Progenitor Stem Cell Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on progenitor stem cell.
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Key words: stem cell; life; gene; DNA; protein; progenitor

Introduction

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into many different types of cells in life bodies, that are exciting to scientists because of their potential to develop into many different cells, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situuition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science. The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" (Stedman's Medical Dictionary, 2002).

Literatures

Aboody, K. S., J. Najbauer, et al. (2008). "Stem and progenitor cell-mediated tumor selective gene therapy." Gene Ther **15**(10): 739-52.

The poor prognosis for patients with aggressive or metastatic tumors and the toxic side effects of currently available treatments necessitate the development of more effective tumor-selective therapies. Stem/progenitor cells display inherent tumor-tropic properties that can be exploited for targeted delivery of anticancer genes to invasive and metastatic tumors. Therapeutic genes that have been inserted into stem cells and delivered to tumors with high selectivity include prodrug-activating enzymes (cytosine deaminase, carboxylesterase, thymidine kinase), interleukins (IL-2, IL-4, IL-12, IL-23), interferon-beta, apoptosis-promoting genes (tumor necrosis factor-related apoptosis-inducing ligand) and metalloproteinases (PEX). We and others have demonstrated that neural and mesenchymal stem cells can deliver therapeutic genes to elicit a significant antitumor response in animal models of intracranial glioma, medulloblastoma, melanoma brain metastasis, disseminated neuroblastoma and breast cancer lung metastasis. Most studies reported reduction in tumor volume (up to 90%) and increased survival of tumor-bearing animals. Complete cures have also been achieved (90% disease-free survival for >1 year of mice bearing disseminated neuroblastoma tumors). As we learn more about the biology of stem cells and the molecular mechanisms that mediate their tumor-tropism and we identify efficacious gene products for specific tumor types, the clinical utility of cell-based delivery strategies becomes increasingly evident.

Adler, E. D., A. Bystrup, et al. (2009). "In vivo detection of embryonic stem cell-derived cardiovascular progenitor cells using Cy3-labeled Gadofluorine M in murine myocardium." JACC Cardiovasc Imaging **2**(9): 1114-22.

OBJECTIVES: The aim of the current study is to test the ability to label and detect murine embryonic stem cell-derived cardiovascular progenitor cells (ES-CPC) with cardiac magnetic resonance (CMR) using the novel contrast agent Gadofluorine M-Cy3 (GdFM-Cy3). BACKGROUND: Cell therapy shows great promise for the treatment of cardiovascular disease. An important limitation to previous clinical studies is the inability to accurately identify transplanted cells. GdFM-Cy3 is a lipophilic paramagnetic contrast agent that contains a perfluorinated side chain and an amphiphilic character that allows for micelle formation in an aqueous

solution. Previous studies reported that it is easily taken up and stored within the cytosol of mesenchymal stem cells, thereby allowing for paramagnetic cell labeling. Investigators in our laboratory have recently developed techniques for the robust generation of ES-CPC. We reasoned that GdFM-Cy3 would be a promising agent for the in vivo detection of these cells after cardiac cell transplantation. METHODS: ES-CPC were labeled with GdFM-Cy3 by incubation. In vitro studies were performed to assess the impact of GdFM-Cy3 on cell function and survival. A total of 500,000 GdFM-Cy3-labeled ES-CPC or control ES-CPC were injected into the myocardium of mice with and without myocardial infarction. Mice were imaged (9.4-T) before and over a 2-week time interval after stem cell transplantation. Mice were then euthanized, and their hearts were sectioned for fluorescence microscopy. RESULTS: In vitro studies demonstrated that GdFM-Cy3 was easily transfectable, nontoxic, stayed within cells after labeling, and could be visualized using CMR and fluorescence microscopy. In vivo studies confirmed the efficacy of the agent for the detection of cells transplanted into the hearts of mice after myocardial infarction. A correspondence between CMR and histology was observed. CONCLUSIONS: The results of the current study suggest that it is possible to identify and potentially track GdFM-Cy3-labeled ES-CPC in murine infarct models via CMR.

Aicher, A., O. Kollet, et al. (2008). "The Wnt antagonist Dickkopf-1 mobilizes vasculogenic progenitor cells via activation of the bone marrow endosteal stem cell niche." Circ Res **103**(8): 796-803.

Therapeutic mobilization of vasculogenic progenitor cells is a novel strategy to enhance neovascularization for tissue repair. Prototypical mobilizing agents such as granulocyte colony-stimulating factor mobilize vasculogenic progenitor cells from the bone marrow concomitantly with inflammatory cells. In the bone marrow, mobilization is regulated in the stem cell niche, in which endosteal cells such as osteoblasts and osteoclasts play a key role. Because Wnt signaling regulates endosteal cells, we examined whether the Wnt signaling antagonist Dickkopf (Dkk)-1 is involved in the mobilization of vasculogenic progenitor cells. Using TOP-GAL transgenic mice to determine activation of beta-catenin, we demonstrate that Dkk-1 regulates endosteal cells in the bone marrow stem cell niche and subsequently mobilizes vasculogenic and hematopoietic progenitors cells without concomitant mobilization of inflammatory neutrophils. The mobilization of vasculogenic progenitors required the presence of functionally active osteoclasts, as demonstrated in PTPepsilon-deficient mice with defective osteoclast function. Mechanistically, Dkk-1 induced the osteoclast differentiation factor RANKL, which subsequently stimulated the release of the major bone-resorbing protease cathepsin K. Eventually, the Dkk-1-induced mobilization of bone marrow-derived vasculogenic progenitors enhanced neovascularization in Matrigel plugs. Thus, these data show that Dkk-1 is a mobilizer of vasculogenic progenitors but not of inflammatory cells, which could be of great clinical importance to enhance regenerative cell therapy.

Aiuti, A., C. Friedrich, et al. (1998). "Identification of distinct elements of the stromal microenvironment that control human hematopoietic stem/progenitor cell growth and differentiation." Exp Hematol **26**(2): 143-57.

Using a novel collection of conditionally immortalized mouse stromal cell clones, we evaluated the role of distinct elements of the hematopoietic microenvironment in supporting and regulating the growth, division, and differentiation of a candidate human stem cell population (CD34+/CD38-). We found functional diversity in the capacity of different stromal cell clones to support the growth of primitive (CD34+/CD38-) and committed (CD34+/CD38+) hematopoietic progenitors and their differentiation into mature hematopoietic cells (CD34-/CD45+). Among the stromal cell clones that supported long-term hematopoiesis, we identified two clones that induced expansion of CD34+ progenitor/stem cells during the first 4 weeks of coculture and that supported the maintenance of this CD34+ population for up to 10 weeks in vitro. However, these two clones appeared to represent two different microenvironments with regard to the signals they provide to the different CD34+ progenitor subpopulations: One stromal clone preserved a pool of undifferentiated, relatively quiescent (CD34+/CD38-) progenitor cells, allowing their differentiation at a low rate into more committed (CD34+/CD38+) progenitors; the other fostered a more extensive and rapid differentiation of all CD34+/CD38- progenitors into CD34+/CD38+ cells, preferentially maintaining this committed population at a higher rate of cell division. These stromal cell clones were also able to support the proliferation and differentiation of CD34+/CD38- cells in conditions in which progenitor-stroma contact was prevented. This collection of stromal cell clones may represent a unique tool for the study of stromal regulators of hematopoiesis as well as for the support of gene transfer into hematopoietic progenitor cells.

Aizawa, Y., N. Leipzig, et al. (2008). "The effect of immobilized platelet derived growth factor AA on

neural stem/progenitor cell differentiation on cell-adhesive hydrogels." Biomaterials **29**(35): 4676-83.

Neural stem/progenitor cells (NSPCs) hold great promise in regenerative medicine; however, controlling their differentiation to a desired phenotype within a defined matrix is challenging. To guide the differentiation of NSPCs, we first created a cell-adhesive matrix of agarose modified with glycine-arginine-glycine-aspartic acid-serine (GRGDS) and then demonstrated the multipotentiality of NSPCs to differentiate to the three primary cell types of the central nervous system on this matrix: neurons, oligodendrocytes and astrocytes. We then examined whether immobilized platelet derived growth factor AA (PDGF-AA) would promote differentiation similarly to the same soluble factor and found similar percentages of NSPCs differentiated to oligodendrocytes as determined by immunohistochemistry (IHC) and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Interestingly, the gene expression of the differentiated oligodendrocytes was similar for 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) but different for myelin oligodendrocyte glycoprotein (MOG) in the presence of soluble PDGF-AA vs. immobilized PDGF-AA. These results demonstrate for the first time, that it is possible to control the differentiation of NSPCs, and specifically to oligodendrocytes, in cell-adhesive matrices with immobilized PDGF-AA.

Akel, S., C. Petrow-Sadowski, et al. (2003). "Neutralization of autocrine transforming growth factor-beta in human cord blood CD34(+)CD38(-)Lin(-) cells promotes stem-cell-factor-mediated erythropoietin-independent early erythroid progenitor development and reduces terminal differentiation." Stem Cells **21**(5): 557-67.

Transforming growth factor (TGF)-beta1 exerts autocrine and paracrine effects on hematopoiesis. Here, we have attempted to evaluate the effect of endogenous TGF-beta1 on early erythroid development from primitive human hematopoietic stem cells (HSCs) and to assess the effects of TGF-beta1 on different phases of erythropoiesis. Cord blood CD34(+)CD38(-) lineage-marker-negative (Lin(-)) cells were cultured in serum-free conditions using various combinations of stem cell factor (SCF), erythropoietin (Epo), and TGF-beta-neutralizing antibody. Generation of erythroid progenitors was assessed using colony assay and flow cytometry. Terminal erythroid differentiation was examined when SCF/Epo-stimulated cells were recultured in the presence of Epo with and without TGF-beta1. Anti-TGF-beta augmented the proliferation of CD34(+)CD38(-)Lin(-) cells (day 21) in SCF-stimulated (6.4-fold +/- 1.5-fold) and SCF/Epo-stimulated (2.9-fold +/- 1.2-fold) cultures. Cells stimulated by SCF/Epo underwent similar levels of erythroid differentiation with and without anti-TGF-beta. While SCF alone stimulated the production of tryptase-positive mast cells, cells stimulated by SCF/anti-TGF-beta were predominantly erythroid (CD36(+)CD14(-) and glycophorin A positive). A distinct expansion of erythroid progenitors (CD34(+)CD36(+)CD14(-)) with the potential to form erythroid colonies was seen, revealing early Epo-independent erythroid development. In contrast, the kinetics of erythroid progenitor generation from primitive HSCs indicate that TGF-beta1 is not inhibitory in late erythropoiesis, but it accelerated the conversion of large BFU-E into colony-forming units-erythroid. Finally, TGF-beta1 accelerated Epo-induced terminal erythroid differentiation and resulted in a greater level of enucleation (22% +/- 6% versus 7% +/- 3%) in serum-free conditions. Serum addition stimulated enucleation (54% +/- 18%), which was lower (26% +/- 14%) with anti-TGF-beta, suggesting that optimal erythroid enucleation is Epo dependent, requiring serum factors including TGF-beta1.

Alam, S., A. Sen, et al. (2004). "Cell cycle kinetics of expanding populations of neural stem and progenitor cells in vitro." Biotechnol Bioeng **88**(3): 332-47.

Neural stem cells (NSCs) are undifferentiated, primitive cells with important potential applications including the replacement of neural tissue lost due to neurodegenerative diseases, including Parkinson's disease, as well as brain and spinal cord injuries, including stroke. We have developed methods to rapidly expand populations of mammalian stem and progenitor cells in neurosphere cultures. In the present study, flow cytometry was used in order to understand cell cycle activation and proliferation of neural stem and progenitor cells in suspension bioreactors. First, a protocol was developed to analyze the cell cycle kinetics of NSCs. As expected, neurosphere cells were found to cycle slowly, with a very small proportion of the cell population undergoing mitosis at any time. Large fractions (65-70%) of the cells were detected in G1, even in rapidly proliferating cultures, and significant fractions (20%) of the cells were in G0. Second, it was observed that different culturing methods influence both the proportion of neurosphere cells in each phase of the cell cycle and the fraction of actively proliferating cells. The results show that suspension culture does not significantly alter the cell cycle progression of neurosphere cells, while long-term culture (>60 days) results in significant changes in cell cycle kinetics. This suggests that when developing a process to produce neural stem cells for clinical

applications, it is imperative to track the cell cycle kinetics, and that a short-term suspension bioreactor process can be used to successfully expand neurosphere cells.

Albo, C., J. de la Fuente, et al. (2004). "Kinetics and immunophenotypic characterization of circulating hematopoietic progenitor cells after peripheral blood stem cell transplantation." Haematologica **89**(7): 845-51.

BACKGROUND AND OBJECTIVES: Hematopoietic progenitor cells (HPC) circulate in the peripheral blood (PB) before and after engraftment following autologous or allogeneic peripheral blood stem cell transplantation (PBSCT), although the characteristics of these cells are not known. CD34 protein is a reliable marker for identifying the fraction of hematopoietic cells in which HPC are contained. The CD34(+) cells represent a heterogeneous cell population consisting of both primitive uncommitted as well as pluripotent committed progenitors. The aim of this study was to investigate the kinetics and immunophenotypic characteristics of these post-transplant circulating progenitor cells. DESIGN AND METHODS: Forty-seven auto-PBSCT and nine allo-PBSCT recipients were selected for this study. Samples of PB were taken from each patient 4, 9, 11, 14, 16 and 18 days after the transplant. Cells were incubated with the following combinations of monoclonal antibodies: CD34-FITC/CD90-PE/CD38-CyCrome; CD34-FITC/CD117-PE/HLA-DR-PerCP; CD34-FITC/CD13-PE/CD33-CyCrome and the cells were then analyzed by flow cytometry. RESULTS: CD34(+) cells were undetectable on day +4; they reappeared from day +9 to day +18 along with neutrophil and platelet recovery. Subsets of CD34(+) HPC enriched in pluripotent stem cells (CD90(+)/CD38(low) or HLADR-) were hardly detected during the very early post-transplant period. HPC that expressed myeloid associated antigens (CD33, CD13, and CD117) increased after engraftment and constituted the largest proportion of the hematopoietic progenitor cells. INTERPRETATION AND CONCLUSIONS: Circulating HPC could be detected in the early period after PBSCT. The qualitative and quantitative composition of these cells is similar to that found among HPC from mobilized PB.

