TISSUE ENGINEERING: Part B
Volume 17, Number 6, 2011
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DOI: 10.1089/ten.teb.2011.0197

# The Niche as a Target for Hematopoietic Manipulation and Regeneration

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Hematopoietic stem cells (HSCs), rare primitive cells capable of reconstituting all blood cell lineages, are the only stem cells currently routinely used for therapeutic purposes. Clinical experience has shown that HSC number is an important limiting factor in treatment success. Strategies to expand HSCs are of great clinical appeal, as they would improve therapeutic use of these cells in stem cell transplantation and in conditions of bone marrow failure. The microenvironment in which HSCs reside, known as the niche, has long been considered a critical regulator of HSCs. Data accumulated over the past decade strongly confirm the importance of the niche in HSC behavior. A number of niche components as well as signaling pathways, such as Notch, have been implicated in the interaction of the microenvironment with HSCs and continue to be genetically evaluated in the hope of defining the critical elements that are required and which, if modified, can initiate HSC behaviors. In this review, we highlight the known characteristics of HSCs, challenges in their expansion, the niche phenomenon, and explain why niche stimulated HSC expansion is of utmost interest in the field, while beginning to bring to the fore potential caveats of niche manipulation. Lastly, the potential pitfalls of avoiding malignancy and controlling self-renewal versus differentiation will be briefly reviewed.

## Introduction

Hematopoietic stem cells (HSCs), rare primitive cells capable of reconstituting all blood cell lineages, are the only stem cells currently routinely used for therapeutic purposes. Clinical experience has shown that HSC number is an important limiting factor in treatment success.[1,2] Strategies to expand HSCs are of great clinical appeal, as they would improve therapeutic use of these cells in stem cell transplantation (SCT) and in conditions of bone marrow failure. Based on seminal observations in the 1970s, the microenvironment in which HSCs reside, known as the niche,[3] has long been considered a critical regulator of HSCs. Accumulated data from over the past decade strongly confirm the importance of the niche in HSC behavior. A number of cellular niche components as well as signaling pathways, such as Notch, have been implicated in the interaction of the microenvironment with HSCs and continue to be genetically evaluated in the hope of defining the critical elements that are required and which, if modified, can initiate HSC behaviors.

Understanding the niche and identifying its components is important, as it is a prerequisite for isolating factors that may be essential in perturbing the cellular micro-environment to produce desired outcomes. Thus, an in-depth knowledge of the bone marrow tissue within the context of hematopoiesis may predict useful strategies to regulate the hematopoietic system, particularly the HSCs and their expansion.

In this article, we highlight the known characteristics of HSCs, challenges to their expansion, the niche phenomenon, and explain why niche stimulated HSC expansion is of upmost interest in the field, while beginning to bring to the fore potential caveats in niche manipulation.

## Hematopoietic Stem Cells

HSCs can be defined as any single cell necessary and sufficient for lifelong sustenance of the normal blood system.[4] HSCs can also be enriched by flow cytometric analysis and can be functionally analyzed with competitive reconstitution assays. Somatic stem cells, in general, can be divided into two populations; both with renewing capacities, but the long-term stem cell (LT-SC) population has an infinite capacity to self-renew, whereas the short-term stem cell (ST-SC) population is limited in regenerative capacities. LT-SCs give rise to ST-SCs, which thereafter give rise to progenitor cells with limited or no renewal capacity, and these cells differentiate to mature populations.[5] Likewise in the hematopoietic system, the HSCs are found at the apex of the hematopoietic hierarchy, which can also be subdivided by using both cell surface markers and functional *in vitro* assays. Extensive research has been directed at understanding the cell surface phenotype of HSCs, which enables identification and prospective isolation of HSCs utilizing flow cytometric analysis and sorting. Specifically, the

