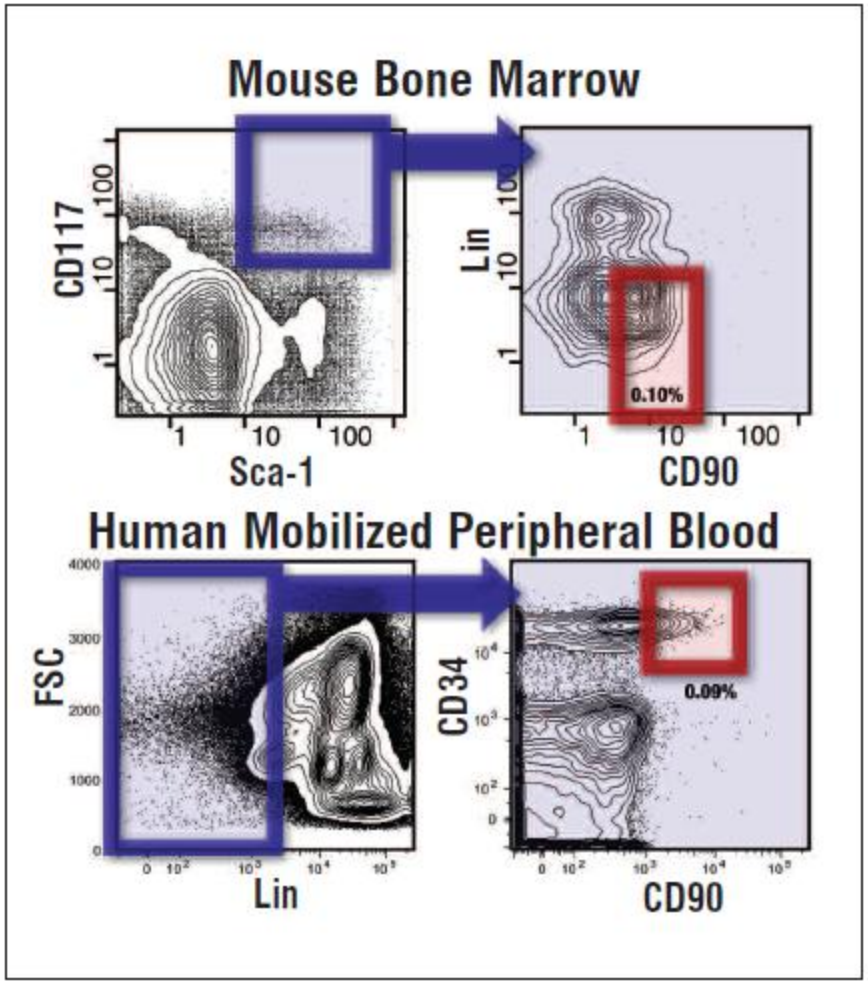

Figure 2.4. Examples of Hematopoietic Stem Cell staining patterns in mouse bone marrow (top) and human mobilized peripheral blood (bottom). The plots on the right show only the cells present in the left blue box. The cells in the right blue box represent HSCs. Stem cells form a rare fraction of the cells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