Al-Shaibi, N. and S. K. Ghosh (2009). "A novel cell-surface protein CSP82 on bone marrow stem cells and a cytosolic phosphoprotein DP58 (ankyrinRD 34B) are involved in promyeloid progenitor induction." Cell Immunol **258**(2): 172-80.

The molecular events associated with the development of common myeloid progenitor (CMP) remain largely unknown. This study reports that a novel glycosylphosphatidylinositol (GPI)-anchored lactoferrin CSP82 on uninitiated mouse bone marrow cells (BMC) may be involved in inducing pro-DC from CMP. By peptide mass fingerprinting, CSP82 has been identified as the mouse lactoferrin precursor, but unlike the latter, it occurs as a GPI-linked cell-surface protein. The GPI-linkage was demonstrated on BMC-derived immunoprecipitates and by other techniques. Furthermore, BMC and hematopoietic stem BM cells following incubation with either CSP82 peptide antibody or purified Reagent A yielded CMP-like progenitors (BM4 cells). These progenitors expressed a previously reported cytosolic phosphoprotein DP58 (AnkRD 34B protein). Continued cultivation of BMC in media containing only anti-CSP82 antibody led to DC-like cells, that bore phenotypic and endocytic resemblance with those obtained using GM-CSF. The results suggest that a receptor lactoferrin on BMC may be an important non-cytokine mechanism for early promyeloid progenitor differentiation.

Andrews, R. G., R. A. Briddell, et al. (1994). "In vivo synergy between recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in baboons enhanced circulation of progenitor cells." Blood **84**(3): 800-10.

Recombinant human stem cell factor (rhSCF) and recombinant human granulocyte colony-stimulating factor (rhG-CSF) are synergistic in vitro in stimulating the proliferation of hematopoietic progenitor cells and their precursors. We examined the in vivo synergy of rhSCF with rhG-CSF for stimulating hematopoiesis in vivo in baboons. Administration of low-dose (LD) rhSCF (25 micrograms/kg) alone did not stimulate changes in circulating WBCs. In comparison, administration of LD rhSCF in combination with rhG-CSF at 10 micrograms/kg or 100 micrograms/kg stimulated increases in circulating WBCs of multiple types up to twofold higher than was stimulated by administration of the same dose of rhG-CSF alone. When the dose of rhG-CSF is increased to 250 micrograms/kg, the administration of LD rhSCF does not further increase the circulating WBC counts. Administration of LD rhSCF in combination with rhG-CSF also stimulated increased circulation of hematopoietic progenitors. LD rhSCF alone stimulated less of an increase in circulating progenitors, per milliliter of blood, than did administration of rhG-CSF alone at 100 micrograms/kg. Baboons administered LD rhSCF together with rhG-CSF at 10, 100, or 250 micrograms/kg had 3.5- to 16-fold higher numbers per milliliter of blood of progenitors cells of multiple types, including colony-forming units

granulocyte/macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E), and colony-forming and burst-forming units-megakaryocyte (CFU-MK and BFU-MK) compared with animals given the same dose of rhG-CSF without rhSCF, regardless of the rhG-CSF dose. The increased circulation of progenitor cells stimulated by the combination of rhSCF plus rhG-CSF was not necessarily directly related to the increase in WBCs, as this effect on peripheral blood progenitors was observed even at an rhG-CSF dose of 250 micrograms/kg, where coadministration of LD rhSCF did not further increase WBC counts. Administration of very-low-dose rhSCF (2.5 micrograms/kg) with rhG-CSF, 10 micrograms/kg, did not stimulate increases in circulating WBCs, but did increase the number of megakaryocyte progenitor cells in blood compared with rhG-CSF alone. LD rhSCF administered alone for 7 days before rhG-CSF did not result in increased levels of circulating WBCs or progenitors compared with rhG-CSF alone. Thus, the synergistic effects of rhSCF with rhG-CSF were both dose- and time-dependent. The doses of rhSCF used in these studies have been tolerated in vivo in humans.(ABSTRACT TRUNCATED AT 400 WORDS)

Andrews, R. G., R. A. Briddell, et al. (1995). "Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in nonhuman primates." Blood **85**(1): 15-20.

We have previously shown that administration of low-dose recombinant human stem cell factor (rhSCF) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) to baboons mobilizes greater numbers of progenitor cells in the blood than does administration of rhG-CSF alone. The purpose of the present study was to determine whether marrow repopulating cells are present in the blood of nonhuman primates administered low-dose rhSCF plus rhG-CSF, and if present, whether these cells engraft lethally irradiated recipients as rapidly as blood cells mobilized by treatment with rhG-CSF alone. One group of baboons was administered low-dose rhSCF (25 micrograms/kg/d) plus rhG-CSF (100 micrograms/kg/d) while a second group received rhG-CSF alone (100 micrograms/kg/d). Each animal underwent a single 2-hour leukapheresis occurring the day when the number of progenitor cells per volume of blood was maximal. For baboons administered low-dose rhSCF plus rhG-CSF, the leukapheresis products contained 1.8-fold more mononuclear cells and 14.0-fold more progenitor cells compared to the leukapheresis products from animals treated with rhG-CSF alone. All animals successfully engrafted after transplantation of cryopreserved autologous blood cells. In animals transplanted with low-dose rhSCF plus rhG-CSF mobilized blood cells, we observed a time to a platelet count of > 20,000 was 8 days +/- 0, to a white blood cell count (WBC) of > 1,000 was 11 +/- 1 days, and to an absolute neutrophil count (ANC) of > 500 was 12 +/- 1 days. These results compared with 42 +/- 12, 16 +/- 1, and 24 +/- 4 days to achieve platelets > 20,000, WBC > 1,000, and ANC > 500, respectively, for baboons transplanted with rhG-CSF mobilized blood cells. Animals transplanted with low-dose rhSCF plus rhG-CSF mobilized blood cells had blood counts equivalent to pretransplant values within 3 weeks after transplant. The results suggest that the combination of low-dose rhSCF plus rhG-CSF mobilizes greater numbers of progenitor cells that can be collected by leukapheresis than does rhG-CSF alone, that blood cells mobilized by low-dose rhSCF plus rhG-CSF contain marrow repopulating cells, and finally that using a single 2-hour leukapheresis to collect cells, the blood cells mobilized by low-dose rhSCF plus rhG-CSF engraft lethally irradiated recipients more rapidly than do blood cells mobilized by rhG-CSF alone.

Aoyama, K., K. Oritani, et al. (1999). "Stromal cell CD9 regulates differentiation of hematopoietic stem/progenitor cells." Blood **93**(8): 2586-94.

CD9 belongs to the transmembrane 4 superfamily, and has been shown to influence cell proliferation, motility, and adhesion. We show here that ligation of CD9 modifies proliferation and/or differentiation of hematopoietic stem/progenitors. Pluripotent EML-C1 hematopoietic cells were cocultured with MS-5 stromal cells in the presence of KMC8.8, an anti-CD9 antibody. Numbers of recovered EML-C1 cells were slightly reduced and the antibody caused the hematopoietic cells to migrate beneath the adherent stromal cell layer. Of particular interest, EML-C1 cells recovered from CD9-ligated cultures had undifferentiated properties. Separate pretreatment of the two cell types with antibody showed that stromal-cell CD9 mediated these responses. Spontaneous expression of erythroid marker was completely blocked and there was a shift towards undifferentiated clonogenic progenitors. Immunoprecipitation studies showed that stromal-cell CD9 associates with the beta1 subunit of integrin, as well as a novel 100 kD protein. Antibody cross-linking of cell surface CD9 increased the amount of 100 kD protein that was subsequently coprecipitated with CD9. These observations show that stromal-cell CD9 influences physical interactions with hematopoietic cells and may be one factor that determines the degree of stem cell differentiation.

Askenasy, N., J. Stein, et al. (2007). "Imaging approaches to hematopoietic stem and progenitor cell function and engraftment." Immunol Invest **36**(5-6): 713-38.

Cell tracking in vivo continues to provide significant insights into hematopoietic cell function and donor cell engraftment after transplantation. The combination of proliferation tracking dyes and induced expression of reporters with advanced imaging modalities has led to better understanding of qualitative and quantitative aspects of hematopoietic cells' homing, seeding and engraftment. Currently, there is no single technique that allows in vivo tracking of cells with molecular resolution, thus several techniques need to be combined. Recent developments promise better implementation of non-invasive imaging modalities to study functional and molecular characteristics of stem cells.

Astori, G., W. Malangone, et al. (2001). "A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use." Blood Cells Mol Dis **27**(4): 715-24; discussion 725-7.

To obtain long-term engraftment and hematopoiesis in myeloablated patients, the cell population used for hematopoietic reconstitution should include a sufficient number of early pluripotent hematopoietic stem cells (HSCs), along with committed cells from the various lineages. For this purpose, the small subset of CD34+ cells purified from different sources must be expanded ex vivo. Since cytokines may induce both proliferation and differentiation, expansion would provide a cell population comprising committed as well as uncommitted cells. Optimization of HSC expansion methods could be obtained by a combination of cytokines able to sustain renewal of pluripotent cells yet endowed with poor differentiation potential. We used variations of the combinations of cytokines described by Brugger et al. [W. Brugger, S. Heimfels, R. J. Berenson, R. Mertelsmann, and L. Kanz (1995) N. Engl. J. Med. 333, 283-287] and Piacibello et al. [W. Piacibello, F. Sanavio, L. Garetto, A. Severino, D. Bergandi, J. Ferrario, F. Fagioli, M. Berger, and M. Aglietta (1997) Blood 89, 2644-2653] to expand UCB CD34+ cells and monitored proliferation rate and phenotype after 14 days of culture. Several hematopoietic lineage-associated surface antigens were evaluated. Our data show that flt3L and thrombopoietin in combination with IL-3, while sustaining a high CD34+ proliferation rate, provide a relatively low enrichment in very early uncommitted CD34+/CD38- cells. Conversely, in the absence of IL-3, they are less effective in inducing proliferation yet significantly increase the number of CD34+/CD38- cells. A combination of the above protocols, applied simultaneously to aliquots of the same sample, would allow expansion of both committed and pluripotent HSC. This strategy may represent a significant improvement for clinical applications.

Ayach, B. B., M. Yoshimitsu, et al. (2006). "Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction." Proc Natl Acad Sci U S A **103**(7): 2304-9.

Inappropriate cardiac remodeling and repair after myocardial infarction (MI) predisposes to heart failure. Studies have reported on the potential for lineage negative, steel factor positive (c-kit+) bone marrow-derived hematopoetic stem/progenitor cells (HSPCs) to repair damaged myocardium through neovascularization and myogenesis. However, the precise contribution of the c-kit signaling pathway to the cardiac repair process has yet to be determined. In this study, we sought to directly elucidate the mechanistic contributions of c-kit+ bone marrow-derived hematopoetic stem/progenitor cells in the maintenance and repair of damaged myocardium after MI. Using c-kit-deficient mice, we demonstrate the importance of c-kit signaling in preventing ventricular dilation and hypertrophy, and the maintenance of cardiac function after MI in c-kit-deficient mice. Furthermore, we show phenotypic rescue of cardiac repair after MI of c-kit-deficient mice by bone marrow transplantation of wild-type HSPCs. The transplanted group also had reduced apoptosis and collagen deposition, along with an increase in neovascularization. To better understand the mechanisms underlying this phenotypic rescue, we investigated the gene expression pattern within the infarcted region by using microarray analysis. This analysis suggested activation of inflammatory pathways, specifically natural killer (NK) cell-mediated mobilization after MI in rescued hearts. This finding was confirmed by immunohistology and by using an NK blocker. Thus, our investigation revealed a previously uncharacterized role for c-kit signaling after infarction by mediating bone marrow-derived NK and angiogenic cell mobilization, which contributes to improved remodeling and cardiac function after MI.

Bakondi, B., I. S. Shimada, et al. (2009). "CD133 identifies a human bone marrow stem/progenitor cell sub-population with a repertoire of secreted factors that protect against stroke." Mol Ther **17**(11): 1938-47.

The reparative properties of bone marrow stromal cells (BMSCs) have been attributed in part to the paracrine action of secreted factors. We isolated

typical human BMSCs by plastic adherence and compared them with BMSC sub-populations isolated by magnetic-activated cell sorting against CD133 (CD133-derived BMSCs, CD133BMSCs) or CD271 [p75 low-affinity nerve growth factor receptor (p75LNGFR), p75BMSCs]. Microarray assays of expressed genes, and enzyme-linked immunosorbent assays (ELISAs) of selected growth factors and cytokines secreted under normoxic and hypoxic conditions demonstrated that the three transit-amplifying progenitor cell populations were distinct from one another. CD133BMSC-conditioned medium (CdM) was superior to p75BMSC CdM in protecting neural progenitor cells against cell death during growth factor/nutrient withdrawal. Intracardiac (arterial) administration of concentrated CD133BMSC CdM provided neuroprotection and significantly reduced cortical infarct volumes in mice following cerebral ischemia. In support of the paracrine hypothesis for BMSC action, intra-arterial infusion of CD133BMSC CdM provided significantly greater protection against stroke compared with the effects of CD133BMSC (cell) administration. CdM from CD133BMSCs also provided superior protection against stroke compared with that conferred by CdM from p75BMSCs or typically isolated BMSCs. CD133 identifies a sub-population of nonhematopoietic stem/progenitor cells from adult human bone marrow, and CD133BMSC CdM may provide neuroprotection for patients with stroke.

Barkho, B. Z., H. Song, et al. (2006). "Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation." Stem Cells Dev **15**(3): 407-21.

Multipotent neural stem/progenitor cells (NSPCs) can be isolated from many regions of the adult central nervous system (CNS), yet neurogenesis is restricted to the hippocampus and subventricular zone in vivo. Identification of the molecular cues that modulate NSPC fate choice is a prerequisite for their therapeutic applications. Previously, we demonstrated that primary astrocytes isolated from regions with higher neuroplasticity, such as newborn and adult hippocampus and newborn spinal cord, promoted neuronal differentiation of adult NSPCs, whereas astrocytes isolated from the nonneurogenic region of the adult spinal cord inhibited neural differentiation. To identify the factors expressed by these astrocytes that could modulate NSPC differentiation, we performed gene expression profiling analysis using Affymetrix rat genome arrays. Our results demonstrated that these astrocytes had distinct gene expression profiles. We further tested the functional effects of candidate factors that were differentially expressed in neurogenesis-promoting and -inhibiting astrocytes using in vitro NSPC differentiation assays. Our results indicated that two interleukins, IL-1beta and IL-6, and a combination of factors that included these two interleukins could promote NSPC neuronal differentiation, whereas insulin-like growth factor binding protein 6 (IGFBP6) and decorin inhibited neuronal differentiation of adult NSPCs. Our results have provided further evidence to support the ongoing hypothesis that, in adult mammalian brains, astrocytes play critical roles in modulating NSPC differentiation. The finding that cytokines and chemokines expressed by astrocytes could promote NSPC neuronal differentiation may help us to understand how injuries induce neurogenesis in adult brains.

