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HEMATOPOIETIC STEM AND PROGENITOR CELLS: Clinical and Preclinical Regeneration of the Hematolymphoid System

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■ **Abstract** A vast literature exists on the biology of blood formation and regeneration under experimental and clinical conditions. The field of hematopoiesis was recently advanced by the capacity to purify to homogeneity primitive hematopoietic stem and progenitor cells. Isolation of cells at defined maturational stages has enhanced the understanding of the fundamental nature of stem cells, including how cell fate decisions are made, and this understanding is relevant to the development of other normal as well as malignant tissues. This review updates the basic biology of hematopoietic stem cells (HSC) and progenitors, the evolving use of purified HSC as grafts for clinical hematopoietic cell transplantation (HCT) including immune tolerance induction, and the application of HSC biology to other stem cell fields.

INTRODUCTION

Stem cells are cells that, at the single-cell level, both self-renew more stem cells and give rise to clonal progeny that continue to differentiate (1). Thus, hematopoietic stem cells (HSC) are multipotent cells that give rise to more HSC and all formed elements in the blood. Progenitor cells are cells that may be multipotent, oligopotent, or unipotent, and they lack significant self-renewal capacity (1). HSC are entirely responsible for the development, maintenance, and regeneration of blood forming tissues for life (1–4). Furthermore, HSC are the most important, if not the only, cells required to engraft in hematopoietic tissue transplants (3–5). The hematopoietic system is arguably the best characterized among all of the tissues of the human body owing to its unique biological properties, which have allowed both experimental manipulation in preclinical studies and its transplantation into patients who have undergone purposeful ablation of their hematopoietic organ.

Isolation of HSC

The first isolation of HSC required quantitative clonal assays for every blood cell progenitor type (T, B, myeloerythroid) (5–8) and methods to sort cells based on their unique gene expression profiles, as manifested by cell surface proteins and glycoproteins (7–9). Using this approach, both mouse and human HSC were prospectively isolated (8, 9). All HSC activity in adult mouse bone marrow (BM) was shown to be contained in a population marked by the composite phenotype of c-Kit$^+$, Thy-1.1^{lo}, lineage marker$^{-/lo}$, and Sca-1$^+$ (designated KTLS) (3, 10). These KTLS HSC in mice transplanted at the single-cell level gave rise to lifelong hematopoiesis, including a steady state of 20 to 100,000 HSC and over 10^9 blood cells produced daily (11–15).

In humans, the combination of positive selection for CD34, Thy-1 and negative selection for lineage marked cells resulted in the purification of a homologous HSC population (9). The critical tests that these candidate human HSC could be applied to clinical HCT—that is, rescue of myeloablated hosts from hematopoietic failure—were performed first in congenic mouse into mouse experiments (7, 8) and subsequently in xenogeneic models into myeloablated immunodeficient mice (9, 16–18). Following the success in rodent models, human HSC were tested in clinical trials (described below) (19–21). In both mouse and human, isolation of HSC defined by the markers shown in Figure 1 led to grafts depleted of contaminating hematopoietic cells, such as T cells, and contaminating resident or metastasized cancer cells (22). This prospective isolation of HSC is, to date, the only effective and complete purge of grafts containing unwanted populations from clinically functional and transplantable HSC populations.

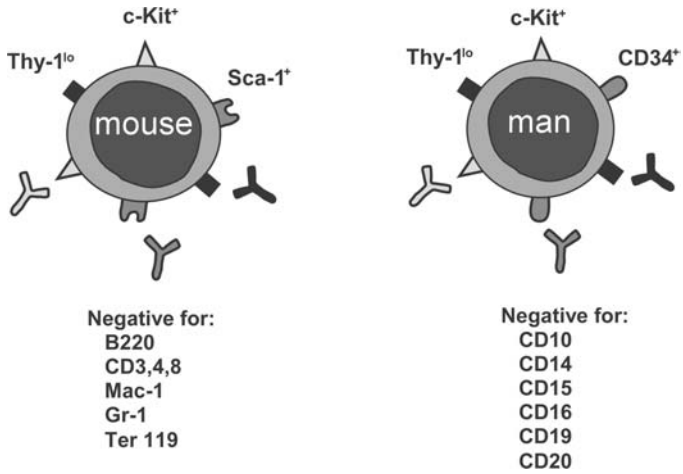

Figure 1 Composite surface phenotype that marks hematopoietic stem cells in mouse (*left*) and humans (*right*). Monoclonal antibodies can be used to select cells on the basis of surface marker expression or to negatively select unwanted populations.

Early Lineage Hematopoietic Progenitors

Development and commitment of progenitors arising from HSC can be followed by surface marker expression and functional readout assays. Mouse HSC that commit to differentiation pass through a phase of being short-term HSC (ST-HSC) that self-renew for six to eight weeks only, then advance to the multipotent progenitor (MPP) stage (23, 24). MPP self-renew for less than two weeks, but neither MPP nor ST-HSC are capable of dedifferentiating to long-term HSC (LT-HSC) under any circumstances yet tested (25). These multipotent stem and/or progenitor cells then commit either to the myeloid or lymphoid lineage by differentiating to common myeloid progenitors (CMP) or common lymphoid progenitors (CLP) (26–33).

Both CMP and CLP are clonal progenitors with little or no detectable self-renewal capacity and limited differentiation as shown in Figure 2 (26–33). CLP give rise to progenitors of at least four classes of cells—the T, B, and natural killer (NK)

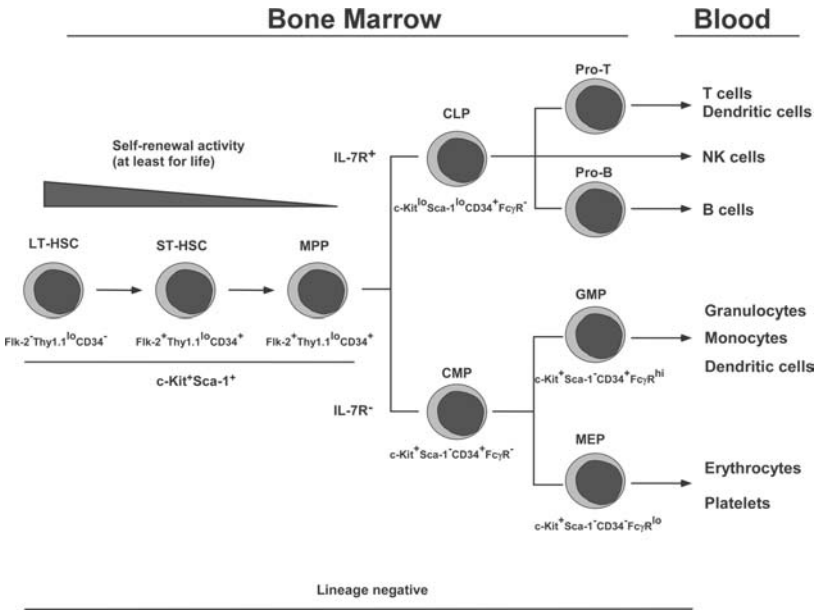

Figure 2 Conceptual hematopoietic tree in adult mice. Long-term hematopoietic stem cells (LT-HSC) self-renew for life, whereas their downstream short-term (ST)-HSC self-renew for six to eight weeks. Further downstream progenitors have been prospectively isolated to phenotypic, functional, and for the more mature cells, gene expression profile homogeneity. MPP, multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte/macrophage progenitor; MEP, megakaryocyte/erythrocyte progenitor. Figure redrawn with permission (39).