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lineage$^-$/Sca-1$^+$/c-Kit$^+$ (or LSK) subset of bone marrow cells is enriched for HSCs.[6–10] However, the LSK compartment is heterogeneous and contains at least three subpopulations of cells with multi-lineage potential, but with progressively more limited self-renewal that have been named LT-HSC, ST-HSC, and multi-potent progenitor cells (MPPs). Initial significant controversy on the phenotypic markers identifying LT-HSCs versus ST-HSCs and MPPs,[6,9,11,12] as well as whether cells within this compartment can contribute to hematopoietic progeny,[6,13] was recently relatively resolved as a combinatorial strategy including both SLAM markers and Flt3 which have been demonstrated as strongly predicting and enriching HSC activities.[14,15] In fact, the presence of heterogeneous subsets and the existence of less quiescent HSCs of limited self-renewal and increased proliferative capacity is well documented in both murine and human hematopoiesis.[16,17] However, it should be highlighted that these complex phenotypic markers still represent a population that is enriched for HSC activity. Moreover, the complexity of these markers limits the ability for specific visualization of HSCs *in vivo*. Therefore, the term *hematopoietic stem and progenitor cells* (HSPCs) more appropriately represents HSC-enriched populations.

The gold standard for quantification of HSCs is the competitive repopulation assay, in which donor HSCs are scored by their ability to contribute to hematopoietic reconstitution.[18] This assay determines multi-lineage repopulation in recipient mice, and typically both short-term and long-term time points are sampled, corresponding to MPP/ST-HSCs and LT-HSCs.

HSCs should balance between self-renewal and differentiation to replenish the entire hematopoietic system and simultaneously survive myeloablative injury throughout the life of an individual.[19] Cellular expansion at the stem-cell level is essential for the current clinical use of HSCs in SCT. Shizuru *et al.* demonstrated in SCT for non-hematopoietic disease that HSC numbers play an important role in graft success and time to engraftment.[9] At HSPC levels below a minimal threshold, there was a significant delay in platelet recovery. Hence, rate of engraftment could be predicted based on a given range of HSC numbers transplanted. Similarly, lethally irradiated recipient mice injected with congenic donor bone marrow HSPCs had dose-dependent kinetic recovery of leukocytes and platelets.[20] By increasing HSPC numbers to high enough doses, clinically relevant outcomes can be achieved in patients requiring SCT.

Although this numeric requirement was initially described in bone-marrow-derived and mobilized peripheral blood stem cells, this issue has become particularly important with the advent of cord blood transplantation. The use of cord blood for SCT is appealing, as it is feasible in patients in whom suitable matched bone marrow or peripheral blood HSPCs cannot be found.[21] However, the limited numbers provided by single or double cord bloods have resulted in delayed engraftment with its associated morbidity.[22] Therefore, the need for HSC expansion has remained an important therapeutic target for SCT-dependent morbidity and mortality. However, as will be discussed next, significant barriers and challenges have been encountered while attempting to expand HSCs.

### Challenges in HSC Expansion

Approaches for HSC expansion *ex vivo* have included cytokine stimulation, use of bioreactors that have attempted to recapitulate the three-dimensional (3D) structure of the bone marrow, and use of systems with physical immobilization of ligands which *in vivo* have been identified in stromal cells supportive of HSCs. A recent excellent review outlines in detail the current advances in *ex vivo* HSC expansion.[23] Briefly, these attempts have met significant obstacles.