Bartkowiak, K., M. Wieczorek, et al. (2009). "Two-dimensional differential gel electrophoresis of a cell line derived from a breast cancer micrometastasis revealed a stem/ progenitor cell protein profile." J Proteome Res **8**(4): 2004-14.

Dissemination of primary cancer cells to distant sites is an early event in breast cancer. These cells can invade the bone marrow, rest there, and many years later disseminated tumor cells (DTC) can grow out to form overt metastases. Epithelium specific cytokeratins are commonly used as marker proteins for sensitive detection of metastatic lesions. However, due to difficulties in the detection of DTC, the question arises if DTC necessarily have the same protein expression profile as advanced tumors. On that account, we analyzed the previously uncharacterized breast cancer DTC cell line BC-M1 by 2-D DIGE. Special protein concentration and purification protocols for 2-DE were developed which resulted in high recovery rates and increased display of alkaline proteins. A broad range reference map of metastasis relevant proteins was compiled including the cytokeratins 5, 7, 8, 17, 18, and 19 and several classes of cytoskeleton proteins involved in metastasis like ezrin, gelsolin, vinculin, or vimentin. BC-M1 shows the rare and highly metastatic vimentin/cytokeratin 5 positive and cytokeratin 8/18 negative breast cancer phenotype and expresses Her-2, which is also found in stem cells/progenitor cells of primary tumors. Supported by the detection of several other epithelium-derived proteins, the example BC-M1 indicates that the protein expression profile of DTC might be reminiscent of the expression profile of the early tumor, which differs from the advanced tumor. Hence, DTC from breast cancer patients' bone marrow expressed cytokeratin 5, which further supports our hypothesis.

Basser, R. L., L. B. To, et al. (1998). "Rapid hematopoietic recovery after multicycle high-dose chemotherapy: enhancement of filgrastim-induced

progenitor-cell mobilization by recombinant human stem-cell factor." J Clin Oncol **16**(5): 1899-908.

PURPOSE: To assess the mobilization potential and safety of recombinant human stem-cell factor (SCF) when coadministered with filgrastim to untreated women with poor-prognosis breast cancer. PATIENTS AND METHODS: Eligible women had breast cancer with 10 or more positive axillary nodes, or estrogen receptor-negative tumor with 4 positive nodes, or stage III disease. Patients were randomized to receive SCF plus filgrastim or filgrastim alone. Filgrastim 12 microg/kg daily was administered for 6 days by continuous subcutaneous infusion. SCF was administered by daily subcutaneous injection at 5, 10, or 15 microg/kg concurrent with filgrastim for 7 days, or 10 microg/kg daily starting 3 days before filgrastim for a total of 10 days (SCF pretreatment). Apheresis was performed on days 5, 6, and 7 of filgrastim administration. Patients then had three cycles of epirubicin 200 mg/m2 and cyclophosphamide 4 g/m2 every 28 days, each supported by one third of the apheresis product. RESULTS: Sixty-two women were treated. Greater yields occurred in patients who received SCF 10 microg/kg daily plus filgastim than those who received filgrastim alone (P=.013 for CD34+ cells; P=.07 for granulocyte-macrophage colony-forming cells [GM-CFCs]). The difference was more marked with SCF-pretreatment than concurrent SCF. Fewer aphereses were required to reach the predetermined target of peripheral-blood progenitor/stem cells (PBPCs) in women who received SCF. SCF was generally well tolerated. Hematologic recovery was rapid after each of the three cycles of chemotherapy. There was no difference in recovery between the different treatment groups. CONCLUSION: Mobilization of PBPCs by filgrastim is significantly enhanced by coadministration of SCF, and commencing SCF before filgrastim can optimize this effect. SCF has the potential to reduce the number of aphereses required to collect a target number of PBPCs.

Baumann, U., H. A. Crosby, et al. (1999). "Expression of the stem cell factor receptor c-kit in normal and diseased pediatric liver: identification of a human hepatic progenitor cell?" Hepatology **30**(1): 112-7.

The stem cell factor (SCF)/c-kit ligand/receptor system has been implicated in stem (oval) cell activation following liver injury in the rat. The aim of this study was to determine the role of the SCF/c-kit system in pediatric human liver during acute and chronic liver injury. Tissue was obtained from hepatectomy specimens of patients undergoing liver transplantation for extrahepatic biliary atresia (EHBA) and fulminant hepatic failure (FHF). Specific expression of mRNA for c-kit and beta-actin was measured by ribonuclease protection and by immunohistochemistry to localize c-kit in tissue sections. Expression of c-kit was detected at relatively consistent levels in normal and cirrhotic (EHBA) livers. However, in FHF, c-kit mRNA levels were elevated in 3 of 6 specimens. Immunolocalization highlighted the presence of small numbers of c-kit-positive cells in the portal tracts of normal livers with increased numbers in cirrhotic livers. The highest c-kit staining, however, was observed in FHF, in which, in addition to the cells in the portal tracts, discrete c-kit-positive cells were also found integrated into bile ducts. Colocalization studies demonstrated some of the c-kit-positive cells to be of mast cell, leukocyte, and hematopoietic cell origin. However, there remained a subset that was also negative for these markers. The up-regulation of c-kit receptor expression in diseased livers suggests an involvement of this receptor/ligand system in hepatic repair mechanisms, and we speculate that c-kit-positive cells may represent a hepatic progenitor cell population. The origin and growth/differentiation potential of these c-kit-positive cells is under investigation.

Baumert, B., K. Grymula, et al. (2008). "An optimization of hematopoietic stem and progenitor cell isolation for scientific and clinical purposes by the application of a new parameter determining the hematopoietic graft efficacy." Folia Histochem Cytobiol **46**(3): 299-305.

The transplantation of hematopoietic stem and progenitor cells (HSPC) is an established lifesaving therapy. Bone marrow (BM), harvested from heparinized cadaveric organ donors, peripheral blood (PB) and cord blood (CB), are important sources of hematopoietic stem cells. HSPCs, which are used for transplantation purposes, are routinely evaluated in terms of number of mononuclear cells (MNCs), CD34+ MNCs count and viability. The efficacy of grafting is determined additionally in clonogenic tests in vitro. These tests deliver important information about the number of HSPCs and their proliferative potential. Unfortunately, they do not give a possibility to evaluate the functional HSPC chemotactic reactivity in the SDF-1 gradient, which is probably the key phenomenon for HSPC homing after transplantation procedure. Thus, the aim of our study was to optimize HSPC isolation according to their chemotactic reactivity in SDF-1 gradient. Using multiparameter cell sorter (FACS Aria, BD) we examined the HSPCs attracted by SDF-1 on a single cell level. The population of cells which participated in the chemotactic process was highly enriched in CXCR4+lin-AC133+CD45+ cells (referred as hematopoietic stem cells) and to our surprise in CXCR4+lin-AC133+CD45- cells (referred as

pluripotent stem cells) in quantitative amounts. Since reactivity of HSPCs may depend on various factors involved in the protocol of their isolation and short-term storage, we tested the most commonly used anticoagulants (ACD, CPDA-1, EDTA and Heparin) and culture media (DME, IMDM, RPMI). HSPCs, harvested from CB, PB and BM, were subsequently investigated for clonogenic growth of CFU-GM in methylcellulose cultures and for the level of apoptosis by employing annexin V staining. Evaluating clonogenic potential, ability of chemotactic reactivity in SDF-1 gradient and intensification of apoptosis of HSPC as the most safe anticoagulant and medium were selected. This study has proved that chemotactic reactivity of HSPCs is a new but very important parameter which should be included in the procedure of their isolation.

Beckmann, J., S. Scheitza, et al. (2007). "Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment: identification of asymmetrically segregating proteins." Blood **109**(12): 5494-501.

The findings that many primitive human hematopoietic cells give rise to daughter cells that adopt different cell fates and/or show different proliferation kinetics suggest that hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) can divide asymmetrically. However, definitive experimental demonstration is lacking due to the current absence of asymmetrically segregating marker molecules within the primitive hematopoietic cell compartment. Thus, it remains an open question as to whether HSCs/HPCs have the capability to divide asymmetrically, or whether the differences that have been observed are established by extrinsic mechanisms that act on postmitotic progenitors. Here, we have identified 4 proteins (CD53, CD62L/L-selectin, CD63/lamp-3, and CD71/transferrin receptor) that segregate differentially in about 20% of primitive human hematopoietic cells that divide in stroma-free cultures. Therefore, this indicates for the first time that HSCs/HPCs have the capability to divide asymmetrically. Remarkably, these proteins, in combination with the surrogate stem-cell marker CD133, help to discriminate the more primitive human cultivated HSCs/HPCs. Since 3 of these proteins, the transferrin receptor and the tetraspanins CD53 and CD63, are endosomal-associated proteins, they may provide a link between the endosomal compartment and the process of asymmetric cell division within the HSC/HPC compartment.

Begley, C. G., R. Basser, et al. (1997). "Enhanced levels and enhanced clonogenic capacity of blood progenitor cells following administration of stem cell factor plus granulocyte colony-stimulating factor to humans." Blood **90**(9): 3378-89.

Administration of hematopoietic growth factors is being used increasingly to obtain populations of blood progenitor/stem cells (PBPC) for clinical transplantation. Here we examined the effect of combining stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) versus G-CSF alone in a randomized clinical study involving 62 women with early-stage breast cancer. In the first patient cohorts, escalating doses of SCF were administered for 7 days with concurrent G-CSF administration. At baseline, levels of progenitor cells in the bone marrow or blood were comparable in the different patient groups. As with administration of G-CSF alone, the combination of SCF plus G-CSF did not alter the wide variation in levels of PBPC observed between individuals and did not alter the selective nature of PBPC release, with preferential release of day-14 granulocyte-macrophage colony-stimulating factor (GM-CFC) versus day-7 GM-CFC. However, SCF acted to sustain the levels of PBPC after cessation of growth factor treatment; levels of PBPC were elevated 100-fold at later timepoints compared with G-CSF alone. In addition, the maximum levels of PBPC observed were increased approximately fivefold at day 5 of growth-factor administration. The increased levels of PBPC resulted in significantly increased levels of PBPC obtained by leukapheresis. In a subsequent patient cohort, 3-days pretreatment with SCF was introduced and followed by 7 days concurrent SCF plus G-CSF. The 3-days pretreatment with SCF resulted in an earlier wave of PBPC release in response to commencement of G-CSF. In addition, maximum PBPC levels in blood and PBPC yield in leukapheresis products were further increased. Unexpectedly however, SCF pretreatment resulted in progenitor cells with enhanced self-generation potential. Recloning assays documented the ability of approximately 30% of primary granulocyte-macrophage (GM) colonies from control cell populations to generate secondary GM colonies (n = 1,106 primary colonies examined). In contrast approximately 90% of GM colonies from PBPC after SCF pretreatment generated secondary clones and 65% generated secondary colonies. The action of SCF was not explicable in terms of altered SCF, GM-CSF, or G-CSF responsiveness, but SCF pretreatment was associated with maximum serum SCF levels at the time G-CSF was commenced. These results show that PBPC populations mobilized by different growth factor regimens can differ in their functional properties and caution against solely considering number of harvested progenitor cells without regard to their function.

Bello, B. C., N. Izergina, et al. (2008). "Amplification of neural stem cell proliferation by intermediate progenitor cells in Drosophila brain development." Neural Dev **3**: 5.

BACKGROUND: In the mammalian brain, neural stem cells divide asymmetrically and often amplify the number of progeny they generate via symmetrically dividing intermediate progenitors. Here we investigate whether specific neural stem cell-like neuroblasts in the brain of Drosophila might also amplify neuronal proliferation by generating symmetrically dividing intermediate progenitors. RESULTS: Cell lineage-tracing and genetic marker analysis show that remarkably large neuroblast lineages exist in the dorsomedial larval brain of Drosophila. These lineages are generated by brain neuroblasts that divide asymmetrically to self renew but, unlike other brain neuroblasts, do not segregate the differentiating cell fate determinant Prospero to their smaller daughter cells. These daughter cells continue to express neuroblast-specific molecular markers and divide repeatedly to produce neural progeny, demonstrating that they are proliferating intermediate progenitors. The proliferative divisions of these intermediate progenitors have novel cellular and molecular features; they are morphologically symmetrical, but molecularly asymmetrical in that key differentiating cell fate determinants are segregated into only one of the two daughter cells. CONCLUSION: Our findings provide cellular and molecular evidence for a new mode of neurogenesis in the larval brain of Drosophila that involves the amplification of neuroblast proliferation through intermediate progenitors. This type of neurogenesis bears remarkable similarities to neurogenesis in the mammalian brain, where neural stem cells as primary progenitors amplify the number of progeny they generate through generation of secondary progenitors. This suggests that key aspects of neural stem cell biology might be conserved in brain development of insects and mammals.

Bender, J. G., S. F. Williams, et al. (1992). "Characterization of chemotherapy mobilized peripheral blood progenitor cells for use in autologous stem cell transplantation." Bone Marrow Transplant **10**(3): 281-5.

Twenty patients were treated with chemotherapy to mobilize progenitors into the blood. Peripheral blood stem cells were quantitated in peripheral blood or leukapheresis products using colony assays and flow cytometric measurement of CD34+ cells. In four patients where complete sets of serial samples were obtained, the appearance of CD34+ cells preceded the increase in CFU-GM by 24-48 h. Peak levels of CD34+ cells ranged from 0.6-5% and coincided with the peak increase in CFU-GM. Mobilized CD34+ cells contained subsets expressing CD33, CD13, CD45RA, CD38, HLA-DR, CD61 and CD41. Subsets of CD34+ cells expressing CD33, CD13, or CD45RA represent committed myeloid progenitors. In contrast to bone marrow CD34+ cells, few mobilized CD34+ cells expressed CD71, CD7, CD19 or CD10. Prompt engraftment of granulocytes greater than 500 x 10(6)/l at a median of 13 days and platelets greater than 50 x 10(9)/l at a median of 15 days was observed in patients reconstituted with mobilized cells. These data indicate that CD34+ cells mobilized during recovery from chemotherapy are predominantly myeloid in phenotype and contain few actively proliferating cells or cells with lymphoid phenotypes.