lymphocyte lineages, and antigen presenting dendritic cells (34, 35). CMP give rise at least to granulocyte monocyte committed progenitors (GMP) or megakaryocyte erythrocyte progenitors (MEP) (26). Recently, we isolated mouse megakaryocyte committed progenitors (MkP) that are likely downstream progeny derived from MEP (36). Figure 2 compares the surface markers known to be present on mouse and human HSC, MPP, and oligolineage progenitors of the myeloid and lymphoid lineages. In mouse transfer studies, oligolineage progenitors give rise to a short but significant burst of daughter cells of their restricted lineages, but because they do not self-renew, by three to four weeks they are no longer significant sources of new blood cells (37). Although the progenitors cannot durably engraft, they nonetheless rescue lethally irradiated mice (38). Thus, infusion of high doses of progenitor cells (ST-HSC, MPP, CMP, MEP) can provide transient hematopoiesis in lethally irradiated mice kept in clean conditions until endogenous host HSC recover (23, 37).

Control of HSC Fates

Prospective isolation and study of mouse HSC has revealed many aspects of their biology and regeneration capacity (39). When an LT-HSC undergoes cell division, it has at least four potential fates—self-renewal, differentiation to ST-HSC, emigration from the BM into the blood, and programmed cell death (apoptosis). Each event involves a regulated shift of the gene expression profile of LT-HSC, including the decision to self-renew. When mouse LT-HSC are incubated in the presence of cytokine(s) and/or ligand(s) for surface receptors, they enact gene expression profiles that reflect the nature of the ligand(s) stimulating their receptor (38). Evidence suggests that signaling through Wnt is a critical pathway utilized by LT-HSC in self-renewing divisions. Wnt proteins are intercellular signaling molecules (40) that regulate development in several organisms (41). Contact of HSC with Wnt results in signaling events such that HSC enter the cell cycle with little differentiation out of the LT-HSC pool (42, 43). The signaled HSC activates a pathway from the surface receptor "frizzled" (Fzd) via a gene product called "disheveled" (Dsh) to separate cytoplasmic β-catenin from a protein complex (APC/axin/GSK3β). In its phosphorphylated form, APC/axin/GSK3β/β-catenin holds β-catenin in the cytoplasm and marks any released phosphorylated β-catenin for rapid degradation in the cytoplasm (for review, see 44). When Wnt signals through Dsh, the complex dissociates and unphosphorylated β-catenin is free to translocate to the nucleus, where it binds to DNA binding proteins such as lymphoid enhancer factor (LEF) and T-cell factor (TCF), converting them from repressors to activators of gene transcription (44). Culture of single LT-HSC in serum-free media with cytokines such as steel factor (SLF), interleukin 11 (IL-11), interleukin 6 (IL-6), thrombopoietin (TPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (GCSF), and macrophage colony-stimulating factor (MCSF) results in most or all LT-HSC entering the cell cycle, transitioning into ST-HSC, and then transitioning into progenitors without measurable HSC

expansion by self-renewal (38). Activation of LT-HSC by purified Wnt 3A results in upregulation of genes such as hoxB4 and Notch 1 (43), genes independently implicated in HSC proliferation and perhaps self-renewal (45–50). The polycomb family gene bmi-1 is also required for self-renewal of LT-HSC in vivo, and also for self-renewal of central and peripheral nervous system cells (51, 51a). Much will be learned about HSC homeostasis by discovering how these pathways regulate HSC activity and their genetically determined frequencies in hematopoietic tissues (52–57).

Expansion of HSC by cell division and exit of a fraction of HSC and HSC progeny into the blood with immigration to distant sites occurs under both steady state and conditions of mobilization (i.e., growth factor ± cyclophosphamide) (11, 18, 58–66). In mice, the average residence time of blood HSC from normal or "mobilized" animals is ~1 min. To maintain the steady state of 100 LT-HSC in the blood, 10^4 to 10^5 LT-HSC must traffic through the blood of a mouse every day (58). Many of these immigrating HSC in physiological or mobilization conditions seed hematopoietic niches at a distance and set up one or more foci of hematopoiesis and HSC self-renewal (58). Thus, unexpectedly, a high flux of HSC traffics through any blood perfused tissue, and the full significance of that flux is not yet understood (58, 60). This flux means that isolation of hematopoietic activity from nonhematopoietic tissue could be derived from itinerant HSC rather than resident tissue-specific stem cells with unexpected hematopoietic activity, whether they are pluripotent or transdifferentiating resident stem cells.

Finally, programmed cell death and chromosome telomere length appear to be one regulators of HSC numbers and functions (66a). The programmed death of HSC can be prevented by enforced expression of the antiapoptotic protein BCL2 in these cells, and such cells and the mice that harbor them have increased HSC frequency and function without developing neoplasms at the level of HSC (38, 67).

HSC Shifts During Ontogeny

HSC change their developmental capacities throughout ontogeny, a process that results in distinct differentiation potentials and gene expression profiles as they progress from multipotent to oligopotent progeny (27, 68). The first defined hematopoietic sites that also contains HSC in mice are the yolk sac blood islands. Hematopoiesis in the yolk sacs precedes the appearance of hematopoietic cells in the embryo proper and in the connection of the vasculature of the yolk sac via the umbilical vein to the embryo proper through the liver (69). Although yolk sac blood island cells transplanted into the yolk sacs of same age allogeneic embryos/fetuses give rise to adult hematopoietic and lymphoid systems (70, 71), at that stage of differentiation these cells do not possess the capability of engrafting adult irradiated hosts (72, 73). HSC found in the fetal liver a few days later possess the capacity to home to hematopoietic sites in irradiated newborn and adult hosts (74–76), and this event requires at least the expression of cell surface homing receptors

(integrin $\alpha 4\beta 1$ and chemokine receptors CXCR4) (65, 77, 78, 44). Thus, there is a fundamental distinction between the yolk sac stage and the fetal liver stage of hematopoiesis; furthermore, the origin of HSC found prior to the establishment of liver hematopoiesis in the dorsal aorta of the aorta-gonads-mesonephros (AGM) region (see 73) might be derived from yolk sac immigrants or from an independent generation of HSC in the embryo proper, and convincing experiments to distinguish between these possibilities have yet to be carried out (70, 71).