Numerous strategies for *ex vivo* expansion by using a number of cytokines *in vitro* have demonstrated that despite initial increases in HSPCs numbers, cells differentiate and lose their multi-potency. Cytokines such as stem cell factor, Flt3L, thrombopoietin (TPO), and interleukin-11 (IL-11) can expand HSPCs *in vitro* but at a loss of self-renewal capabilities. Numerous trials demonstrate the safety of these approaches, but their overall lack of effectiveness is revealed by lack of improvement in platelet and neutrophil numbers after transplantation.[23] In *ex vivo* systems, LT-HSCs expansion is especially complex.[24] Most two-dimensional *in vitro* approaches have not been able to expand immature HSPCs without the presence of a stromal cell population. 3D porous scaffolds are a potential approach with perfusion of cell suspension through scaffold pores in alternate directions in bioreactor-based systems. The 3D system allows perfusion of human nucleated bone marrow cells and culturing for 3 weeks, followed by demonstration of human HSC activity by transplantation to nude mice.[24] 3D scaffold-based approaches can recapitulate matrix formation, cellular heterogeneity, and, with cytokine addition, produce increased hematopoietic progenitors. This system exemplifies a platform for future capabilities for modulating parameters identified by studying the *in vivo* HSC niche, which can result in effective *ex vivo* HSC expansion.[24]

The identification of important HSC cell autonomous molecular signals that regulate self-renewal has provided potential targets for HSC expansion. However, manipulation of such targets has also met significant obstacles, including depletion of the HSC pool, as well as the potential for initiation of malignancy. For example, genetic deletion of the cell-cycle regulator p21 achieves initial expansion of the phenotypic HSPC pool, which is eventually exhausted.[25] Similarly, genetic deletion of PTEN, an inhibitor of the PI3K-Kinase/AKT pathway, initially expands phenotypic HSPCs, which are depleted, and results in a myeloproliferative phenotype.[26] Therefore, caution is needed particularly in the manipulation of molecular targets with potential tumor-suppressor capabilities. In this context, definition of the regulatory components in the HSC microenvironment can provide guidance in the development of *ex vivo* expansion strategies. Moreover, *in vivo* targeting of the HSC niche may provide a safer approach that could stimulate the HSC in a more physiologic fashion, thus avoiding issues with oncogenesis.

### The Concept of the Niche

In trying to overcome the many challenges encountered in HSC expansion, a better understanding of the microenvironment and its regulatory roles is essential. Certainly, cell autonomous signals that regulate stem cell behavior have been described. However, definition of the HSC regulatory

components in the microenvironment could provide additional targets that could be manipulated to influence stem cells to different fates such as asymmetric cell division, differentiation, quiescence, self-renewal, and apoptosis.

Schofield described the regulatory microenvironment in which true HSCs reside as a niche. This nomenclature was based on the idea that the niche included cells and factors which would retain the stem cell in a fixed state of restricted differentiation.[3] The stem cell would replicate mainly for self-renewal, one of the daughter cells would be the first progenitor colony forming cell and unlike the stem cell, the colony forming cell would have a limited number of divisions. The life span of the colony forming unit would lead to proliferation, maturation, differentiation, and, eventually, apoptosis of the cells. On the other hand, if the daughter cell occupied the stem cell niche, it would also become fixed and acquire stem cell characteristics.[3] This interpretation of the niche would entail the concept that niche components stipulate the fate of stem cells. Schofield's conceptual model was first experimentally demonstrated *in vivo* in the *Drosophila* gonad, where somatic niche cells had been first identified.[27–31]

### Cell Populations Implicated in the HSC Niche

Several different anatomical sites for hematopoietic ontogeny and development have been defined, possibly representing the requirement for a niche that designates the fate of the HSC as it develops.[32] In the adult mammal, the bone marrow is the site of normal hematopoiesis, and the location to which infused immature HSPCs home. However, the bone marrow microenvironment is a highly complex system of matrix embedded-cells, bone forming and resorbing cells, hard tissue, and endothelial structures, as well as a complex hierarchy of hematopoietic cells. In addition, it has been well established that the skeleton is continuously repairing and remodeling, under hormonal control, and mobilizing calcium and phosphate. Therefore, if the HSC niche is present within the bone marrow, then it is likely also under constant remodeling, and should not be considered physiologically static.