Benet, I., B. F. Prosper, et al. (1999). "Mobilization of peripheral blood progenitor cells (PBPC) in patients undergoing chemotherapy followed by autologous peripheral blood stem cell transplant (SCT) for high risk breast cancer (HRBC)." Bone Marrow Transplant **23**(11): 1101-7.

We have determined the effect of delayed addition of G-CSF after chemotherapy on PBPC mobilization in a group of 30 patients with high risk breast cancer (HRBC) undergoing standard chemotherapy followed by high-dose chemotherapy (HDCT) and autologous SCT. Patients received FAC chemotherapy every 21 days followed by G-CSF at doses of 5 microg/kg/day starting on day +15 (groups 1 and 2) or +8 (group 3) after chemotherapy. PBPC collections were performed daily starting after 4 doses of G-CSF and continued until more than 2.5 x 10(6) CD34+ cells had been collected. In group 1, steady-state BM progenitors were also harvested and used for SCT. Groups 2 and 3 received PBPC only. The median number of collections was three in each group. Significantly more PB CD34+ cells were collected in patients receiving G-CSF starting on day 8 vs day 15 (9.43 x 10(6)/kg and 6.2 x 10(6)/kg, respectively) ($P < 0.05$). After conditioning chemotherapy all harvested cells including BM and PBPC were reinfused. Neutrophil and platelet engraftment was significantly faster in patients transplanted with day 8 G-CSF-mobilized PBPC ($P < 0.05$) and was associated with lower transplant related morbidity as reflected by days of fever, antibiotics or hospitalization ($P < 0.05$). Both schedules of mobilization provided successful long-term engraftment with 1 year post-transplant counts above 80% of pretransplant values. In conclusion, we demonstrate that delayed addition of G-CSF results in successful mobilization and collection of PBPC with significant advantage of day 8 G-CSF vs day 15. PBPC collections can be scheduled on a fixed day instead of being guided by the PB counts which

provides a practical advantage. Transplantation of such progenitors results in rapid short-term and long-term trilineage engraftment.

Beretta, F., S. van den Bosch, et al. (1998). "Intrapatient comparison of an intermittent and a continuous flow cell separator for the collection of progenitor and stem cells from the blood." Vox Sang **75**(2): 149-53.

BACKGROUND AND OBJECTIVES: Continuous-flow and intermittent-flow blood cell separators (CFCS and IFCS) are both used to collect stem cells from the blood to rescue patients undergoing myeloablative treatment for cancer. MATERIALS AND METHODS: We designed a study to compare the collection efficiency of the two systems. The continuous-flow Cobe Spectra and the intermittent-flow Haemonetics MCS-3P were used to collect cells on consecutive days from 9 patients mobilised with G-CSF with or without chemotherapy. Blood obtained before leukapheresis and the leukapheresis product were analysed for their content of red and white cells, platelets, CD34-positive cells, GM-CFC, CFC-E, and BFU-E. An extraction ratio was calculated. RESULTS: We found that the CFCS extracted about 4 times more mononuclear cells per unit time, 3 times more CD34-positive, and 4 times more clonogenic cells than the IFCS. The subject acceptability of the two systems was similar. CONCLUSION: The CFCS is a more efficient system for stem cell collection. IFCS requires a longer harvesting time for the same result.

Boecker, W. and H. Buerger (2003). "Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept." Cell Prolif **36 Suppl 1**: 73-84.

Although experimental data clearly confirm the existence of self-renewing mammary stem cells, the characteristics of such progenitor cells have never been satisfactorily defined. Using a double immunofluorescence technique for simultaneous detection of the basal cytokeratin 5, the glandular cytokeratins 8/18 and the myoepithelial differentiation marker smooth muscle actin (SMA), we were able to demonstrate the presence of CK5+ cells in human adult breast epithelium. These cells have the potential to differentiate to either glandular (CK8/18+) or myoepithelial cells (SMA+) through intermediary cells (CK5+ and CK8/18+ or SMA+). We therefore proceeded on the assumption that the CK5+ cells are phenotypically and behaviourally progenitor (committed adult stem) cells of human breast epithelium. Furthermore, we furnish evidence that most of these progenitor cells are located in the luminal epithelium of the ductal lobular tree. Based on data obtained in extensive analyses of proliferative breast disease lesions, we have come to regard usual ductal hyperplasia as a progenitor cell-derived lesion, whereas most breast cancers seem to evolve from differentiated glandular cells. Double immunofluorescence experiments provide a new tool to characterize phenotypically progenitor (adult stem) cells and their progenies. This model has been shown to be of great value for a better understanding not only of normal tissue regeneration but also of proliferative breast disease. Furthermore, this model provides a new tool for unravelling further the regulatory mechanisms that govern normal and pathological cell growth.

Boecker, W., R. Moll, et al. (2002). "Usual ductal hyperplasia of the breast is a committed stem (progenitor) cell lesion distinct from atypical ductal hyperplasia and ductal carcinoma in situ." J Pathol **198**(4): 458-67.

Current classification systems in proliferative mammary gland pathology are based on a two-cell system, recognizing only glandular and myoepithelial lines of differentiation. A third cell type has recently been characterized in normal breast tissue by double-immunofluorescence analysis to express cytokeratin 5 (Ck5) only. These cells were shown to represent progenitor or adult stem cells that give rise to the glandular and myoepithelial cell lineage. The double-labelling technique has been applied to characterize a spectrum of intraductal epithelial proliferations, namely benign usual ductal hyperplasia, atypical ductal hyperplasia, and ductal carcinoma in situ, all of which are thought to represent the gradual steps of a sequence in the development of breast cancer. Immunofluorescence studies with specific antibodies against Ck5, Ck8/18/19, and smooth muscle actin were complemented by western blotting analysis of Ck5 and Ck8/18/19 expression in normal breast tissue and in proliferative lesions. Usual ductal hyperplasia appears to be a Ck5-positive committed stem (progenitor) cell lesion with the same differentiation potential as seen in the normal breast. This is in sharp contrast to atypical ductal hyperplasia/ductal carcinoma in situ, which display the differentiated glandular immunophenotype (Ck8/18/19-positive, but Ck5-negative). These data require the abandonment of the idea of an obligate biological continuum of intraductal proliferations from benign to malignant. This study provides evidence that cells undergoing malignant transformation tend to be fairly advanced in the glandular lineage of differentiation. The committed stem (progenitor) cell model may contribute to a better understanding of both benign

proliferative breast disease and breast cancer development.

Bongiorno, M. R., S. Doukaki, et al. (2008). "Identification of progenitor cancer stem cell in lentigo maligna melanoma." Dermatol Ther **21 Suppl 1**: S1-5.

The potential role of stem cells in neoplasia has aroused considerable interest over the past few years. A number of known biologic characteristics of melanomas support the theory that they may originate in a mutated stem cell. Melanocytic stem cell markers have been described recently. Moreover, the CD133 cells that show surface markers for CD34 are stem cells primitive. These stem cells are capable of differentiating into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. The identification of cancer stem/initiating cells with a crucial role in tumor formation may open up new pharmacologic perspectives. The purpose of this study is to detect the expression of CD133 and CD34, two putative markers of cancer stem cells in the lentigo maligna melanoma. Thirty cases of lentigo maligna melanoma were analyzed using indirect immunohistochemical staining. The vast majority of the samples analyzed showed the presence of rare cells, which were clearly positive for CD133 and CD34. Strong CD133 and CD34 staining was found in the outer root sheath of the mid-lower hair follicles, intermixed with atypical melanocytes extending along layers of the hair follicles. A number of these staminal cells were adjacent and intermixed with melanoma cells. This study supports the stem cell origin of this tumor and suggests that the precursor of the melanoma in question is a stem-like cell rather than the primitive melanoblast committed to be exclusively involved in melanocytic differentiation.

Bonig, H., K. L. Watts, et al. (2009). "Concurrent blockade of alpha4-integrin and CXCR4 in hematopoietic stem/progenitor cell mobilization." Stem Cells **27**(4): 836-7.

The important contributions of the alpha4 integrin VLA-4 and the CXCR4/SDF-1 axis in mobilization have been demonstrated and thereby, these pathways can be suggested as rational targets for clinical stem cell mobilization in the absence of cytokine use. alpha4-blockade alone (in humans, macaques and mice), or genetic ablation of alpha4-integrin in mice, provides reproducible, but modest mobilization. Similarly, CXCR4 blockade with small-molecule antagonists mobilizes hematopoietic stem cells in all three species, but at least with the established single-injection schedule, the mobilization efficiency is marginally sufficient for clinical purposes. Hypothesizing that the different molecular targets (alpha4-integrin vs. CXCR4) might allow for additive mobilization effects, we therefore tested the efficacy of the combination of alpha4-integrin blockade with anti-functional antibodies and CXCR4 blockade with the small-molecule inhibitor AMD3100 in macaques, or the combination of conditional alpha4-integrin ablation and AMD3100 in mice. Mobilization was at least additive. While the prolonged effects of alpha4-blocking antibodies may not be suitable for clinical mobilization, future availability of small-molecule alpha4-antagonists in combination with AMD3100 could provide an alternative to granulocyte colony-stimulating factor.

Borgs, L., P. Beukelaers, et al. (2009). "Period 2 regulates neural stem/progenitor cell proliferation in the adult hippocampus." BMC Neurosci **10**: 30.

BACKGROUND: Newborn granule neurons are generated from proliferating neural stem/progenitor cells and integrated into mature synaptic networks in the adult dentate gyrus of the hippocampus. Since light/dark variations of the mitotic index and DNA synthesis occur in many tissues, we wanted to unravel the role of the clock-controlled Period2 gene (mPer2) in timing cell cycle kinetics and neurogenesis in the adult DG. RESULTS: In contrast to the suprachiasmatic nucleus, we observed a non-rhythmic constitutive expression of mPER2 in the dentate gyrus. We provide evidence that mPER2 is expressed in proliferating neural stem/progenitor cells (NPCs) and persists in early post-mitotic and mature newborn neurons from the adult DG. In vitro and in vivo analysis of a mouse line mutant in the mPer2 gene (Per2Brdm1), revealed a higher density of dividing NPCs together with an increased number of immature newborn neurons populating the DG. However, we showed that the lack of mPer2 does not change the total amount of mature adult-generated hippocampal neurons, because of a compensatory increase in neuronal cell death. CONCLUSION: Taken together, these data demonstrated a functional link between the constitutive expression of mPER2 and the intrinsic control of neural stem/progenitor cells proliferation, cell death and neurogenesis in the dentate gyrus of adult mice.

Boswell, H. S., P. M. Wade, Jr., et al. (1984). "Thy-1 antigen expression by murine high-proliferative capacity hematopoietic progenitor cells. I. Relation between sensitivity to depletion by Thy-1 antibody and stem cell generation potential." J Immunol **133**(6): 2940-9.

Hematopoietic stem cells of high proliferative potential such as the giant macrophage colony-forming cell HPP-CFC, were present in the

marrow of mice treated with high dose 5-fluorouracil (5Fu) (150 mg/kg i.v.), whereas most committed granulocyte-macrophage progenitors, GM-CFU-C, were depleted. Enrichment of primitive stem cells in post 5-Fu bone marrow (5FuBM) was reflected in an enhanced capacity to proliferate in suspension cultures stimulated by the mixture of lymphokines present in Con A spleen-conditioned medium supernatant (Con A CM) when compared to normal bone marrow. The population of blast-like cells harvested at 5 days from suspension cultures of 5FuBM with Con A CM showed marked increases in stem cells GM-CFU-C and HPP-CFC. For this reason, 5FuBM was utilized to study the cell surface characteristics of putative pluripotential stem cells capable of giving rise to committed stem cells in suspension cultures. Treatment of 5FuBM (BDF1 mice) before suspension culture with a high concentration of either of two cytotoxic monoclonal antibodies directed against the Thy-1.2 surface antigen in the presence of rabbit complement reduced or abrogated the generation of stem cells HPP-CFC and GM-CFU-C in suspension cultures, even though the input content of HPP-CFC and GM-CFU-C in treated 5FuBM compared with control 5FuBM showed little reduction by the antibody plus complement treatment. The Thy-1+ cell required for generation of stem cells was not a T cell, because reconstitution of Thy-1.2-depleted 5FuBM with spleen nylon nonadherent (T) cells did not reconstitute the generation of stem cells, even though T cells did grow in the suspension cultures. In addition, depletion from 5FuBM of cells expressing Lyt-1 and Lyt-2 antigens, unambiguous markers of T cell-thymocyte differentiation, did not ablate the generation of HPP-CFC and GM-CFU-C. Rather, performance of Thy-1 cell depletion at lower efficiency, which still abrogated T cell function, ablated generation of HPP-CFC but did not affect the generation of GM-CFU-C. It was concluded that 5FuBM contains distinct Thy-1+ primitive stem cells expressing different amounts of Thy-1 antigen correlating with their respective generation potentials. Some of these Thy-1+ progenitor cells may be pluripotential.

Boyd, N. L., K. R. Robbins, et al. (2009). "Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells." Tissue Eng Part A **15**(8): 1897-907.

Human embryonic stem cells (hESC) have the potential to produce all of the cells in the body. They are able to self-renew indefinitely, potentially making them a source for large-scale production of therapeutic cell lines. Here, we developed a monolayer differentiation culture that induces hESC (WA09 and BG01) to form epithelial sheets with mesodermal gene expression patterns (BMP4, RUNX1, and GATA4). These E-cadherin+ CD90low cells then undergo apparent epithelial-mesenchymal transition for the derivation of mesenchymal progenitor cells (hESC-derived mesenchymal cells [hES-MC]) that by flow cytometry are negative for hematopoietic (CD34, CD45, and CD133) and endothelial (CD31 and CD146) markers, but positive for markers associated with mesenchymal stem cells (CD73, CD90, CD105, and CD166). To determine their functionality, we tested their capacity to produce the three lineages associated with mesenchymal stem cells and found they could form osteogenic and chondrogenic, but not adipogenic lineages. The derived hES-MC were able to remodel and contract collagen I lattice constructs to an equivalent degree as keloid fibroblasts and were induced to express alpha-smooth muscle actin when exposed to transforming growth factor (TGF)-beta1, but not platelet derived growth factor-B (PDGF-B). These data suggest that the derived hES-MC are multipotent cells with potential uses in tissue engineering and regenerative medicine and for providing a highly reproducible cell source for adult-like progenitor cells.