Yolk sac hematopoiesis in mice initiates at day 7.5 postconception (DPC), which is four days after the mouse blastocyst implants in the uterus (79). Interestingly, embryonic stem (ES) cell lines are derived from the DPC 3.5 blastocyst inner cell mass, and under various culture conditions give rise to ES-derived in vitro HSC on day 4 or 5 (80). This timing is important when one considers the ability of ES cells to give rise to transplantable HSC (see below).

TRANSLATION OF HSC TRANSPLANTATION TO CLINICAL PRACTICE

The two major types of clinical HCT procedures, autologous and allogeneic, are defined by the donor graft source. Most patients that undergo HCT have a malignancy that either has proven resistant to standard chemotherapy or is considered to be high risk for relapse even though complete remission (CR) may have been achieved. The principle behind the two approaches differs. In autologous HCT, myeloablative doses of chemotherapy and/or radiation are administered to optimize tumor kill. The dose limiting toxicity is death to the hematopoietic organ. Thus, prior to HCT, patients undergo harvesting of their hematopoietic cells. Compared to BM harvest, significantly higher numbers of stem and progenitor cells can be obtained from the blood after growth factor injection—a process termed mobilization. Thus, the current practice is to collect autologous mobilized peripheral blood (MPB) grafts.

Allogeneic HCT is similarly applied to patients with high risk, relapsed, or chemotherapy refractory malignancies. However, the spectra of diseases treated differ, as do the potential benefits and complications. For hematologic malignancies thought to evolve from early hematopoietic progenitors (i.e., chronic myelogenous leukemia) or for BM failure states (i.e., aplastic anemia), replacement with healthy donor cells is required. In addition, allograft recipients benefit from an effect termed graft-versus-tumor (GVT), which is conferred by virtue of the donor's nongenetic identity. Under standard conditions, donors and recipients are matched at the major histocompatibility complex (MHC), which in humans are the genes of the human leukocyte antigens (HLA). However, more recently, increasing numbers of haploidentical (one HLA chromosomal region shared, the other different) HCT are being performed. Compared to autologous HCT, allogeneic HCT is higher risk, primarily because allografts contain mature immune cells that can respond against host-specific antigens and cause the syndrome called graft-versus-host disease

(GVHD). The non-HLA antigens that induce GVHD are not yet known, although candidate genes such as alleles of CD31 in humans (81–83) and H60 (H-2K^b restricted) in mice have been proposed. All allogeneic HCT patients that receive unmodified grafts are given GVHD prophylaxis with immunosuppression, and despite this prophylaxis, ~20–30% of such patients develop an acute form of GVHD, and ~50% develop a chronic form of the disease (84, 85). Taken together with the risks of GVHD, immunosuppression, and the potential for engraftment failure, the transplant-related mortality for an allogeneic HCT is ~10–15%.

Autologous HSC Transplantation: Lessons from Mice

Isolation of human HSC was challenging owing to the rarity of the population and lack of suitable assays such as the reconstitution of lethally irradiated animals. Surrogate in vitro and in vivo assays were used to screen for biological activity, and by employing the strategy of fluorescence-activated cell sorting (FACS)–based separation as developed in mice, a candidate human HSC with the surface phenotype of CD34$^+$Thy-1$^+$Lin$^-$ was identified (9, 86, 87).

One concern in translating the HSC studies from mouse to human was the view that because HSC are primitive, engraftment times would be prohibitively delayed compared to standard MPB grafts. Thus, the kinetics of reconstitution were tested in mice comparing KTLS HSC engraftment with BM (88). Engraftment times were shown to be a direct function of the dose of purified HSC infused (4) and were nearly identical for HSC versus BM. Correlate results from these experiments included the realization that the rapidity of BM or MPB engraftment is solely a property of its HSC content, and that a biologic threshold exists for HSC engraftment, with ~9–10 days being the soonest mice engraft with even very high HSC doses. The latter observations agree with a common finding after human autologous HCT, that is, the earliest time to engraftment, even in patients given mega-doses of MPB, is ~8–9 days. Assuming the average mouse weighs 25 g, the studies showed that the equivalent human HSC dose that should give rapid engraftment is $2–4 \times 10^5$ HSC/kg, and as described below, this dose was predictive of engraftment kinetics in the clinical trials.

Clinical Trials of Purified Autologous HSC

The clinical trials of HSC transplantation formally validated the biologic principles developed in mice and confirmed the efforts behind the isolation of the human HSC. Three separate trials were carried out for patients with multiple myeloma (21), non-Hodgkin's lymphoma (NHL) (20), and metastatic breast cancer (19). The goal was to purify HSC, and thereby reduce the burden of occult malignant cells. It is known for all three diseases that a significant percentage of harvested BM or MPB are contaminated with malignant cells. Analysis of graft purity documented that HSC isolation resulted in excellent removal of tumor cells.

These HSC clinical trials presented technical challenges, primarily because of the rarity of HSC in BM and MPB. In human BM, ~5–10% of cells are CD34$^+$, and

of these cells, 10–20% express the $CD34^+Thy\text{-}1^+Lin^-$ phenotype (9). In steady state, peripheral blood HSC are even rarer. Mobilization with either growth factors such as granulocyte-colony stimulating factor (GCSF) or chemotherapy plus GCSF results in a time-dependent proliferation and efflux of HSC out of the BM into the blood (89). The HSC-enriched mononuclear cell fraction can then be collected from the blood by apheresis and processed for purification. Large numbers of non-HSC are recovered from an apheresis procedure, and for technical considerations, a debulking step using $CD34^+$ magnetic beads is required prior to cell sorting on the FACS (89).

ASSESSMENT OF TUMOR CONTAMINATION OF $CD34^+THY\text{-}1^+$ HSC In all three trials, tumor reduction following $CD34^+Thy\text{-}1^+$ isolation was assessed by sensitive assays of malignant cells including PCR for clonal immunoglobulin (Ig) or t (14; 18) gene rearrangements for patients with multiple myeloma (21) and NHL (20), respectively. For breast cancer patients, the investigators used an immunofluorescence-based assay using antibodies against cytokeratin (present in malignant breast cancer cells). This assay was capable of detecting one malignant cell in 10^6 normal cells. In most $CD34^+Thy\text{-}1^+$ samples analyzed, tumor reduction of >3 logs was routinely achieved. In the trial of patients with metastatic breast cancer, 37% of patient MPB samples had detectable tumor cells. Following HSC selection, all of the positive samples were rendered negative (19). For patients with NHL, 30% had PCR evidence of minimal residual disease (MRD) in MPB, all of whom were successfully rendered negative following HSC selection—a >3.26 to >5.68-fold log 10 reduction (20). In patients with multiple myeloma, clonal plasma cells were frequently detected prior to selection. Following $CD34^+Thy\text{-}1^+Lin^-$ HSC isolation, up to 5 logs of tumor removal was achieved (21, 90). Thus, most of the patients transplanted with $CD34^+Thy\text{-}1^+$ HSC received grafts without any detectable malignant cells.