Multiple reports have described the visualization of HSPCs as solitary cells in the bone marrow along the endosteum.[33–38] This localization and the presence of single cells provide additional support to the concept that specific signals in the bone marrow could be responsible for HSC regulation. However, as others have recently observed,[39] the endosteal location does not necessarily implicate osteoblastic cells, and the presence of sinusoidal cells as well as primitive mesenchymal cells at this location has been recently highlighted, as will be discussed next.

Initial *in vitro* data demonstrate that high levels of cytokines known to be important for HSC support, such as granulocyte colony-stimulating factor (G-CSF), GM-CSF, IL-6, tumor necrosis factor α, and transforming growth factor β, were produced by osteoblastic cultures, and, in turn, the osteoblastic monolayer increased cell recovery of CD34$^+$ hematopoietic progenitor cells.[40] Further, the addition of donor osteoblastic cells improved engraftment in allogenic bone marrow transplant.[41]

The first mammalian cells to be genetically defined *in vivo* as cells capable of HSC regulation were cells of the osteoblastic lineage.[42,43] A number of molecules have since been implicated in HSC-osteoblastic interactions. In fact, it has become evident that osteoblastic cells can both stimulate[42,43] and limit HSC expansion,[44,45] promote quiescence,[46–48] initiate HSC mobilization,[49] and integrate sympathetic nervous system and HSC regulation.[50] Osteoblastic cells are also key regulators of osteoclasts, cells of hematopoietic origin which resorb bone, and that can regulate HSPC mobilization.[51] In addition, osteoblastic cells appear to be mediators of the anemia induced by graft versus host disease after bone marrow transplantation,[52] and bone progenitor dysfunction is sufficient to induce myelodysplasia and secondary leukemia.[53] Therefore, increasing evidence points to cells of the osteoblastic lineage as key regulators of HSC behavior. However, there is currently significant controversy on the association and role of osteoblasts within the niche.[54,55] Abundant data attest to the proximity of osteoblasts to HSCs, for example, histological analyses showing proximity of LT-HSCs and spindle-shaped N-cadherin$^+$ osteoblastic cells in bone.[43] However, close anatomic location of stem and niche cells is but one criterion for the identity of a regulatory niche. Data have suggested that there exists a population of marrow cells capable of generating both HSCs and osteogenic cells,[56,57] hence the proximity of the osteoblasts and HSC. However, functional data also implicate osteoblastic cells, for example, osteoblasts in the marrow are the sole source of thrombopoietin (THPO), a critical regulator of HSC quiescence and, therefore, potentially vital in HSC niche.[47,58] Additional controversies exist on the differentiation stage within the osteoblastic lineage, which is important for HSC support. Two studies have suggested, based on immunofluorescence, that HSC supporting cells are osteocalcin$^+$ osteoblastic cells, based on data demonstrating their proximity to HSC.[43,46] Data on the effects of osteoblastic deficiency induced in a transgenic mouse model expressing herpes virus thymidine kinase gene under the control of a 2.3-kilobase fragment of the rat collagen alpha1 type I gene promoter suggest that osteoblastic cells identified by this promoter, as well as their osteoblastic and osteocytic progeny, comprise the pool of osteoblastic cells capable of HSC regulation and support.[59] On the other hand, more primitive skeletal cells have been demonstrated as possessing HSC-regulatory properties. Self-renewing osteoprogenitors in the bone marrow sinusoids can initiate the bone marrow microenvironment.[60] Moreover, it has recently been demonstrated that Nestin$^+$ putative mesenchymal stem cells (MSCs) present in the bone marrow, which are involved in the neuro-reticular complex linked to adrenergic nerve fibers and are also spatially coupled to HSPCs, are important for HSC regulation. These cells express high levels of HSC maintenance genes. Stimulation of Nestin$^+$ MSCs enables HSPCs to home to the bone marrow, whereas depletion of these cells results in mobilization of HSPCs.[61] However, MSC progeny includes both osteoblastic and endothelial cells, and, therefore, effects of Nestin$^+$ cells, which are likely solely enriched and still heterogeneous, may include HSC effects by other MSC progeny cells.