Brice, P., J. P. Marolleau, et al. (1996). "Hematologic recovery and survival of lymphoma patients after autologous stem-cell transplantation: comparison of bone marrow and peripheral blood progenitor cells." Leuk Lymphoma **22**(5-6): 449-56.

Autologous stem-cell transplantation is widely used as part of the treatment of poor prognosis lymphoma patients. Since 1986, peripheral blood progenitor cells (PBPC) mobilized by chemotherapy and/or hematopoietic growth factors have progressively been used instead of autologous bone marrow (BM) cells. Toxicity, engraftment and long-term outcome were compared in a population of relapsing or refractory lymphoma patients given high-dose therapy. During 1986 to 1993, 150 patients with refractory or relapsed non-Hodgkin's lymphomas (n = 93) or Hodgkin's disease (n = 57) received intensive therapy followed by the reinjection of BM (n = 72) or PBPC (n = 78). PBPC were collected by aphereses during the phase of hematologic recovery after mobilization by chemotherapy alone (n = 36) or associated with GCSF (n = 43). Conditioning regimens included chemotherapy alone in 77%, associated with total body irradiation (TBI) in 23%. After stem-cell reinfusion, 55% of the PBPC group received GCSF versus 24% in the BM group. Results show that the median time to neutrophil counts > 500/microliters and platelets > 50,000/microliters was significantly shorter in the PBPC than the BM group, respectively 13 versus 23 days and 18 versus 26 days ($P < 0.05$). This difference remained significant ($P <$

0.05) when patients were stratified according to the administration or not of GCSF after transplantation. PBPC grafting after high-dose therapy was associated with a median reduction of the hospital stay of 10 days. The majority of patients (90%) maintained normal blood counts at 3 months, and no secondary graft failure was observed in either group. The use of TBI in the conditioning regimen was the only significant factor affecting long-term hematologic recovery. For relapsing patients with histologically aggressive lymphomas, overall survival and failure-free survival were similar in both groups. In conclusion, PBPC transplantation is a safe procedure associated with improvement of hematopoietic recovery and a shortened hospital stay.

Briddell, R. A., C. A. Hartley, et al. (1993). "Recombinant rat stem cell factor synergizes with recombinant human granulocyte colony-stimulating factor in vivo in mice to mobilize peripheral blood progenitor cells that have enhanced repopulating potential." Blood **82**(6): 1720-3.

Splenectomized mice treated for 7 days with pegylated recombinant rat stem cell factor (rrSCF-PEG) showed a dose-dependent increase in peripheral blood progenitor cells (PBPC) that have enhanced in vivo repopulating potential. A dose of rrSCF-PEG at 25 micrograms/kg/d for 7 days produced no significant increase in PBPC. However, when this dose of rrSCF-PEG was combined with an optimal dose of recombinant human granulocyte colony-stimulating factor (rhG-CSF; 200 micrograms/kg/d), a synergistic increase in PBPC was observed. Compared with treatment with rhG-CSF alone, the combination of rrSCF-PEG plus rhG-CSF resulted in a synergistic increase in peripheral white blood cells, in the incidence and absolute numbers of PBPC, and in the incidence and absolute numbers of circulating cells with in vivo repopulating potential. These data suggest that low doses of SCF, which would have minimal, if any, effects in vivo, can synergize with optimal doses of rhG-CSF to enhance the mobilization of PBPC stimulated by rhG-CSF alone.

Brooks, Y. S., G. Wang, et al. (2009). "Functional pre-mRNA trans-splicing of coactivator CoAA and corepressor RBM4 during stem/progenitor cell differentiation." J Biol Chem **284**(27): 18033-46.

Alternative splicing yields functionally distinctive gene products, and their balance plays critical roles in cell differentiation and development. We have previously shown that tumor-associated enhancer loss in coactivator gene CoAA leads to its altered alternative splicing. Here we identified two intergenic splicing variants, a zinc finger-containing coactivator CoAZ and a non-coding transcript ncCoAZ, between CoAA and its downstream corepressor gene RBM4. During stem/progenitor cell neural differentiation, we found that the switched alternative splicing and trans-splicing between CoAA and RBM4 transcripts result in lineage-specific expression of wild type CoAA, RBM4, and their variants. Stable expression of CoAA, RBM4, or their variants prevents the switch and disrupts the embryoid body formation. In addition, CoAA and RBM4 counter-regulate the target gene Tau at exon 10, and their splicing activities are subjected to the control by each splice variant. Further phylogenetic analysis showed that mammalian CoAA and RBM4 genes share common ancestry with the Drosophila melanogaster gene Lark, which is known to regulate early development and circadian rhythms. Thus, the trans-splicing between CoAA and RBM4 transcripts may represent a required regulation preserved during evolution. Our results demonstrate that a linked splicing control of transcriptional coactivator and corepressor is involved in stem/progenitor cell differentiation. The alternative splicing imbalance of CoAA and RBM4, because of loss of their common enhancer in cancer, may deregulate stem/progenitor cell differentiation.

Brown, J., M. F. Greaves, et al. (1991). "The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts." Int Immunol **3**(2): 175-84.

The human haemopoietic cell surface antigen, CD34, is a 105 - 120 kd cell surface glycoprotein whose stage-specific expression by stem cells and lineage-specific progenitor cells suggests a role in regulating early events in blood cell differentiation. A murine gene and cDNA encoding a closely homologous protein have been isolated. The gene is organized in eight exons in 22 kb of DNA. The first exon lies in a GC- and CpG-rich island. The sequence of the gene and the cDNA predict a 382 amino acid-long protein containing an N-terminal signal peptide and one transmembrane region 73 amino acids from the C-terminus. The extracellular part of the protein contains: a 140 amino acid-long-N-terminal region, 40% of whose residues are serine or threonine potential attachment sites for O-linked carbohydrate, as well as five potential attachment sites for N-linked carbohydrate. Proximal to the extracellular membrane there is a 79 amino acid-long cysteine-rich region. The homology with the human sequence is highest in the intracellular domain (90% amino acid identity) and lowest in the N-terminal region (43% amino acid identity). The protein is not homologous with any other proteins currently in the databases. The expression of the murine gene by a

number of haemopoietic progenitor cell lines suggests that the CD34 function in haemopoiesis may be conserved between man and mouse. The high level of expression in a number of embryonic fibroblast cell lines and in brain imply a function outside of haemopoiesis.

Broxmeyer, H. E. (1995). "Cord blood as an alternative source for stem and progenitor cell transplantation." Curr Opin Pediatr **7**(1): 47-55.

Blood collected from the umbilical cord and placenta at the birth of a child is a rich source of immature blood cell elements and has been used clinically as an alternative source of transplantable stem and progenitor cells. Studies on the proliferative and replating capacities of cord blood stem and progenitor cells have documented their extensive capacity for division and self renewal. Studies on the immune cells in cord blood have shown them to be less immunologically reactive in a number of situations. These characteristics are consistent with the experience in children receiving HLA-matched sibling cord blood cells, in which these cells have been transplantable in a large number of clinical disorders with low or absent graft-versus-host disease. Stem and progenitor cells from cord blood are efficiently transduced with new genetic material by retroviral and adeno-associated viral vectors and may be of efficacy in the future for autologous gene therapy approaches to treat disease. Efforts in banking of cryopreserved cord blood cells have been undertaken, and a number of such stored samples have been used for fully and partially HLA-matched unrelated transplantation. Efforts to better understand the cells in cord blood and their clinical utility are continuing.

Broxmeyer, H. E., L. Kohli, et al. (2003). "Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells." J Leukoc Biol **73**(5): 630-8.

Stromal cell-derived factor-1 (SDF-1/CXCL12) enhances survival of myeloid progenitor cells. The two main questions addressed by us were whether these effects on the progenitors were direct-acting and if SDF-1/CXCL12 enhanced engrafting capability of competitive, repopulating mouse stem cells subjected to short-term ex vivo culture with other growth factors. SDF-1/CXCL12 had survival-enhancing/antiapoptosis effects on human bone marrow (BM) and cord blood (CB) and mouse BM colony-forming units (CFU)-granulocyte macrophage, burst-forming units-erythroid, and CFU-granulocyte-erythroid-macrophage-megakaryocyte with similar dose responses. The survival effects were direct-acting, as assessed on colony formation by single isolated human BM and CB CD34(+++) cells. Effects were mediated through CXCR4 and G(alpha)i proteins. Moreover, SDF-1/CXCL12 greatly enhanced the engrafting capability of mouse long-term, marrow-competitive, repopulating stem cells cultured ex vivo with interleukin-6 and steel factor for 48 h. These results extend information on the survival effects mediated through the SDF-1/CXCL12-CXCR4 axis and may be of relevance for ex vivo expansion and gene-transduction procedures.

Brugger, W., W. Mocklin, et al. (1993). "Ex vivo expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, interleukin-1 beta (IL-1 beta), IL-6, IL-3, interferon-gamma, and erythropoietin." Blood **81**(10): 2579-84.

To provide sufficient numbers of peripheral blood progenitor cells (PBPCs) for repetitive use after high-dose chemotherapy, we investigated the ability of hematopoietic growth factor combinations to expand the number of clonogenic PBPCs ex vivo. Chemotherapy plus granulocyte colony-stimulating factor (G-CSF) mobilized CD34+ cells from 18 patients with metastatic solid tumors or refractory lymphomas were cultured for up to 28 days in a liquid culture system. The effects of interleukin-1 beta (IL-1), IL-3, IL-6, granulocyte-macrophage-CSF (GM-CSF), G-CSF, macrophage-CSF (M-CSF), stem cell factor (SCF), erythropoietin (EPO), leukemia inhibitory factor (LIF), and interferon-gamma, as well as 36 combinations of these factors were tested. A combination of five hematopoietic growth factors, including SCF, EPO, IL-1, IL-3, and IL-6, was identified as the optimal combination of growth factors for both the expansion of total nucleated cells as well as the expansion of clonogenic progenitor cells. Proliferation peaked at days 12 to 14, with a median 190-fold increase (range, 46- to 930-fold) of total clonogenic progenitor cells. Expanded progenitor cells generated myeloid (colony-forming unit-granulocyte-macrophage), erythroid (burst-forming unit-erythroid), as well as multilineage (colony-forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte) colony-forming units. The number of multilineage colonies increased 250-fold (range, 33- to 589-fold) as compared with pre-expansion values. Moreover, the absolute number of early hematopoietic progenitor cells (CD34+/HLA-DR-; CD34+/CD38-), as well as the number of 4-HC-resistant progenitors within expanded cells increased significantly. Interferon-gamma was shown to synergize with the 5-factor combination, whereas the addition of GM-CSF significantly decreased the number of total clonogenic progenitor cells. Large-scale expansion of PB CD34+ cells (starting cell number, 1.5 x 10(6) CD34+ cells)

in autologous plasma supplemented with the same 5-factor combination resulted in an equivalent expansion of progenitor cells as compared with the microculture system. In summary, our data indicate that chemotherapy plus G-CSF-mobilized PBPCs from cancer patients can be effectively expanded ex vivo. Moreover, our data suggest the feasibility of large-scale expansion of PBPCs, starting from small numbers of PB CD34+ cells. The number of cells expanded ex vivo might be sufficient for repetitive use after high-dose chemotherapy and might be candidate cells for therapeutic gene transfer.

Bruns, I., A. Czibere, et al. (2009). "The hematopoietic stem cell in chronic phase CML is characterized by a transcriptional profile resembling normal myeloid progenitor cells and reflecting loss of quiescence." Leukemia **23**(5): 892-9.

We found that composition of cell subsets within the CD34+ cell population is markedly altered in chronic phase (CP) chronic myeloid leukemia (CML). Specifically, proportions and absolute cell counts of common myeloid progenitors (CMP) and megakaryocyte-erythrocyte progenitors (MEP) are significantly greater in comparison to normal bone marrow whereas absolute numbers of hematopoietic stem cells (HSC) are equal. To understand the basis for this, we performed gene expression profiling (Affymetrix HU-133A 2.0) of the distinct CD34+ cell subsets from six patients with CP CML and five healthy donors. Euclidean distance analysis revealed a remarkable transcriptional similarity between the CML patients' HSC and normal progenitors, especially CMP. CP CML HSC were transcriptionally more similar to their progeny than normal HSC to theirs, suggesting a more mature phenotype. Hence, the greatest differences between CP CML patients and normal donors were apparent in HSC including downregulation of genes encoding adhesion molecules, transcription factors, regulators of stem-cell fate and inhibitors of cell proliferation in CP CML. Impaired adhesive and migratory capacities were functionally corroborated by fibronectin detachment analysis and transwell assays, respectively. Based on our findings we propose a loss of quiescence of the CML HSC on detachment from the niche leading to expansion of myeloid progenitors.

Brunt, K. R., S. R. Hall, et al. (2007). "Endothelial progenitor cell and mesenchymal stem cell isolation, characterization, viral transduction." Methods Mol Med **139**: 197-210.

Endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) have emerged as potentially useful substrates for neovascularization and tissue repair and bioengineering. EPCs are a heterogeneous group of endothelial cell precursors originating in the hematopoietic compartment of the bone marrow. MSCs are a rare population of fibroblast-like cells derived from the bone marrow stroma, constituting approximately 0.001-0.01% of the nucleated cells in the marrow. Both cells types have been isolated from the bone marrow. In addition, EPC can be isolated from peripheral blood as well as the spleen, and MSC has also been isolated from peripheral adipose tissue. Several approaches have been used for the isolation of EPC and MSC, including density centrifugation and magnetic bead selection. Phenotypic characterization of both cell types is carried out using immunohistochemical detection and fluorescence-activated cell sorting analysis of cell-surface molecule expression. However, the lack of specific markers for each cell type renders their characterization difficult and ambiguous. In this chapter, we describe the methods that we use routinely for isolation, characterization, and genetic modification of EPC and MSC from human, rabbit, and mouse peripheral blood and bone marrow.