TIME TO ENGRAFTMENT OF $CD34^+THY\text{-}1^+$ HSC Following high-dose myeloablative therapy, all patients received $CD34^+Thy\text{-}1^+$ HSC only. However, additional MPB products were collected and stored without manipulation as "backup" (19–21). In the metastatic breast cancer trial (19), engraftment was prompt and occurred with neutrophil recovery (>500 cells/μl) in a median of 10 days (range 8–15 days). Platelet recovery was also rapid, with patients achieving an unsupported platelet count in a median of 14 days (range 9–42 days). The relationship between the number of HSC/kg infused and time to neutrophil and platelet recovery for patients in this trial is shown in Figure 3. Over the dose range tested, all patients recovered neutrophil counts in a timely fashion. The lowest dose administered was 4.7×10^5 $CD34^+Thy\text{-}1^+$ cells/kg. Engraftment kinetics were as predicted by the mouse HSC studies (wherein a dose of $2\text{–}4 \times 10^5$ HSC/kg predicted rapid engraftment) and were similar to engraftment times for patients who undergo HCT with unmanipulated MPB. The data further suggested that the minimal engraftment HSC dose is substantially lower. Comparable engraftment results were obtained in the trials of

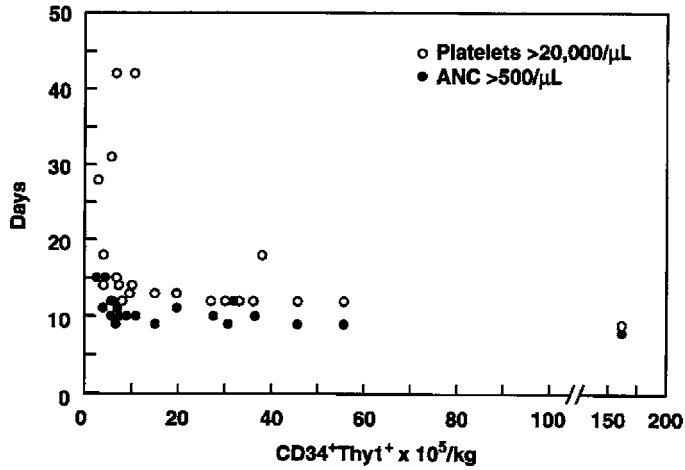

Figure 3 Engraftment times in patients with metatastic breast cancer that underwent myeloablative transplantation and rescue with purified autologous HSC. Shown are days to neutrophil (*solid circles*) and platelet engraftment (*open circles*) based on the number of $CD34^+Thy\text{-}1^+$ cells infused. Each dot represents an individual patient. Reprinted with permission (19).

HSC for patients with multiple myeloma and NHL. Thus, HSC isolation is feasible on a clinical scale, and sufficient numbers of cells can be obtained to perform autologous HCT using high dose preparative regimens.

In all three studies, some patients did not mobilize sufficiently to proceed to HSC isolation. For example, two mobilization regimens were used for the metastatic breast cancer patients–high dose (24 μg/kg/d) G-CSF and cyclophosphamide (4 gm/m^2) plus standard dose (10 μg/kg/d) G-CSF. Among the 28 enrolled patients, 4 patients (14%) did not have sufficient HSC in their MPB products to undergo the HSC purification process (19). A median of two aphereses (range 2–4) were required to obtain a dose of $CD34^+Thy\text{-}1^+$ cells of 11.3 (range 4.7–163.1) $\times$ 10^5/kg. Purity was 95.3% (range 91.1–98.3%) and viability of 98.6% (range 96.5–100%). Although it is difficult to measure recovery yields of $CD34^+Thy\text{-}1^+$ cells during the purification process because of the low and sometimes inaccurate value of the $CD34^+Thy\text{-}1^+$ cells obtained prior to HSC selection, yields can be estimated to be in the range of 15–40%.

IMMUNE RECONSTITUTION POST-HSC TRANSPLANTATION A potential concern using HSC grafts was that delayed immune reconstitution might result in increased opportunistic infections. Indeed, as compared with patients that receive MPB, lymphocyte recovery times were slower. T cell recovery was delayed, especially $CD4^+$ T cells, which took up to 3–6 months to achieve >100 $CD4^+$ T cells/μl (19). However, HSC engrafted patients rarely developed opportunistic infections

and the frequency and severity of these infections was not significantly different from standard HCT using MPB. But it should noted that a relatively small number of patients were evaluated and that a minority developed unusual infections, especially in the NHL trial (i.e., severe cases of influenza, respiratory syncytial virus, and an episode of *Pneumocystis* pneumonia) (20), thus raising caution. To date, there are no studies showing how HSC dose impacts immune recovery.

DISEASE OUTCOMES Because of the small patient sample size, none of the studies were powered to evaluate event-free and overall survival. However, most patients appeared to fare well. Favorable results were particularly evident in the metastatic breast cancer study, where 73% of patients (16 of 22) were alive and 41% (9 of 22) disease free in the initial report (19). Further follow-up continues to be encouraging (R.S. Negrin, unpublished observations). Because of the small number of patients studied, it is not yet possible to determine whether purified HSC result in improved outcome due to the reduction in reinfused metastatic malignant cells, although the results obtained to date are provocative and deserve follow-up with larger studies.

ALLOGENEIC HSC TRANSPLANTATION

The in vivo studies in mice on the basic biology of HSC and progenitor function were largely done using congenic donor/recipient pairs. Because congenic pairs share genetic identity at all except one or a few nonhistocompatibility loci, such transplants are analogous to autologous HCT and relatively straightforward to transition into clinical trials. By contrast, transplantations of allogeneic HSC differ substantially from congenic procedures, and they can be both advantageous and problematic to the allograft recipient. For example, allogeneic HSC, which are devoid of T cells, eliminate the major clinical problem of GVHD and the requirement for immunosuppression. However, the beneficial GVT effect is lost, immune reconstitution is likely to be delayed, and engraftment failure may occur. These issues have prevented the translation of allogeneic HSC transplants from bench to clinical practice and are discussed in the next sections.

Resistance to Engraftment

Concern over engraftment failure has presented a major obstacle to the translation of purified allogeneic HSC transplants to human studies. In the 1980s, attempts to reduce GVHD by T cell depletion resulted in significantly increased and unacceptable incidences of engraftment failure (91, 92). These failures were attributed to loss of a facilitating cell population(s) eliminated by manipulation of the BM grafts and, in retrospect, may also relate to a relatively low dose of $CD34^+$ cell content contained in the transferred manipulated BM product. Regarding this latter point, purified HSC transplantations using low doses in mice similarly demonstrate increased levels of resistance to engraftment (93).