In addition to osteoblastic lineage cells, other cellular components of the HSC niche have been identified. Osteoclasts participate in HSC mobilization by producing cleaving enzymes which decrease local CXCL12.[51,62]

Sinusoidal endothelial cells along the endosteum were demonstrated as sites of HSPC residence.[23,63] Models of an

endosteal quiescent niche and an active perivascular niche more recently incorporate several different cells in HSC regulation, with the sinusoidal endothelial cells being common to both niche models.[23] The exact role of sinusoidal endothelial cells is still being elucidated; however, some studies have begun uncovering evidence of endothelial cell regulation of LT-HSC during myeloablative recovery via angiocrine factors.[64,65] Moreover, *in vivo* real-time imaging suggests that HSPC localize to areas where a combination of osteoblastic and endothelial cells are in close proximity.[36]

Adipocytes have an inverse relationship with HSPCs. Although well characterized as a component of the bone marrow, bone marrow adipocytic cells possess unique characteristics compared with their extramedullary counterparts.[66] Genetic models have recently suggested that adipocytes inhibit HSCs.[67] Additionally, brown fat, although understudied, is known to generate hypoxic conditions that stimulate differentiation of stem cells to chondrocytes, and, hence, may also have an effect on the HSC niche.[68,69]

Monocytes/macrophages have recently emerged as HSC regulators.[70] These cells, particularly the macrophage population, are critical in HSPC retention within the niche, as loss of the phagocytes increases HSPC mobilization, although whether this effect involves decreased osteoblasts and/or Nestin$^+$ cells is unclear.[71–73] Nevertheless, increased HSC mobilization accompanied by decreased niche cells and niche retention factors due to G-CSF manipulation of macrophages highlights phagocytic cells as crucial members of the bone marrow HSC microenvironment.[71–73]

Other understudied cell components of the niche exist that are slowly being unraveled along with their regulatory roles within the microenvironment. The sympathetic nervous system down regulates the level of chemokine (C-X-C motif) ligand 12 (CXCL12), which attracts HSCs to the marrow site in response to G-CSF.[50] Several cell types derive from neural crest stem cells including osteogenic cells of the face,[74] and neural cells might similarly contribute to the osteoblastic population in the bone marrow. Altogether, continuous probing of the bone marrow niche components will enable a better understanding and identification of the essential players within the micro-environment.

### Multiple Niches for Different HSC Cell Fates

As others have also recently proposed,[39,70] it is unlikely that a single cell type is responsible for the many fates of the HSC. In fact, a model has emerged in which different niches engaging the HSC, indeed, determine its different behaviors, which include quiescence, self-renewal, differentiation, mobilization, and apoptosis. The continuous demands posed on the HSC by the need for its progeny are obvious: the hematopoietic system is responsible for the massive expansion in cell numbers which are needed to generate >400 billion blood cells daily to replace those that are lost to senescence and attrition. The skeletal system would seem, by contrast, very static; however, it is in constant flux, as it undergoes continuous bone remodeling in response to mechanical stimuli and micro-fracture repair, reacts to hormonal stimuli, including estrogen and parathyroid hormone, and maintains minute to minute calcium and phosphate homeostasis.[75,76] In this context, the HSC emerges as a relatively peripatetic cell, which migrates over time to different available niches and acquires fates based on the niches in which it becomes engaged. In fact, support to the claim that HSCs frequently and spontaneously mobilize is found in experiments using parabiosis to demonstrate that HSPCs rapidly and constitutively migrate through the blood and re-engraft.[77] The importance of calcium in the local HSC milieu is highlighted by experiments which suggest that the ionic mineral content of the niche may determine the preferential localization of adult mammalian hematopoiesis in bone, as HSC lacking the Calcium Sensing Receptor could not effectively localize to the endosteal niche.[78] Moreover, the presence of at least two functional pools of HSCs with distinctive cell cycle kinetics has been demonstrated, and interestingly even the resident, dormant HSPCs have been described at both the endosteum and in more central locations within the bone marrow.[38] As essential micro-environmental signals required for specific HSC behaviors are identified, careful analysis using genetic means will likely be necessary to define which niche component and/or which subset of the osteoblastic lineage population is necessary and sufficient for individual HSC fates.