Buhring, H. J., S. Kuci, et al. (2004). "CDCP1 identifies a broad spectrum of normal and malignant stem/progenitor cell subsets of hematopoietic and nonhematopoietic origin." Stem Cells **22**(3): 334-43.

CUB-domain-containing protein 1 (CDCP1) is a novel transmembrane molecule that is expressed in metastatic colon and breast tumors as well as on the surface of hematopoietic stem cells. In this study, we used multiparameter flow cytometry and antibodies against CDCP1 to analyze the expression of CDCP1 on defined hematopoietic cell subsets of different sources. In addition, CDCP1 expression on leukemic blasts and on cells with nonhematopoietic stem/progenitor cell phenotypes was determined. Here we demonstrate that a subset of bone marrow (BM), cord blood (CB), and mobilized peripheral blood (PB) CD34+ cells expressed this marker and that CDCP1 was detected on CD34(+)CD38- BM stem/progenitor cells but not on mature PB cells. Analysis of leukemic blasts from patients with acute lymphoblastic leukemia, acute myeloid leukemia, and chronic myeloid leukemia in blast crisis revealed that CDCP1 is predominantly expressed on CD34(+)CD133+ myeloid leukemic blasts. However, CDCP1 was not strictly correlated with CD34 and/or CD133 expression, suggesting that CDCP1 is a novel marker for leukemia diagnosis. Stimulation of CD34+ BM cells with CDCP1-reactive monoclonal antibody CUB1 resulted in an increased (approximately twofold) formation of erythroid colony-forming units, indicating that CDCP1 plays an important role in early hematopoiesis. Finally, we show that CDCP1 is also

expressed on cells phenotypically identical to mesenchymal stem/progenitor cells (MSCs) and neural progenitor cells (NPCs). In conclusion, CDCP1 is not only a novel marker for immature hematopoietic progenitor cell subsets but also unique in its property to recognize cells with phenotypes reminiscent of MSC and NPC.

Bull, N. D. and P. F. Bartlett (2005). "The adult mouse hippocampal progenitor is neurogenic but not a stem cell." J Neurosci **25**(47): 10815-21.

The aim of this investigation was to characterize the proliferative precursor cells in the adult mouse hippocampal region. Given that a very large number of new hippocampal cells are generated over the lifetime of an animal, it is predicted that a neural stem cell is ultimately responsible for maintaining this genesis. Although it is generally accepted that a proliferative precursor resides within the hippocampus, contradictory reports exist regarding the classification of this cell. Is it a true stem cell or a more limited progenitor? Using a strict functional definition of a neural stem cell and a number of in vitro assays, we report that the resident hippocampal precursor is a progenitor capable of proliferation and multipotential differentiation but is unable to self-renew and thus proliferate indefinitely. Furthermore, the mitogen FGF-2 stimulates proliferation of these cells to a greater extent than epidermal growth factor (EGF). In addition, we found that BDNF was essential for the production of neurons from the hippocampal progenitor cells, being required during proliferation to trigger neuronal fate. In contrast, a bona fide neural stem cell was identified in the lateral wall of the lateral ventricle surrounding the hippocampus. Interestingly, EGF proved to be the stronger mitogenic factor for this cell, which was clearly a different precursor from the resident hippocampal progenitor. These results suggest that the stem cell ultimately responsible for adult hippocampal neurogenesis resides outside the hippocampus, producing progenitor cells that migrate into the neurogenic zones and proliferate to produce new neurons and glia.

Burchfield, J. S. and S. Dimmeler (2008). "Role of paracrine factors in stem and progenitor cell mediated cardiac repair and tissue fibrosis." Fibrogenesis Tissue Repair **1**(1): 4.

A new era has begun in the treatment of ischemic disease and heart failure. With the discovery that stem cells from diverse organs and tissues, including bone marrow, adipose tissue, umbilical cord blood, and vessel wall, have the potential to improve cardiac function beyond that of conventional pharmacological therapy comes a new field of research aiming at understanding the precise mechanisms of stem cell-mediated cardiac repair. Not only will it be important to determine the most efficacious cell population for cardiac repair, but also whether overlapping, common mechanisms exist. Increasing evidence suggests that one mechanism of action by which cells provide tissue protection and repair may involve paracrine factors, including cytokines and growth factors, released from transplanted stem cells into the surrounding tissue. These paracrine factors have the potential to directly modify the healing process in the heart, including neovascularization, cardiac myocyte apoptosis, inflammation, fibrosis, contractility, bioenergetics, and endogenous repair.

Bussolati, B., C. Grange, et al. (2009). "Endothelial cell differentiation of human breast tumour stem/progenitor cells." J Cell Mol Med **13**(2): 309-319.

Breast tumour stem cells have been reported to differentiate in the epithelial lineage but a cross-lineage potential has not been investigated. We aimed to evaluate whether breast tumour stem cells were able to differentiate also into the endothelial lineage. We isolated and cloned a population of breast tumour stem cells, cultured as mammospheres that expressed the stem markers nestin and Oct-4 and not epithelial and endothelial differentiation markers, and formed serially transplantable tumours in SCID mice. When cultured in the presence of serum, mammosphere-derived clones differentiated in the epithelial lineage. When cultured in the presence of VEGF, the same clones were also able to differentiate in the endothelial lineage acquiring endothelial markers and properties, such as the ability to organize in Matrigel into capillary-like structures. In the transplanted tumours, originated from mammospheres, we demonstrate that some of the intratumour vessels were of human origin, suggesting an in vivo endothelial differentiation of mammosphere-derived cells. Finally, endothelial cell clones originated from mammospheres were able, when implanted in Matrigel in SCID mice, to form after 7 days a human vessel network and, after 3-4 weeks, an epithelial tumour suggesting that in the endothelial-differentiated cells a tumourigenic stem cell population is maintained. In conclusion, the results of the present study demonstrate that stem cells of breast cancer have the ability to differentiate not only in epithelial but also in endothelial lineage, further supporting the hypothesis that the tumour-initiating population possesses stem cell characteristics relevant for tumour growth and vascularization.

Cairo, M. S., P. Law, et al. (1992). "The in vitro effects of stem cell factor and PIXY321 on myeloid

progenitor formation (CFU-GM) from immunomagnetic separated CD34+ cord blood." Pediatr Res **32**(3): 277-81.

Two novel cytokines, stem cell factor (SCF) and PIXY321 (a fusion protein, granulocyte macrophage colony-stimulating factor+IL-3), have recently been demonstrated to enhance in vitro adult myelopoiesis. In this study, we compared the success of separating very early hematopoietic progenitor cells (CD34+) from both cord blood (CB) and adult bone marrow (ABM) and their differential response to SCF, PIXY321, and other later-acting colony-stimulating factors (CSF). Briefly, CD34+ cells were isolated from CB and ABM with an anti-CD34 MAb, HPCA-1, and incubated with various combinations of SCF, PIXY321, and other CSF. The percentage of CD34+ cells was decreased in CB compared to ABM before separation (0.54 versus 1.71%) ($p = 0.05$). Isolated CD34+ cells from CB and ABM were similar in lineage with respect to CD38, HLA-DR, CD33, and CD5, but decreased in CB with respect to B-lineage expression (CD19, CD10, and CD22) ($p = 0.05$). SCF increased colony forming unit-granulocyte-macrophage (CFU-GM) formation from CB CD34+ cells compared to unconditioned media and had a significant additive increase with IL-3 ($p = 0.006$) and granulocyte colony-stimulating factor ($p = 0.03$). SCF also had an additive increase in CB CFU-GM formation with PIXY321 ($p = 0.007$). PIXY321 had a similar increase in CFU-GM formation from both CB and ABM CD34+ cells compared to the combination granulocyte macrophage colony-stimulating factor + IL-3. When SCF was added to IL-3, PIXY321, or PIXY321 + IL-6, there was an increase in CFU-GM from CB versus ABM CD34+ cells.(ABSTRACT TRUNCATED AT 250 WORDS)

Campbell, T. B., S. Basu, et al. (2009). "Overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation." Blood **114**(16): 3392-401.

Molecular mechanisms preserving hematopoietic stem cell (HSC) self-renewal by maintaining a balance between proliferation, differentiation, and other processes are not fully understood. Hyperactivation of the mammalian target of rapamycin (mTOR) pathway, causing sustained proliferative signals, can lead to exhaustion of HSC repopulating ability. We examined the role of the novel ras gene Rheb2, an activator of the mTOR kinase, in colony-forming ability, survival, and repopulation of immature mouse hematopoietic cells. In a cell line model of mouse hematopoietic progenitor cells (HPCs), we found enhanced proliferation and mTOR signaling in cells overexpressing Rheb2. In addition, overexpression of Rheb2 enhanced colony-forming ability and survival of primary mouse bone marrow HPCs. Expansion of phenotypic HSCs in vitro was enhanced by Rheb2 overexpression. Consistent with these findings, Rheb2 overexpression transiently expanded phenotypically defined immature hematopoietic cells after in vivo transplantation; however, these Rheb2-transduced cells were significantly impaired in overall repopulation of primary and secondary congenic transplantation recipients. Our findings suggest that HPCs and HSCs behave differently in response to growth-promoting signals stimulated by Rheb2. These results may have value in elucidating mechanisms controlling the balance between proliferation and repopulating ability, a finding of importance in clinical uses of HPCs/HSCs.

Capuco, A. V., C. M. Evock-Clover, et al. (2009). "In vivo expansion of the mammary stem/ progenitor cell population by xanthosine infusion." Exp Biol Med (Maywood) **234**(4): 475-82.

Mammary stem cells provide for growth and maintenance of the mammary gland and are therefore of considerable interest as determinants of productivity and efficiency of dairy animals and as targets of carcinogenesis in humans. Xanthosine treatment was previously shown to promote expansion of hepatic stem cells in vitro. The objective of this study was to determine if in vivo treatment with xanthosine can increase the mammary stem cell population. Xanthosine was infused into the right mammary glands of four female Holstein calves for 5 consecutive days. Immediately after each xanthosine treatment, calves were injected intravenously with 5-bromo-2-deoxyuridine (BrdU). Forty days after the final treatment, calves were euthanized and mammary tissue harvested. BrdU-label retaining epithelial cells (LREC) were detected immunohistochemically and quantified. Retention of BrdU was used as a marker for putative bovine mammary stem cells. Infusion of xanthosine into the bovine mammary gland significantly increased the number of LREC in treated glands compared to contralateral control glands ($P < 0.05$). LREC averaged 0.4% of epithelial cells in control glands and 0.8% in xanthosine-treated glands. The increase in LREC in xanthosine-treated glands was supported by a concomitant increase in telomerase activity ($P < 0.01$) and a correlation between LREC and telomerase ($P < 0.05$; $r(2) = 0.7$). Data indicate that in vivo treatment with xanthosine can be used to increase the number of mammary stem cells. This is the first demonstration of an in vivo treatment to increase the endogenous population of mammary stem cells, with utility for biomedical research and dairy management.

Care, A., M. Valtieri, et al. (1999). "Enforced expression of HOXB7 promotes hematopoietic stem cell proliferation and myeloid-restricted progenitor differentiation." Oncogene **18**(11): 1993-2001.

Hematopoietic progenitor/stem cells (HPCs/HSCs) purified from human adult peripheral blood (PB) were triggered into cycling, retrovirally transduced with HOXB7 and then functionally assayed in vitro. HPCs were assayed in multi- and unilineage differentiation cultures in either liquid phase or semisolid medium, primitive HPCs in the high proliferative potential colony-forming cell (HPP-CFC) evaluation system and putative HSCs in Dexter type long-term culture (LTC) as LTC initiating cells (LTC-ICs). Control experiments ensured that the exogenous HOXB7 gene was constantly expressed, while the endogenous one was barely or not transcribed. Enforced expression of the gene markedly modulated the proliferation/differentiation program of the entire HSC/HPC population. Enforced HOXB7 expression exerted a potent stimulatory effect on the proliferation of the primitive HPC and putative HSC subsets, assayed as HPP-CFCs and LTC-ICs respectively. While not modifying the total number of HPCs, exogenous HOXB7 induced an increase of the number of granulo-monocytic (GM) HPCs [colony-forming unit GM (CFU-GM) CFU-GM, CFU-G and CFU-M, as evaluated by clonogenic assays] and markedly amplified the progeny of both CFU-G and CFU-M, which showed a sustained proliferation through at least 1-2 months (as evaluated in liquid suspension culture). The prolonged proliferative stimulus induced by HOXB7 transfer into LTC, primitive and GM oriented HPC culture was characterized by persistent proliferation of a discrete population of blast cells and a large pool of differentiated myeloid precursors. Altogether, these results suggest the hypothesis that the proliferative stimulus exerted by exogenous HOXB7 in primitive and GM-oriented HPCs may represent a preleukemic immortalization step. Consistent with the functional role of HOXB7 in the initial ontogenetic phase, these studies indicate that ectopic HOXB7 expression in early HPCs and HSCs from adult PB stimulates their self renewal, sustained proliferation and myeloid differentiation.

Carpentino, J. E., N. W. Hartman, et al. (2008). "Region-specific differentiation of embryonic stem cell-derived neural progenitor transplants into the adult mouse hippocampus following seizures." J Neurosci Res **86**(3): 512-24.

Embryonic stem (ES) cells can generate neural progenitors and neurons in vitro and incorporate into the adult central nervous system (CNS) following transplantation, suggesting their therapeutic potential for treating neurological disorders. However, our understanding of the conditions that direct ES-derived neural progenitor (ESNP) migration and differentiation within different regions of the adult CNS is incomplete. Rodents treated with the chemoconvulsant kainic acid (KA) experience seizures and display hippocampal sclerosis, as well as enhanced hippocampal neurogenesis, similar to pathological findings in patients with temporal lobe epilepsy (TLE). To examine the potential for ESNPs to incorporate into the adult hippocampus and differentiate into hippocampal neurons or glia following seizure-induced damage, we compared the fates of ESNPs after they were transplanted into the CA3 region or fimbria 1 week following KA-induced seizures. After 4-8 weeks, ESNPs grafted into the CA3 region had migrated to the dentate gyrus (DG), where a small subset adopted neural stem cell fates and continued to proliferate, based on bromodeoxyuridine uptake. Others differentiated into neuroblasts or dentate granule neurons. In contrast, most ESNPs transplanted into the fimbria migrated extensively along existing fiber tracts and differentiated into oligodendrocytes or astrocytes. Hippocampal grafts in mice not subjected to seizures displayed a marked tendency to form tumors, and this effect was more pronounced in the DG than in the fimbria. Taken together, these data suggest that seizures induce molecular changes in the CA3 region and DG that promote region-specific neural differentiation and suppress tumor formation.