Engraftment resistance can be quantitated by the dose of HSC required to rescue lethally irradiated mice from hematopoietic failure. The barrier to engraftment was overcome between most strains by escalating the dose of HSC transferred by 10- to 60-fold of the congenic dose (93). The highest levels of resistance are observed when mice are mismatched at MHC plus minor histocompatibility loci. Interestingly, at low HSC doses (100–500), several times the congenic dose is required to engraft in allogeneic recipients, whereas at higher doses (i.e., >5000), the difference in engraftment kinetics diminishes, such that in some allogeneic strain combinations, only two-fold higher the congenic dose is required for rapid engraftment (4). These latter observations suggest that even fully allogeneic barriers can be traversed with high doses of HSC, and if this threshold dose level is reached, it is possible to obtain rapid engraftment comparable to those obtained following autologous HCT.

Disparity at the MHC contributes significantly to resistance, reflecting the important role that NK cells play in rejection of hematopoietic grafts (94, 95). NK cells express a complex array of recognition molecules that have both stimulatory and inhibitory activity, and families of NK cell inhibitory receptors interact with certain MHC molecules. BM transplantations into NK-deficient mice (96, 97) or treatment of wild-type mice with agents that eliminate NK cells (98, 99) results in decreased engraftment resistance. Even in the most highly resistant mouse strain combinations, wherein lethal irradiation and high dose HSC (i.e., 10,000 HSC) are insufficient to overcome the engraftment barrier, addition of anti-NK antibody to the conditioning regimen markedly reduces the barrier, allowing successful HSC engraftment (C. Scheffold, Y.C. Scheffold, T.M. Cao, J. Gworek, J.A. Shizuru, unpublished observations).

In human HCT, hidden polymorphisms in MHC molecules compromise engraftment outcome (100, 101). However, MHC disparities are not the only determinants of hematopoeitic resistance. Mouse strains matched at the MHC show marked variability in resistance to HSC (102). DNA sequencing of the class I and class II MHC alleles of these strains revealed exact sequence homology, eliminating the possibility that serologically silent MHC polymorphisms were responsible for the variability and underscoring the role that non-MHC background genes can play in hematopoietic resistance (102).

Cells that Facilitate HSC Engraftment

The role of BM-derived non-HSC populations in enhancing engraftment has long been appreciated by BM transplant physicians since it was observed that T cell depletion can lead to increased graft failures. Quantitation of engraftment facilitating capacity of non-HSC cells can be performed by comparing engraftment of a given BM dose with the number of HSC contained within that amount of BM (93). Using this approach, the identity of engraftment facilitating cells that do not cause GVHD was pursued (103–105). An extended analysis of HSC facilitating cells showed that one salient feature of the candidate cells was the

expression of the CD8 molecule. Interestingly, the $CD8^+$ cells were heterogeneous and two morphologically and phenotypically distinct engraftment enhancing populations were identified: one that is likely a conventional α/β^+ T cell, and a second α/β^- T cell receptor negative population that resembles $CD8^+$ dendritic cells (103). In the future, HSC transplants will undoubtedly include such graft facilitating cells.

Loss of GVT Activity

The importance of GVT as a therapeutic benefit following allogeneic HCT for hematologic malignancies is well established. Because GVT is mediated by mature immune cells, HSC grafts or grafts depleted of mature cells are expected to fail to confer this benefit (106, 107). Indeed, mouse studies of fully allogeneic (MHC plus minor histocompatibility mismatched) HSC versus BM transplants into mice bearing a B cell lymphoma showed a loss of GVT activity in the former group (106). Co-transfer of immune cell subsets isolated from BM revealed that the GVT activity could be recovered and that all of the antitumor activity was contained in the $CD8^+$ cell fraction. At the $CD8^+$ cell doses infused, GVT was separable from GVHD. Other candidate populations have restored GVT without GVHD when coinfused with HSC, including an in vitro expanded $CD8^+$NK-T cell derived from peripheral lymphoid cells. Of note, a homologous NK-T population can be expanded from human peripheral blood. Thus, although grafts composed solely of HSC are devoid of GVT, HSC can serve as platform to engineer grafts that contain cell populations with defined activities (107).

Immune Reconstitution Following HSC Transplantation

The elimination of differentiated blood and marrow cells from a hematopoietic graft also affects defense from infectious pathogens. Indeed, mice transplanted with HSC only are more susceptible to lethal infections caused by bacterial, fungal (108), or viral (109) pathogens. This immune deficit is overcome, however, by coinfusion of lineage-specific progenitor cells. Mice infected with pathogenic strains of human *Aspergillus fumigatus* or *Pseudomonas aeruginosa,* which would otherwise die if HSC only were transplanted, demonstrated improved survival when CMP and GMP myeloid progenitors were coinfused (108, 110). In fact, a combination of myeloid progenitor injection and G-CSF treatment completely protected against otherwise lethal *Aspergillus* fungal and *Pasteurella* bacterial infections. Whereas CMP and GMP can protect mice against fungal and bacterial pathogens, CLP confer protection against a viral pathogen, murine cytomegalovirus (MCMV) (109). It is interesting and important to note that progenitor populations derived from third-party donors that are allogeneic to both the recipient and HSC donor may be capable of conferring protection against pathogenic agents (C. Arber, A. BitMansour, J.M. Brown, unpublished observations). These latter observations in mice may find utility in clinical HCT as well as protection of immunodeficient and/or neutropenic patients.

IMMUNE TOLERANCE INDUCTION WITH HSC

Beyond its current uses for the treatment of malignancies and BM failure states, HCT has potential applications in other areas of medicine, including the induction of donor-specific tolerance to solid organs and the treatment of severe autoimmune diseases (ADs) (111, 112). To date, however, HCT has not been routinely applied to tolerance induction, primarily because of concerns regarding the morbidity and mortality of the allografting procedure. The continued improvements in the control of regimen-related toxicities, including development of nonmyeloablative protocols that permit engraftment of donor cells with attenuated conditioning regimens (113, 114), are rapidly changing the view, and eventually HCT may be applied to these other areas.

HCT for the Treatment of Autoimmune Disease (AD)

Preclinical studies and clinical case reports show that hematopoietic cells can transfer ADs from affected donors to unaffected hosts (115–117), and conversely, they can ameliorate disease if transferred from normal donors to disease-afflicted hosts (117–120). ADs arise as complex polygenic traits that can be influenced by environmental elements (121, 122). Most ADs arise from dysregulated immune responses resulting in the generation of self-reactive T lymphocytes. The observations that the genotypic origins of the hematopoietic cells confer disease resistance or susceptibility led to the hypothesis that allogeneic HCT cures ADs because the defective HSC and mature autoreactive cells are eliminated and replaced by the donors. This formulation may be too simple, however.