### Signaling Pathways Involved in Niche-HSC Interactions

A comprehensive review of all signals implicated in the interaction between HSC and their niche is beyond the scope of this review. However, we highlight next the signals identified by multiple groups as critical for HSC maintenance of quiescence, HSC self-renewal, and recovery of HSCs from myeloablation. Frisch *et al.*, provides a point of reference for some of the signaling pathways involved.[79]

Micro-environmental regulation of HSC quiescence has been described. The tight adhesive binding of osteoblastic cells to HSPCs via the Angiopoetin-1 (Ang-1) ligand and Tie 2 receptor, respectively[46] allows for a specific population of HSCs to maintain quiescence even in the presence of mobilizing factors such as G-CSF. Although Ang-1 is abundant in a number of cells within the niche, it is produced by osteoblastic cells, and its stimulation triggers maintenance of the long-term repopulating HSCs.[46] Similarly, THPO is produced by osteoblastic cells, and the THPO receptor is found in a quiescent population of LT-HSCs, and these quiescent stem cells are found adhering to THPO$^+$ osteoblastic cells. Stimulation of this pathway increases the number of quiescent HSCs, and inhibition leads to a decrease in LT-HSC frequency.[47]

The Wnt signaling pathway has been implicated in regulation of HSC balance between proliferation and self-renewal. Data have demonstrated that over expression of β-catenin expands the HSPC pool and that HSCs *in vivo* respond to Wnt signaling.[19] Moreover, different experimental models of inhibition of the Wnt pathway resulted in decreased quiescence and decreased reconstituting ability in serial reconstitution experiments.[19,48] Additionally, Wnt activation in HSCs responds to prostaglandin E2 modulation, which results in HSC expansion.[80] However, the role of Wnt signaling in the hematopoietic niche remains controversial. *In vivo* loss of function of β- catenin results in early embryonic lethality, whereas inducible Cre targeted deletion of β-catenin yields no observable effects on the hematopoietic system.[81] Therefore, the Wnt signaling pathway remains an

important signal that can participate in microenvironmental regulation of HSCs.

The Notch signaling pathway is activated by direct cell-cell contact of the Notch receptor with one of its ligands. In the absence of Notch signaling, lineage specific gene expression occurs, and cells undergo differentiation. In contrast, when a Notch ligand such as Jagged1,2 or Delta-like1,3,4 binds the Notch receptor, through γ-secretase enzymatic activity, an activated Notch intracytoplasmic domain (NICD) is released. Nuclear translocation of activated NICD results in transcriptional suppression, with inhibition of differentiation and primitive cell self-renewal.[82]

Notch signaling regulates cell-fate specification in a wide variety of systems including HSC self-renewal.[83–87] By changing the balance of daughter cell self-renewal versus differentiation, activation of Notch signaling can increase stem-cell numbers without expanding mature cells.[83–86] Indeed, previous studies demonstrated that Jagged1 is expressed by bone-marrow stromal cells[87,88] and murine osteoblastic cells,[89] and that increased stromal Jagged1 is sufficient to expand HSC.[88,90] Although some studies have suggested that Jagged1 and Notch1 are not necessary for HSC maintenance in homeostasis,[90,91] our work has implicated osteoblastic Jagged1 in HSC regulation by the niche.[42,92] Recent data have suggested that Notch ligands expressed by bone marrow sinusoidal cells are essential for HSC self-renewal in the setting of recovery from myeloablative injury.[64,65] Moreover, use of immobilized Notch ligands has emerged as a promising strategy for HSC expansion *ex vivo*.[23]

Interestingly, integration of the Notch and Wnt signaling systems has been demonstrated in HSC maintenance, where Notch signaling has a dominant function in inhibition of differentiation, and Wnt is required for induction of self-renewal,[93] again speaking to the complexity of niche signals to HSCs.