Cartron, G., O. Herault, et al. (2002). "Quantitative and qualitative analysis of the human primitive progenitor cell compartment after autologous stem cell transplantation." J Hematother Stem Cell Res **11**(2): 359-68.

The aim of this study was to examine whether the severe prolonged deficiency in marrow clonogenic progenitor cells reported after autologous stem cell transplantation (ASCT) is associated with impairment of the primitive progenitor cell compartment. We performed Dexter-type marrow cultures and limiting dilution assays with CD34(+) cells from patients 1 year and/or later after autografting with peripheral blood stem cells for non-Hodgkin's lymphoma (NHL). Flow cytometric analysis was used to assess the CD38 antigen expression and apoptotic state (7-ADD(-)/annexin-V(+) cells) of the CD34(+) cell population. We found a dramatic decrease in both clonogenic progenitor cell production and frequency of long term culture-initiating cells (LTC-IC) in all the patients tested at 1 year, even in those displaying normal progenitor cell frequency. Surprisingly, the clonogenic capacity of each LTC-IC was not increased. Flow cytometric

analysis of the CD34(+) cell population confirmed this quantitative defect, with a reduction in the CD38(dim/neg) cell population but no increase in apoptosis. This defect did not improve over time up to 4 years after transplantation. In addition, qualitative abnormalities were revealed, demonstrated by decreased CD34 antigen expression, together with impaired differentiating properties of LTC-IC toward erythroid lineage at 1 year. This study indicates that both quantitative and qualitative abnormalities of the primitive progenitor cell compartment are a constant feature up to 4 years after autologous stem cell transplantation.

Cesana, C., C. Carlo-Stella, et al. (1997). "In vitro growth of mobilized peripheral blood progenitor cells is significantly enhanced by stem cell factor." Stem Cells **15**(3): 207-13.

The existence of primitive hematopoietic progenitors in mobilized peripheral blood is suggested by clinical, phenotypic and in vitro cell culture evidences. In order to quantify primitive progenitors, 32 leukaphereses from 15 patients with lymphoid malignancies were investigated for the growth of multilineage colony-forming units (CFU-Mix), erythroid burst-forming units (BFU-E) and granulocyte-macrophage colony-forming units (CFU-GM) in the absence or presence of recombinant stem cell factor (SCF), a cytokine which selectively controls stem cell self-renewal, proliferation and differentiation. Primitive progenitors were also quantitated by means of a long-term assay which allows the growth of cells capable of initiating and sustaining hematopoiesis in long-term culture (LTC-IC). Addition of SCF (50 ng/ml) to methyl-cellulose cultures stimulated with maximal concentrations of G-CSF, GM-CSF, interleukin 3 and erythropoietin significantly increased the growth (mean +/- SE) of CFU-Mix (7.7 +/- 1.7 versus 2.4 +/- 0.6, p < or = 0.0001), BFU-E (47 +/- 10 versus 32 +/- 6, p < or = 0.002) and CFU-GM (173 +/- 31 versus 112 +/- 20, p < or = 0.0001). Mean (+/- SE) percentages of SCF-dependent CFU-Mix, BFU-E and CFU-GM were 60 +/- 5%, 19 +/- 5%, and 33 +/- 4%, respectively. Mean (+/- SE) LTC-IC growth per 2 x 10(6) nucleated cells was 221 +/- 53 (range, 2 to 704). Linear regression analysis demonstrated a statistically significant correlation (r = .87; p < or = 0.0001) between LTC-IC and SCF-dependent progenitors. In conclusion, our data suggest that: A) the optimal quantification of mobilized progenitors requires supplementation of methylcellulose cultures with SCF, and B) in vitro detection of SCF-dependent progenitors might represent a reliable and technically simple method to assess the primitive progenitor cell content of blood cell autografts. Such in vitro evaluation of immature hematopoietic progenitors might be clinically relevant for predicting the reconstituting potential of autografts.

Chan, K. M., S. Bonde, et al. (2008). "Hematopoiesis and immunity of HOXB4-transduced embryonic stem cell-derived hematopoietic progenitor cells." Blood **111**(6): 2953-61.

The ability of embryonic stem (ES) cells to form cells and tissues from all 3 germ layers can be exploited to generate cells that can be used to treat diseases. In particular, successful generation of hematopoietic cells from ES cells could provide safer and less immunogenic cells than bone marrow cells, which require severe host preconditioning when transplanted across major histocompatibility complex barriers. Here, we exploited the self-renewal properties of ectopically expressed HOXB4, a homeobox transcription factor, to generate hematopoietic progenitor cells (HPCs) that successfully induce high-level mixed chimerism and long-term engraftment in recipient mice. The HPCs partially restored splenic architecture in Rag2(-/-)gamma(c)(-/-)-immunodeficient mice. In addition, HPC-derived newly generated T cells were able to mount a peptide-specific response to lymphocytic choriomeningitis virus and specifically secreted interleukin-2 and interferon-gamma upon CD3 stimulation. In addition, HPC-derived antigen presenting cells in chimeric mice efficiently presented viral antigen to wild-type T cells. These results demonstrate for the first time that leukocytes derived from ES cells ectopically expressing HOXB4 are immunologically functional, opening up new opportunities for the use of ES cell-derived HPCs in the treatment of hematologic and immunologic diseases.

Chaussain, C., A. S. Eapen, et al. (2009). "MMP2-cleavage of DMP1 generates a bioactive peptide promoting differentiation of dental pulp stem/progenitor cell." Eur Cell Mater **18**: 84-95.

Dentin Matrix Protein 1 (DMP1) plays a regulatory role in dentin mineralization and can also function as a signaling molecule. MMP-2 (matrix metalloproteinase-2) is a predominant protease in the dentin matrix that plays a prominent role in tooth formation and a potential role during the carious process. The possibility that MMP-2 can cleave DMP1 to release biologically active peptides was investigated in this study. DMP1, both in the recombinant form and in its native state within the dentin matrix, was shown to be a substrate for MMP-2. Proteolytic processing of DMP1 by MMP-2 produced two major peptides, one that contains the C-terminal region of the protein known to carry both the

ASARM (aspartic acid and serine rich domain) domain involved in biomineralization and the DNA binding site of DMP1. In vitro experiments with recombinant N- and C-terminal polypeptides mimicking the MMP-2 cleavage products of DMP1 demonstrated an effect of the C-polypeptide on the differentiation of dental pulp stem/progenitor cells to a putative odontoblast phenotype. In vivo implantation of this peptide in a rat injured pulp model induced a rapid formation of a homogeneous dentin bridge covered by a palisade of orientated cells expressing dentin sialoprotein (DSP) and DMP1, attesting an efficient repair process. These data suggest that a peptide generated through the proteolytic processing of DMP1 by MMP-2 can regulate the differentiation of mesenchymal cells during dentinogenesis and thus sustain reparative dentin formation in pathological situations such as carious decay. In addition, these data open a new therapeutic possibility of using this peptide to regenerate dentin after an injury.

Chen, J., N. Hersmus, et al. (2005). "The adult pituitary contains a cell population displaying stem/progenitor cell and early embryonic characteristics." Endocrinology **146**(9): 3985-98.

A side population (SP) has been identified in a number of tissues, where it typically represents a small population enriched in stem/progenitor cells. In this study we show that the adult mouse anterior pituitary (AP) also contains a characteristic SP displaying verapamil-sensitive Hoechst dye efflux capacity. A majority of the SP cells express stem cell antigen 1 at a high level (Sca1high). Using (semi)quantitative RT-PCR and immunofluorescence, we characterized the Sca1high SP as a population enriched in cells expressing stem/progenitor cell-associated factors and components of the Notch, Wnt, and sonic hedgehog signaling pathways, functional in stem cell homeostasis as well as in early pituitary embryogenesis. Lhx4, a transcription factor pivotal for early embryonic development of the AP, was only detected in the Sca1high SP, whereas Lhx3, in contrast to Lhx4 not down-regulated after AP development, was only found in the main population. The Sca1high SP was depleted from cells expressing phenotypic markers of differentiated AP cells (hormones), but contained a small proportion of folliculo-stellate cells. Stem cells of many tissues can clonally expand to nonadherent spheres in culture. Clonal spheres also developed in AP cell cultures. Spheres showed an expression pattern resembling that of Sca1high SP cells. Moreover, the sphere-initiating cells of the pituitary segregated to the SP and not to the main population. In conclusion, we show that the adult pituitary contains a hitherto undescribed population of cells with SP phenotype and clonal expansion capacity. These cells express (signaling) molecules generally found in stem/progenitor cells and/or operative during pituitary early embryonic development. These characteristics are supportive of a stem/progenitor cell phenotype.

Cheng, T. and D. T. Scadden (2002). "Cell cycle entry of hematopoietic stem and progenitor cells controlled by distinct cyclin-dependent kinase inhibitors." Int J Hematol **75**(5): 460-5.

The therapeutic promise of hematopoietic stem cells in medicine has been expanded as broader differentiation potential of the cells has gained experimental support. However, hurdles for stem cell manipulation in vitro and tissue regeneration in vivo remain because of lack of the molecular biology of the stem cells. In particular, elucidating the molecular control of cell cycle entry is necessary for rational stem cell expansion strategies. Understanding how the stem and progenitor cell populations are controlled by negative regulators of cell cycle entry may provide one basis for manipulating these cells. In this mini-review, we focus on the rationale of targeting the cyclin-dependent kinase inhibitors (CKIs) in stem cell biology. Two CKI members, p21(Cip1/Waf1) (p21) and p27kip1 (p27), have been shown to govern the pool sizes of hematopoietic stem and progenitor cells, respectively. Of note, their inhibitory roles in primitive hematopoietic cells are distinct from the action of the inhibitory cytokine, transforming growth factor-beta1 (TGF-beta1). Therefore, the distinct roles of p21, p27, and TGF-beta1 in hematopoietic cells offer attractive targets for specific manipulation of the stem or progenitor cell populations in therapeutic strategies.

Christophersen, N. S., X. Meijer, et al. (2006). "Induction of dopaminergic neurons from growth factor expanded neural stem/progenitor cell cultures derived from human first trimester forebrain." Brain Res Bull **70**(4-6): 457-66.

Multipotent stem/progenitor cells derived from human first trimester forebrain can be expanded as free-floating aggregates, so called neurospheres. These cells can differentiate into neurons, astrocytes and oligodendrocytes. In vitro differentiation protocols normally yield gamma-aminobutyric acid-immunoreactive neurons, whereas only few tyrosine hydroxylase (TH) expressing neurons are found. The present report describes conditions under which 4-10% of the cells in the culture become TH immunoreactive (ir) neurons within 24h. Factors including acidic fibroblast growth factor (aFGF) in combination with agents that increase intracellular cyclic AMP and activate protein kinase C, in addition to a substrate that promotes neuronal differentiation

appear critical for efficient TH induction. The cells remain THir after trypsinization and replating, even when their subsequent culturing takes place in the absence of inducing factors. Consistent with a dopaminergic phenotype, mRNAs encoding aromatic acid decarboxylase, but not dopamine-beta-hydroxylase were detected by quantitative real time RT-PCR. Ten weeks after the cells had been grafted into the striatum of adult rats with unilateral nigrostriatal lesions, only very few of the surviving human neurons expressed TH. Our data suggest that a significant proportion of expandable human neural progenitors can differentiate into TH-expressing cells in vitro and that they could be useful for drug and gene discovery. Additional experiments, however, are required to improve the survival and phenotypic stability of these cells before they can be considered useful for cell replacement therapy in Parkinson's disease.

Chung, I. J., C. Dai, et al. (2003). "Stem cell factor increases the expression of FLIP that inhibits IFNgamma -induced apoptosis in human erythroid progenitor cells." Blood **101**(4): 1324-8.

Interferon gamma (IFNgamma) acts on human erythroid colony-forming cells (ECFCs) to up-regulate Fas, without a demonstrable change of Fas ligand (FasL) or Fas-associated DD-containing protein (FADD) expression and activates caspase-8 plus caspase-3, which produce apoptosis. Our previous data showed that stem cell factor (SCF) reduced the inhibitory effect of IFNgamma on human ECFCs when both factors were present in the cultures. However, the mechanism by which SCF prevents IFNgamma-induced apoptosis in ECFCs is unclear. In this study we used highly purified human ECFCs to investigate the mechanism of the effect of SCF on IFNgamma-induced apoptosis. Because the binding of FasL to Fas is the first step of the apoptosis cascade and IFNgamma strongly up-regulates Fas expression, we added FasL (50 ng/mL) to the cultures with IFNgamma to accentuate the IFNgamma-induced activation of caspase-8 and caspase-3 plus subsequent apoptosis. SCF (100 ng/mL) clearly inhibited the activation of caspase-8 and caspase-3 induced by IFNgamma and/or FasL, and it also reduced apoptosis as measured by the terminal dUTP nick-end labeling (TUNEL) assay. SCF did not decrease the surface expression of Fas on the ECFCs. FADD-like interleukin 1 beta (IL-1beta)-converting enzyme (FLICE)-inhibitory protein (FLIP) has been reported to interact with FADD and/or caspase-8 at the death-inducing signaling complex (DISC) level following Fas stimulation and acts as a dominant-negative caspase-8. SCF increased FLIP mRNA and protein expression, concomitant with reduced apoptosis, whereas IFNgamma and/or FasL did not change FLIP expression. Reduction of FLIP expression with antisense oligonucleotides decreased the capacity of SCF to inhibit IFNgamma-induced apoptosis, demonstrating a definite role for FLIP in the SCF-induced protection of ECFCs from IFNgamma-initiated apoptosis.

Cicuttini, F. M., C. G. Begley, et al. (1992). "The effect of recombinant stem cell factor (SCF) on purified CD34-positive human umbilical cord blood progenitor cells." Growth Factors **6**(1): 31-9.