Studies using syngeneic or autologous HCT demonstate that it may not be necessary to replace host hematopoiesis to obtain therapeutic benefit for ADs. The rationale for the autologous approach is that high dose therapy will ablate the patient's defective immune system, and the patient can be rescued from the ablative conditioning with an immunologically naive graft. Such reasoning is analogous to the treatment of cancer, wherein the conditioning regimen results in cytoreduction of malignant cells and patients receive grafts with none or few passenger cancer cells. In some rodent models of ADs induced by antigen immunization, significant proportions of affected animals were cured by high dose treatment and transplantation of syngeneic (autologous) T cell–depleted BM (111, 112). However, we tested syngeneic transplantation of purified KTLS HSC in a mouse model of spontaneous autoimmune diabetes mellitus and found no benefit (123). At the time of transplant, the autoimmune prone nonobese diabetic (NOD) mice were not yet hyperglycemic, but they had ongoing inflammation of their pancreatic islets. The mice were conditioned with lethal radiation with or without T cell–depleting antibodies. Despite this rigorous myelo- and lymphoablative conditioning and rescue with HSC that contained negligible contaminating NOD T cells, NOD recipients developed hyperglycemia in a time frame similar to that of untreated controls (123). Furthermore, when congenic NOD mice that carry the severe

combined immune deficiency (SCID) mutation were used as donors, which are capable of rescuing myeloid but not lymphoid lineages (124), diabetes still developed in NOD recipients. This result showed that even low levels of T cells that persist after lethal conditioning could mediate the autoimmune process (123). The reason for the discrepancies encountered in the different animal models is not known, although it has been proposed (119) that certain diseases may be more amenable to treatment with syngeneic HCT—for example, rodent diseases induced by exogenous antigen rather than spontaneously arising ones. Despite the observations that the preclinical models do not consistently demonstrate blockade of AD pathology, clinical trials are nonetheless underway to study the efficacy of autologous HCT for severe human ADs (111, 112). In the past five years, more than 500 AD patients worldwide have undergone autologous HCT. These patients constitute a heterogeneous group with different diseases and were transplanted at a number of different centers. As might be expected from the preclinical studies, some patients show excellent and long-lived clinical remissions, whereas others enjoy initial benefit but subsequently relapse. Transplant-related mortality ranged from 0% to 12.5% depending on the disease entity and reporting institution (111, 112). Whether or not autologous HCT will yield long-term cures of autoimmune affected patients will not be known until there is longer follow-up from the current clinical trials.

In contrast to the conflicting results on the success of autologous HCT to treat ADs, the literature on allogeneic HCT is more uniform. Since the seminal studies by Morton and Siegel in 1974 (115), multiple reports have shown that rodent ADs, both spontaneous and antigen-induced, are cured with BM allografting (118, 119). Significant variability exists among the studies, including the degree of donor-host genetic mismatching, the type of hematopoietic grafts, the preparative regimens, and the timing of the HCT procedure relative to the onset of disease manifestations, making this body of evidence even more striking. The superior outcomes in allogeneic versus autologous studies suggest that donor cells effectively modify recipient immune responses (125). A known effect of transplantation of unmanipulated allogeneic BM is the elimination of host T cells with relatively rapid conversion of recipients full T cell donor chimerism (93). In contrast, HSC allografts do not contain facilitating populations that mediate this antihost T cell effect, thus HSC engrafted mice remain as partial T cell chimeras. In tests of KTLS HSC transplantation in the NOD model, we noted that all allografted mice were protected, despite the fact that significant levels of recipient NOD T cell persisted for many months after transplantation. The level of host T cells that remained following allogeneic HSC versus NOD-SCID transplantation (described above) was equivalent, and yet mice in the former group were disease protected whereas those in the latter were not. Thus, it can be concluded that allogeneic hematopoietic grafts can alter autoreactive responses by mechanisms other than complete replacement of host T cells. The precise mechanism(s) by which allogeneic hematopoietic grafts alter autoimmune responses in susceptible recipients remains to be defined.

Transplants of MHC-matched HSC were equally protective of NOD disease as MHC-mismatched HSC, a finding of particular clinical relevance. The only firmly established genetic association for susceptibility to ADs is the genotype of the MHC, and there is a well known association of NOD diabetes with its MHC-class II allele (126). In addition, standard clinical HCT utilizes MHC-matched donors to minimize the complications of the HCT procedure. Thus, in the NOD model, disease protection was not dependent upon replacement of a nonsusceptible MHC allele expressed on donor cells. However, it is possible that not all MHC-matched donor/host combinations will confer protection from ADs unless critical background genes are expressed in the hematopoietic cells. The next important step is to identify the background genes expressed in the hematopoietic lineages that might uniformly confer protection from AD.

HSC Transplantation in the Induction of Immune Tolerance to Solid Organs

One long-held ambition of solid organ transplantation has been to apply the 50-year-old concept of allogeneic BM engraftment to induce a state of donor-specific immune tolerance (127, 128). Concerns regarding the morbidity of HCT has been a major limitation in the realization of this goal. Because GVHD can be eliminated by transplantation of HSC or grafts debulked of T cells, the remaining obstacle is to achieve durable chimerism with less morbid conditioning. Recently clinical regimens have been developed that do not ablate recipient hematopoiesis to achieve donor hematopoietic cell engraftment (129). These nonmyeloablative regimens are much less toxic than the standard myeloablative protocols and are now routinely performed in the outpatient setting, and on patients who previously were not considered candidates for HCT because of older age or medical cormorbidities (114). Thus, the way is now open to safely transition the field of HCT to the application of organ tolerance induction.

Recent studies tested if purified allogeneic HSC could, like BM grafts, induce tolerance to donor-matched hearts (130, 131). Engraftment of HSC permitted long-term survival of donor-matched neonatal heart grafts, and third-party heart grafts were rejected. The timing of the heart transplantation procedure relative to the HSC procedure appears to be flexible, because near simultaneous heart plus HSC transplantation results in long-term graft acceptance (130) as does HSC transplantation performed months prior to the introduction of a donor-matched heart (132). The next step in the translation of HCT to human organ transplantation is combining nonmyeloablative preparative regimens with purified grafts. Such studies are emerging in the clinical literature. However, in the patient studies reported to date, although the preparative regimens are well-tolerated, durable chimerism that permits long-term organ tolerance has not yet been achieved.

HSC AND BEYOND

Developmental Plasticity of HSC

Many experiments wherein BM or MPB was transplanted in mice or human show donor markers in diverse cell populations of the irradiated host, including liver, brain, gut epithelium, lung, heart, and skeletal muscle (133–145). Although it seems natural to assume that this activity came by transdifferentiation of HSC into other tissue-specific stem and progenitor cell types, BM contains not only HSC but other primitive populations. The other cell types include mesenchymal stem cells (MSC)—that may be the same as or different from multipotent adult progenitor cells (MAPC), stromal cells derived from BM and grown under particular conditions in vitro—and perhaps even independent endothelial progenitors (146–148). It has been reported that populations exist in the BM capable of differentiation into not only blood, but also cells of the liver and lung (139, 146). The comparison of these populations with HSC and other progenitors in the marrow has yet to be carried out thoroughly.