## Malignancy and the HSC Niche

Much attention has recently focused on the concept of cancer stem cells (CSCs).[94] Normal and malignant cells retain shared properties of self-renewal and quiescence. It has been proposed that CSCs may play an essential role in cancer metastasis and recurrence. In leukemia, where leukemic stem cells (LSCs) have been identified in both murine models and humans,[95] it has been shown that residual disease lining the endosteum surface in the bone marrow after standard chemotherapy for acute myelogenous leukemia (AML) is comprised of cells positive for stem cell markers. In turn, collections of these cells for secondary transplant successfully engraft, thus recapitulating the leukemic phenotype. Hence, LSCs identify a quiescent population resistant to cell-cycle dependent chemotherapies.[96] Therefore, it has been recently proposed that niches may also support CSCs.[97] It is currently controversial whether there is identity between the benign and malignant niche, and studies have suggested that malignancies induce malignant microenvironments at the expense of normal niches.[98] In prostate cancer, data have suggested that metastatic cells occupy normal HSC niches, and that expansion of the niche increases metastatic disease, whereas mobilization from the normal niche also mobilizes normal cells.[99]

The existence of the CSC niche, regardless of whether complete or no identity exists between these malignant microenvironments and the HSC niche, is critical for at least two reasons. First, *in vivo* HSC expansion may be employed in the setting of recovery from iatrogenic injury inflicted during cancer treatment; therefore, it is essential to determine whether attempts at normal HSC expansion would be at the expense of recurrence of malignancy. Second, identification of components of the malignant niches may provide additional therapeutic targets in cancers where the microenvironment plays an important role and where outcomes remain poor, such as in the treatment of adult AML.

## Conclusions

Investigation of components of the HSC niche has yielded many discoveries in the past decade that have highlighted its complexity. However, potential niche manipulations have already resulted in measurable favorable outcomes, at least in murine models.[100] Understanding the signaling pathways, specific growth factors, and cytokines involved in both maturation and self-renewal of the hematopoietic system will contribute to effectively expanding HSCs. For example, currently, *ex vivo* expansion yielding clinically relevant results stems from knowledge of the notch signaling pathway.[23] Moreover, advances in the understanding of HSC niche components have contributed to the refinement of *ex vivo* HSC expansion strategies.

A number of obstacles still lie ahead in the expansion of HSCs, namely, increasing HSPCs without loss of multipotency and identifying all necessary and sufficient factors required to achieve clinical success. The HSC niche remains an attractive target for hematopoietic regeneration, even as we face these obstacles. It is our strong contention that ascertaining niche factors necessary and sufficient for HSC expansion is required to ensure safe and efficient HSC expansion, both *in vivo* and *in vitro*.

Although discovery of the CSC and of the potential role for the HSC niche as a foothold for metastatic disease highlights the potential risk of micro-environmental manipulation, it also suggests a very exciting and novel approach to cancer treatment. Continued refinement of our understanding of the highly interactive and plastic bone marrow microenvironment, therefore, holds much promise not only for tissue regeneration, but also for cancer therapeutics.

## Acknowledgments

This work was supported by the National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Diseases (R01 DK 076876 and R01 DK 081843 to L.M.C.). R.A.L is a trainee in the Medical Scientist Training Program, NIH T32 GM-07356. The authors thank Julianne N. Smith, Rebecca L. Porter, and Benjamin J. Frisch for their helpful discussion.

## Disclosure Statement

No competing financial interests exist.

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*Received: April 4, 2011*
*Accepted: August 8, 2011*
*Online Publication Date: September 26, 2011*