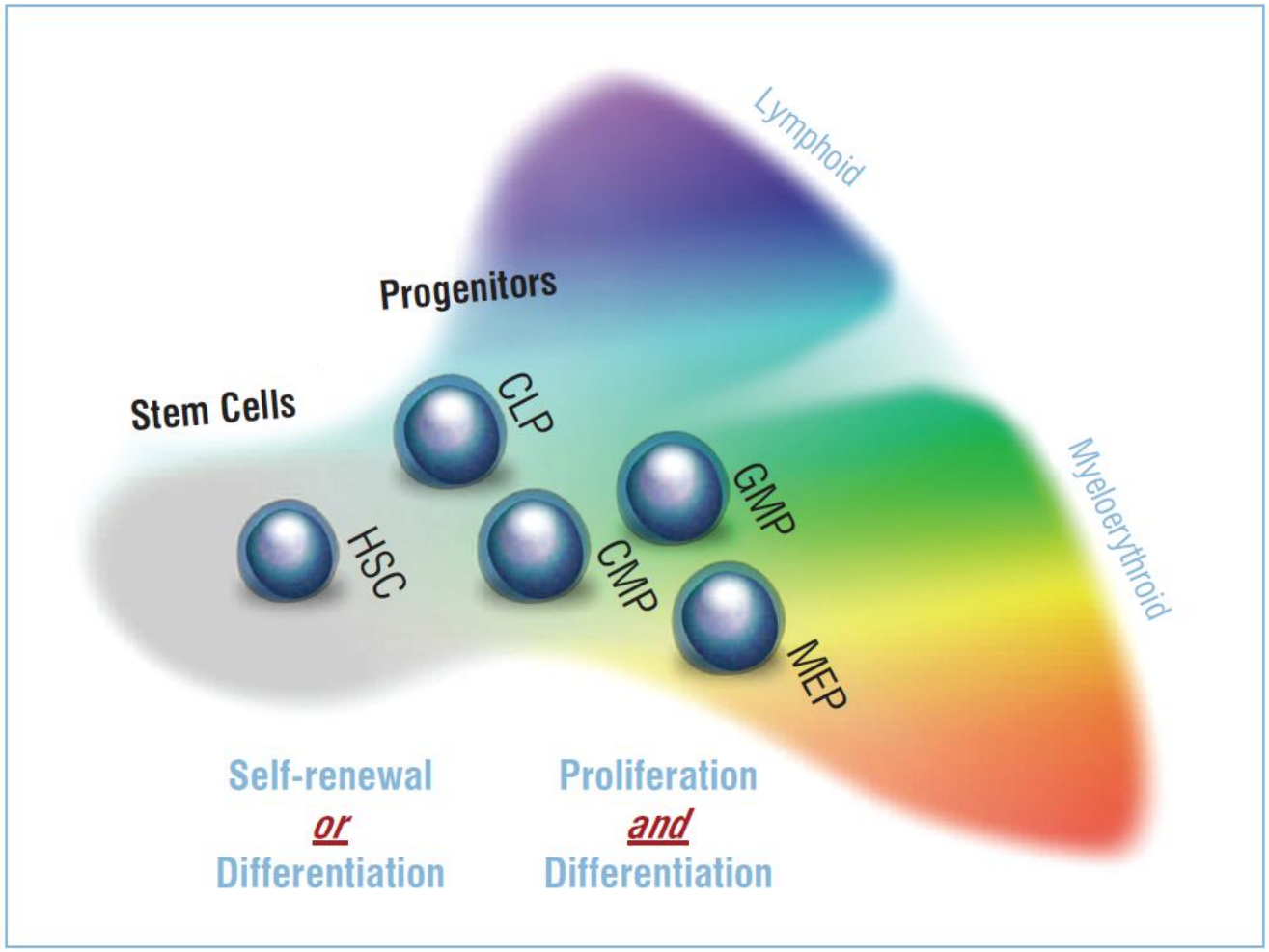

Figure 2.5. Relationship between several of the characterized hematopoietic stem cells and early progenitor cells. Differentiation is indicated by colors; the more intense the color, the more mature the cells. Surface marker distinctions are subtle between these early cell populations, yet they have 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.

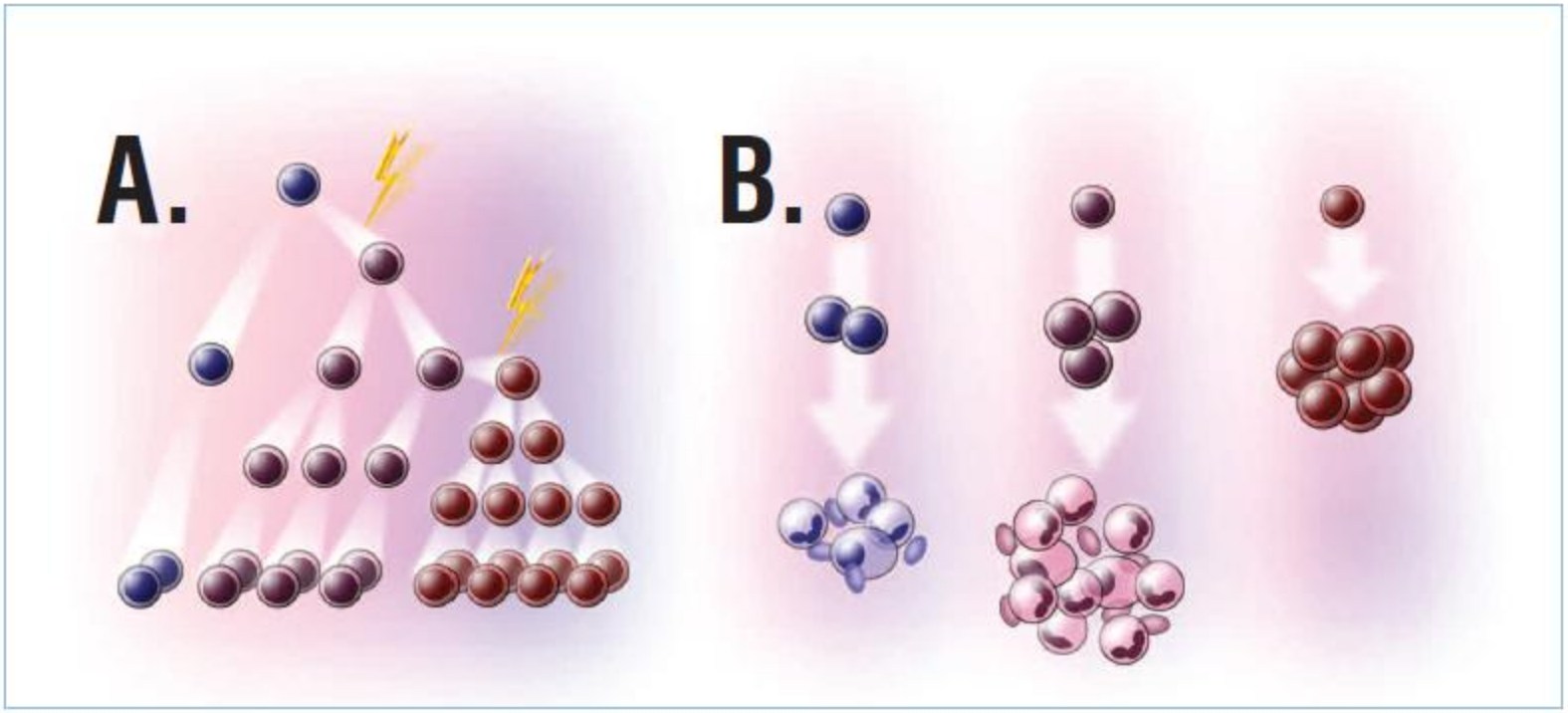

Figure 2.6. Leukemic progression at the hematopoietic stem cell level. Self-renewing HSCs are the cells present long enough to accumulate the many activating events necessary for full transformation into tumorigenic cells. Under normal conditions, half of the offspring of HSC cell divisions would be 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.