The phenomenon of HSC transdifferentiation was interrogated using mice reconstituted with single, prospectively isolated LT-HSC. With the exception of one rare Purkinje cell in the cerebellum and some liver cells, none of the irradiation-injured tissues other than blood showed donor markers (15). The rare positive findings in the brain and liver were almost certainly due to fusion of an HSC daughter cell with a resident injured cell that, in the absence of injury, could not have been detected (149, 150). Repeated injury of muscle in HSC-transplanted or parabiotic mice resulted in cells fusing with muscle cell or muscle cell progenitors, providing donor cell markers in host skeletal muscle (150a). Fusion events were more frequent when BM was transplanted, and they were rare when HSC were transplanted in numbers equivalent to the BM dose. Thus, certain circumstances such as muscle injury may be more permissive for BM and HSC to participate in muscle fusion events, but the nature of the cells that under these extreme circumstances participate in muscle fusion remains to be determined. Furthermore, whether such fusion is biologically and clinically significant—or is simply an experimental artifact—also is yet to be determined. We have recently isolated to near or total homogeneity the mouse resident skeletal muscle stem cell, a subset of cells known morphologically as satellite cells (RI Sherwood, JL Christensen, IL Weissman, AJ Wagers, manuscript submitted). These cells provide robust myogenesis at the single-cell level at $>50\%$ efficiency, although no other muscle resident cell, whether derived from the circulation, BM, or HSC, show any activity. These candidate skeletal muscle stem cells also provide robust muscle regeneration in vivo.

Transplantation of HSC or BM directly into areas of myocardial infarction, using the protocols of Orlic et al. (138), did not result in donor markers in cardiomyocytes or coronaries (endothelial or smooth muscle components) and did not improve cardiac regeneration or function (151). Thus, even this example of proposed transdifferentiation, one that has led to extensive human clinical trials, does not meet the criteria of transdifferentiation or functional restoration on repeat studies.

Isolation and Manipulation of Stem and Progenitor Cells from Other Tissues

The study of HSC and oligopotent progenitors has provided a strong basis for the development and practice of regenerative medicine, which can be translated to other tissue systems. The methods by which HSC were isolated and transplanted have already served as a model for the isolation or enrichment of other stem cell populations, including rat peripheral nervous system stem cells (152, 153) and human central nervous system stem cells (154), as well as the enrichment of populations of skin stem cells and a few other cell types. Although the isolation of MSC did not occur through prospective separation of cells from BM or other sites, it was dependent on development of culture methods for these cells and limit dilution cloning of such cell types (148). The latter approach was also applied to the isolation of the MAPC population found by Verfaillie and colleagues (146) in mouse and human. However, it is still unknown if the MAPC cell exists as a pluripotent stem cell in vivo, or whether it is derived by the kinds of factors added to it in vitro, and finally whether or not these MAPC will prove to be an efficient source of tissue regenerating cells in a clinical setting.

The isolation of pluripotent embryonic stem (ES) cell lines from the inner cell mass of mouse blastocysts developed from the finding that leukemia inhibitory factor (LIF) prevented these cells from proliferating (155, 156). Mouse ES cells, in the absence of LIF, give rise to embryoid bodies containing elements of virtually every tissue type. Human ES cells derived from blastocysts obtained from in vitro fertilization (IVF) clinics, demonstrate formation of embryoid tissue–like mouse ES cells (157–159). The fact that potentially all tissue types can be generated from ES cells has led to the hope that human cell lines will be used in the future to generate stem and progenitor cells for transplantation in clinical settings. However, the derivation of such clinically relevant transplantable tissue from ES cells will be a hard road. For example, mouse ES–derived hematopoietic progenitors, roughly at the same age ontogenetically as yolk sac blood island cells, cannot by themselves engraft into lethally or sublethally irradiated syngeneic hosts (160, 161). Thus, it seems likely that these ES–derived progenitors require the kind of differentiation that occurs when these embryonic stage HSC differentiate to the liver stage (70–73). The failure of directly transplanted ES–derived hematopoietic cells in mice to transplant may be a special case of HSC that must navigate the bloodstream to find their differentiation sites. In vitro generation of CNS type cells can, upon transplantation into mice or chickens, result in apparently site-specific and appropriate regeneration of cell types important in human diseases (162, 163).

Nuclear Transplantation

Nuclear transfer (NT) as a method to produce pluripotent cells is another iteration of the technologies that lay ahead for stem cell biologists. In a demonstration experiment, new pluripotent cell lines were produced by transfer of a somatic cell nucleus derived from a mouse with a monogenetic disorder (severe combined

immunodeficiency) into an enucleated egg (164). Stimulation of the transfer entity was done to produce an embryoid blastocyst from which stem cell lines were obtained; these NT-derived pluripotent stem cell lines faithfully replicated this immune deficiency. Correction of the disorder in these pluripotent cells was carried out in vitro (164). To test if gene-corrected nuclear transfer cells could be used therapeutically (therapeutic cloning), the hematopoietic cells derived from these pluripotent cells with genetic modification were capable of transplantation into adults, partially correcting the immunodeficiency (47). Two important principles derive from these and other experiments (165): (*a*) The genotype of the pluripotent stem cell derived by nuclear transfer is determined by the genes of the donor nucleus, including those that carry a genetic disorder; and (*b*) maturation of ES stage hematopoietic cells to fetal or adult stage HSC was achieved, and as more is learned about the genetic program for such maturation, the efficiency should improve. The important lesson for medicine is that it may be someday achievable, if funded and legal, to produce pluripotent stem cell lines drawn from the nucleus of patients with authentic monogenic and polygenic disorders and to use such pluripotent lines to study development and pathogenesis of these human diseases in vitro and/or by transfer into mice xenografted with human blood cells (SCID-hu mice) (154, 166–168). Gene correction techniques may eventually be used to determine more directly the role of such genes in disease pathogenesis (162).

Researchers should continue the search for both multipotent tissue-specific stem cells (such as HSC) and pluripotent stem cells, obtained either from adults as in MAPC (146) or from blastocysts as in the generation of true ES cells. Of course, it shall be of great medical relevance to produce pluripotent embryoid stem cell lines by nuclear transfer from defined genetic donors (168). It is conceivable that pluripotent stem cell lines derived from an individual's somatic cell nucleus could be used to regenerate multipotent stem cells for that individual. This type of "therapeutic cloning" should lessen the risk of immune destruction of such cells, although it is clear that the host egg recipient of the donor nucleus carries its own mitochondrial DNA. Mitochondrial genes are highly polymorphic in a species, and thus mitochondrial encoded proteins might be presented as minor histocompatibility antigens for rejection (169, 170).

Cancer Stem Cells

The development of cancers and leukemias involves a long progression of genetic and epigenetic events for a single cell to give rise, clonally, to a neoplastic lineage (for review, see 171). In models of tumorigenesis, these events include avoidance of programmed cell death by various pathways such as the BCL2-inhibited or FAS receptor–mediated pathways (169) and avoidance of death induced by innate and adoptive immune cells (172). Cancers must also contain populations with properties possessed by stem cells, namely self-renewal, but in this case in a poorly or unregulated fashion (44). Finally, cancer cells must be capable of extending their cell division–limited life spans, and one way that this extension is achieved is by

providing active telomerase complex genes for the lengthening of telomeres in these cells and their daughters (173). Properties such as invasion and metastasis may also require the elaboration of new genetic programs, but it is still possible that the properties of invasion and metastasis are those found in clonal progenitors of the cancer cell (174, 175). Because it is obvious that cancers do not come from Mars, but rather derive from resident cells undergoing defined genetic and epigenetic events, it is important for each cancer and leukemia to place the neoplastic population in the context of the normal differentiation sequence. Such an approach should test whether or not the true "cancer stem cells"—cells capable of self-renewal as well as some differentiation—arise in the tissue's own stem cell population or in some population downstream of the stem cells, for example, oligolineage or oligopotent progenitors (44).

Many years ago, it was proposed that leukemias and lymphomas might follow a stem/progenitor model similar to normal tissues. It was shown that only a low frequency of cells within a lymphoma in mice could form viable colonies in vivo (44). Those experiments, however, did not test whether the colony-forming cells were capable of both self-renewal and differentiation. Several other examples demonstrate that not all cells within a tumor cell are capable of spreading the cancer. In 1977, Lynch and colleagues observed that when mouse myeloma cell populations are placed in diffusion chambers in the peritoneal cavity of mice, there is first a die-off of the plasma cell stage of the myeloma with retention largely of blast cells with cell surface but not secreted immunoglobulin (176). This cell correlated with the phenotype of germinal center B cells, whose progeny also include plasma cells (177, 178). On the basis of these observations, it is reasonable to propose that if surface Ig-positive, nonsecreting blast cells could be isolated from these myelomas, such cells may in fact be the myeloma stem cells.

Within the past ten years, Dick and colleagues have carried out a remarkable series of experiments to identify leukemia stem cells. Subsets of cells from patients with acute myelogenous leukemia were transplanted into immunodeficient mice, and the cell types that gave rise to transplanted leukemias were recorded (179). Only the $CD34^{+}38^{-}$ population of cells were capable of transmitting the leukemia, although the other cells, which were presumably derived from those clonal leukemic cells, could not (179). A few years later, Miyamoto et al. showed that BM from patients whose acute myelogenous leukemia (AML) contained the cytogenetic abnormality, translocation AML-1/Eto, contained $CD34^{+}38^{-}Lin^{-}$ $Thy\text{-}1^{+}$ HSC also that had the translocation AML-1/Eto, but these HSC gave rise only to normal hematopoietic progeny in vitro (180). Remarkably, such HSC bearing the translocation but having a normal differentiation outcome are retained in the marrows of surviving patients who have not relapsed for many years (181). However, $CD34^{+}38^{-}Lin^{-}Thy1^{-}$ cells from these marrows have the capacity, in vitro, of producing leukemia blast cell clones (180). The concept that non-HSC can function as leukemia stem cells is consistent with more recent studies, which demonstrated in mouse models of AML that the leukemia stem cells are limited to the progenitor (I.L. Weissman, unpublished observations) GMP

population and that neither HSC nor their progeny can transfer the disease (172, 182, 183).

Finally, identification of a putative breast cancer stem cell population was recently described by Al-Hajj et al. They showed that human breast cancers contain a small proportion (3–5%) of $CD44^+CD24^-$ cells, which are the only cells within the largely cytokeratin-positive tumor that can transfer mammary tumorigenic potential to the mammary gland of NOD-SCID mice (184). The tumors that arise in these mice regenerate all phenotypic subsets, but only those in the relatively rare $CD44^+CD24^-$ cells are capable of a second breast cancer transplant, whereas the $CD44^-$ and/or $CD24^+$ dominant populations do not participate (184).

The studies described above highlight the growing evidence that tumors contain relatively rare populations primarily responsible for mediating the devastating effects of malignant transformations. These cancer stem cells exist, perhaps at the level of tissue-specific stem cells, and others at the level of defined progenitors (44). These cells share the property of poorly regulated self-renewal, and they share the events that have led them to be immortal and capable of avoiding internal and external programs for cell death. It is fundamentally important to determine if in the malignant progression, cells that normally do not self-renew (i.e., MPP or GMP) gain the property of self-renewal and to determine whether or not they have simply reacquired the pathway that their tissue-specific stem cells (HSC) use (44). In that regard, it is important to recall that the Wnt pathway was first discovered as genes that were activated in mammary tumorigenesis in mice by the oncogene-lacking mouse mammary tumor virus (MMTV) (40, 185). The MMTV promoter activated the gene then called int-1. The complex of MMTV promoter/enhancer and int-1 (Wnt) introduced as a transgene complex was sufficient to transfer the property of tissue-specific oncogenesis in the breast gland in transgenic mice (186). Furthermore, the Wnt β-catenin pathway has been implicated in progression to colon cancer, breast cancer, and perhaps other tissues (for review, see 43). Thus, we propose that there may be for many tissues a common program for self-renewal that is normally regulated, and that the cancer stem cells that arise—no matter what stage of differentiation they come from—utilize that program and regulate it poorly (44). It is apparent that in those systems, as in the generation of myeloid cells from HSC, the only cells that self-renew are HSC. Because the progression to leukemia requires four, five, or more rare genetic or epigenetic events, it is virtually impossible that all of those events could have occurred in a progenitor cell; instead, stem cells (in this case HSC) are the repositories of cells that self-renew and carry forward clonal lines capable of the the next steps of progression (44). Thus, it is not surprising that HSC in leukemic patients contain genetic events such as translocations that are necessary but not sufficient for leukemia generation, and it is likely in these cases that the order of events resulting in the leukemia may be mapped by exhaustively examining clones of HSC and their progeny for such events. In this context, of course, the presence of an easily recognized and important marker such as a translocation does not mean, as is currently believed, that the patient has minimal residual disease. Rather, the detection of such a marker may

mean that one or more of the steps in the preleukemic pathway are still resident in hematopoiesis, and that the patient may enjoy a very long life with only a low probability that the events to progression will occur again. In fact, we have recently shown that while HSC contain the leukemia stem cells in the chronic phase of chronic myelogenous leukemia (CML), the myeloid blast crisis is made up of leukemic stem cells at the stage of the GMP. These leukemic GMP both self-renew in vitro and have activated the β-catenin pathway (187).

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