We describe the effect of soluble c-kit ligand (stem cell factor, SCF) on highly purified CD34-positive hemopoietic progenitors from human umbilical cord blood. Progenitor cells were purified from cord blood mononuclear cells by immune rosetting with lineage specific antibodies and subsequent sorting of the rosette-negative population for CD34(BI3C5)-positive cells. This procedure enriched greater than 100-fold for colony forming cells (CFC). Using optimal concentrations of colony-stimulating factors (CSF) without added SCF approximately 2.5% of cells formed colonies. SCF also had CSF activity on this population, up to 0.5% of cells forming small colonies in response to SCF alone. In contrast, the addition of SCF to optimal concentrations of the other growth factors produced a greater than 10-fold increase in colony number. However, the most notable effect was an approximately 100-fold increase in the number of cells in each colony. Equally striking was the very high proportion (50-80%) of mixed colonies (CFU-MIX). These findings suggest the progenitor cell pool in cord blood is skewed towards very early cells. However, when day 14 colonies formed in response to SCF and other factors were assessed for their re-cloning potential they did not contain significant numbers of CFC, implying that SCF did not support the self-renewal of these CD34 positive cord blood progenitor cells. These findings support a role for SCF as an enhancing factor for hemopoietic progenitor cells but it does not promote self-renewal in these populations.

Clore, J. N., A. R. Sharpe, et al. (1988). "Thyrotropin-induced hyperthyroidism: evidence for a common progenitor stem cell." Am J Med Sci **295**(1): 3-5.

A 36-year-old woman with hyperthyroidism, elevated blood thyroid-stimulating hormone (TSH) and alpha-subunit levels, amenorrhea, hyperprolactinemia and no evidence of acromegaly, was found to have a pituitary adenoma containing TSH, alpha-subunit and growth hormone by immunohistochemistry. Preoperative testing revealed elevated TSH and alpha-subunit with no response to

thyrotropin-releasing hormone (TRH) but a normal response in prolactin to TRH. Culture of the pituitary cells showed release of TSH, alpha-subunit and prolactin. In vitro, TRH failed to cause TSH discharge; however, it increased prolactin concentrations in the culture medium. Triiodothyronine, added to the pituitary cell culture, resulted in no inhibition of TSH and prolactin discharge. By electron microscopy, the adenoma cells showed features of thyrotrophs. However, some adenoma cells contained fibrous bodies characteristic of some growth hormone cell tumors and acidophil stem cell adenomas, suggesting that the adenoma originated in a common progenitor cell.

Coles, B. L., D. J. Horsford, et al. (2006). "Loss of retinal progenitor cells leads to an increase in the retinal stem cell population in vivo." Eur J Neurosci **23**(1): 75-82.

Retinal stem cells [with the potential to produce either neural retinal progenitors or retinal pigment epithelial (RPE) progenitors] exist in the mammalian eye throughout life, and indeed the greatest absolute increase in the stem population occurs postnatally. The stem cells proliferate embryonically and thus may help to build the retina initially, but in postnatal mammals they clearly do not proliferate to regenerate the retina in response to injury. Using Chx10(orJ/orJ) and Mitf(mi/mi) mice, with small eye phenotypes due to the reduction of the neural retinal progenitor population and the retinal pigmented epithelial progenitor population, respectively, we now report that the retinal stem cell population, when assayed from the ciliary margin, increases 3-8-fold in both mutants. These findings suggest that the mammalian retinal stem cell population may be capable of responding to genetically induced signals from the progenitor populations.

Collis, S. J., S. Neutzel, et al. (2004). "Hematopoietic progenitor stem cell homing in mice lethally irradiated with ionizing radiation at differing dose rates." Radiat Res **162**(1): 48-55.

It has recently been shown that specific lineage-depleted murine hematopoietic stem cells that home to the bone marrow 2 days after transplantation of ablated primary recipients are capable of long-term engraftment and repopulation of secondary recipients. We were interested in determining whether the rate at which the ablating radiation dose was delivered to the mice affected the homing of lineage-depleted stem cells to the bone marrow and/or sites of tissue damage. Fractionated, lineage-depleted donor marrow cells were isolated and labeled with the membrane dye PKH26. Recipient mice were lethally irradiated with 11 Gy ionizing radiation using varying dose rates and were immediately injected with PKH26-labeled progenitor stem cells. With the exception of the lowest dose-rate group, all irradiated mice had an approximately fivefold (P = 0.014 to 0.025) reduction in stem cell homing to the bone marrow compared to unirradiated control animals. A fivefold reduction of stem cell homing to the spleen compared to unirradiated animals was also observed, though this was not statistically significant for any dose-rate group (P = 0.072 to 0.233). This difference in homing could not be explained by increased stem cell apoptosis/necrosis or non-marrow tissue homing to the intestine, lung or liver. We show that the dose rate at which a lethal dose of total-body radiation is delivered does not augment hematopoietic progenitor stem cell homing to the bone marrow, spleen or sites of early radiation-mediated tissue damage at either 2 or 5 days postirradiation/transplantation. The observation that greater homing was seen in unirradiated control mice calls into question the concept that adequate bone marrow stem cell homing requires radiation-induced "space" to be made in the marrow, certainly for the enriched early progenitor hematopoietic stem cells used for this set of experiments. Further experiments will be needed to determine whether these homed cells are as capable of giving rise to long-term engraftment/repopulation of the marrow of secondary recipients as they are in irradiated recipients.

Colvin, G. A., J. F. Lambert, et al. (2004). "Intrinsic hematopoietic stem cell/progenitor plasticity: Inversions." J Cell Physiol **199**(1): 20-31.

Traditional concepts indicate that stem cells give rise to progenitor cells in a hierarchical system. We studied murine engraftable stem cells (ESCs) and progenitors in in vitro and found that ESC and progenitors exist in a reversible continuum, rather then a hierarchy. B6.SJL and BALB/c marrow cells were serially cultured with thrombopoietin (TPO), FLT-3 ligand (FLT-3L), and steel factor through cell cycle. Progenitors (high-proliferative potential colony-forming cells (HPP-CFC) and colony-forming unit culture (CFU-c)) and ESC capacity was determined. The cell cycle status of purified lineage(negative)rhodamine(low)Hoechst(low) stem cells was determined under the same conditions using tritiated thymidine incorporation and cell counts. We found an inverse relationship between progenitors and ESC, which occurred during the first cell cycle transit and was reversible. We have termed these progenitor/stem cell inversions and found that these inversions were consistently seen at 28-32 h of culture, representing early S-phase. We observed 13 major reversible increases in progenitor numbers from one time-point to another during the first cell cycle

transit; this was coupled with 11 major ESC decreases and in 2 instances ESC were at baseline. These studies indicate that primitive marrow cells reversibly shift from ESC to progenitors without differentiation occurring. They exist as a fluctuating continuum.

Corrales, C. E., L. Pan, et al. (2006). "Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti." J Neurobiol **66**(13): 1489-500.

Hearing loss in mammals is irreversible because cochlear neurons and hair cells do not regenerate. To determine whether we could replace neurons lost to primary neuronal degeneration, we injected EYFP-expressing embryonic stem cell-derived mouse neural progenitor cells into the cochlear nerve trunk in immunosuppressed animals 1 week after destroying the cochlear nerve (spiral ganglion) cells while leaving hair cells intact by ouabain application to the round window at the base of the cochlea in gerbils. At 3 days post transplantation, small grafts were seen that expressed endogenous EYFP and could be immunolabeled for neuron-specific markers. Twelve days after transplantation, the grafts had neurons that extended processes from the nerve core toward the denervated organ of Corti. By 64-98 days, the grafts had sent out abundant processes that occupied a significant portion of the space formerly occupied by the cochlear nerve. The neurites grew in fasciculating bundles projecting through Rosenthal's canal, the former site of spiral ganglion cells, into the osseous spiral lamina and ultimately into the organ of Corti, where they contacted hair cells. Neuronal counts showed a significant increase in neuronal processes near the sensory epithelium, compared to animals that were denervated without subsequent stem cell transplantation. The regeneration of these neurons shows that neurons differentiated from stem cells have the capacity to grow to a specific target in an animal model of neuronal degeneration.

Couillard, M. and M. Trudel (2009). "C-myc as a modulator of renal stem/progenitor cell population." Dev Dyn **238**(2): 405-14.

The role of c-myc has been well-studied in gene regulation and oncogenesis but remains elusive in murine development from midgestation. We determined c-myc function during kidney development, organogenesis, and homeostasis by conditional loss of c-myc induced at two distinct phases of nephrogenesis, embryonic day (e) 11.5 and e17.5. Deletion of c-myc in early metanephric mesenchyme (e11.5) led to renal hypoplasia from e15.5 to e17.5 that was sustained until adulthood (range, 20-25%) and, hence, reproduced the human pathologic condition of renal hypoplasia. This phenotype resulted from depletion of c-myc-positive cells in cap mesenchyme, causing a approximately 35% marked decrease of Six2- and Cited1-stem/progenitor population and of proliferation that likely impaired self-renewal. By contrast, c-myc loss from e17.5 onward had no impact on late renal differentiation/maturation and/or homeostasis, providing evidence that c-myc is dispensable during these phases. This study identified c-myc as a modulator of renal organogenesis through regulation of stem/progenitor cell population.

da Silva, C. L., R. Goncalves, et al. (2009). "Differences amid bone marrow and cord blood hematopoietic stem/progenitor cell division kinetics." J Cell Physiol **220**(1): 102-11.

Human hematopoietic stem/progenitor cells (HSC) isolated based upon specific patterns of CD34 and CD38 expression, despite phenotypically identical, were found to be functionally heterogeneous, raising the possibility that reversible expression of these antigens may occur during cellular activation and/or proliferation. In these studies, we combined PKH67 tracking with CD34/CD38 immunostaining to compare cell division kinetics between human bone marrow (BM) and cord blood (CB)-derived HSC expanded in a serum-free/stromal-based system for 14 days (d), and correlated CD34 and CD38 expression with the cell divisional history. CB cells began dividing 24 h earlier than BM cells, and significantly higher numbers underwent mitosis during the time in culture. By d10, over 55% of the CB-cells reached the ninth generation, whereas BM-cells were mostly distributed between the fifth and seventh generation. By d14, all CB cells had undergone multiple cell divisions, while 0.7-3.8% of BM CD34(+) cells remained quiescent. Furthermore, the percentage of BM cells expressing CD34 decreased from 60.8 +/- 6.3% to 30.6 +/- 6.7% prior to initiating division, suggesting that downmodulation of this antigen occurred before commencement of proliferation. Moreover, with BM, all primitive CD34(+)CD38(-) cells present at the end of culture arose from proliferating CD34(+)CD38(+) cells that downregulated CD38 expression, while in CB, a CD34(+)CD38(-) population was maintained throughout culture. These studies show that BM and CB cells differ significantly in cell division kinetics and expression of CD34 and CD38, and that the inherent modulation of these antigens during ex vivo expansion may lead to erroneous quantification of the stem cell content of the expanded graft.

da Silva, M. G., P. Pimentel, et al. (2004). "Ancestim (recombinant human stem cell factor, SCF) in association with filgrastim does not enhance chemotherapy and/or growth factor-induced peripheral blood progenitor cell (PBPC) mobilization in patients with a prior insufficient PBPC collection." Bone Marrow Transplant **34**(8): 683-91.

Up to a third of autologous transplantation candidates fail to mobilize hematopoietic progenitors into the peripheral blood with chemotherapy and/or growth factor treatment, thus requiring innovative mobilization strategies. In total, 20 cancer patients unable to provide adequate PBPC products after a previous mobilization attempt were treated with ancestim (20 microg/kg/day s.c.) and filgrastim (10 microg/kg/day s.c.). In 16 patients, the pre-study mobilization was with filgrastim alone. Eight patients underwent single large volume leukapheresis (LVL) and 12 multiple standard volume leukaphereses (SVL) in both mobilizations. Pairwise comparison of peripheral blood CD34(+) cell concentrations on the day of first leukapheresis failed to document synergism - median CD34(+)/microl of 3.2 (<0.1 to 15.4) and 4.5 (1-28.56) for the pre-study and on-study mobilizations (P = 0.79, sign test), and 4.2 (<0.1-15.4) and 5 (1-28.56), respectively, for the 16 patients previously mobilized with filgrastim alone (P = 1, sign test). The number of CD34(+) cells/kg collected per unit of blood volume (BV) processed was similar in both mobilizations - median 0.1 x 10(6)/kg/BV and 0.09 x 10(6)/kg/BV, respectively (P = 1, sign test). In this phase II study, the combination of ancestim and filgrastim did not allow adequate PBPC mobilization and collection in patients with a previous suboptimal PBPC collection.

Dahl, L., K. Richter, et al. (2008). "Lhx2 expression promotes self-renewal of a distinct multipotential hematopoietic progenitor cell in embryonic stem cell-derived embryoid bodies." PLoS One **3**(4): e2025.

The molecular mechanisms regulating the expansion of the hematopoietic system including hematopoietic stem cells (HSCs) in the fetal liver during embryonic development are largely unknown. The LIM-homeobox gene Lhx2 is a candidate regulator of fetal hematopoiesis since it is expressed in the fetal liver and Lhx2(-/-) mice die in utero due to severe anemia. Moreover, expression of Lhx2 in embryonic stem (ES) cell-derived embryoid bodies (EBs) can lead to the generation of HSC-like cell lines. To further define the role of this transcription factor in hematopoietic regulation, we generated ES cell lines that enabled tet-inducible expression of Lhx2. Using this approach we observed that Lhx2 expression synergises with specific signalling pathways, resulting in increased frequency of colony forming cells in developing EB cells. The increase in growth factor-responsive progenitor cells directly correlates to the efficiency in generating HSC-like cell lines, suggesting that Lhx2 expression induce self-renewal of a distinct multipotential hematopoietic progenitor cell in EBs. Signalling via the c-kit tyrosine kinase receptor and the gp130 signal transducer by IL-6 is necessary and sufficient for the Lhx2 induced self-renewal. While inducing self-renewal of multipotential progenitor cells, expression of Lhx2 inhibited proliferation of primitive erythroid precursor cells and interfered with early ES cell commitment, indicating striking lineage specificity of this effect.

Dai, C. H., S. B. Krantz, et al. (1994). "Polycythaemia vera. IV. Specific binding of stem cell factor to normal and polycythaemia vera highly purified erythroid progenitor cells." Br J Haematol **88**(3): 497-505.

Polycythaemia vera (PV) patients' blood burst-forming units-erythroid (BFU-E) have an enhanced sensitivity to stem cell factor (SCF) compared to normal BFU-E. To characterize SCF receptors on erythroid progenitors from normal individuals and PV patients, we performed binding experiments using radioiodinated recombinant SCF (rSCF), day 1 BFU-E and day 8 erythroid colony-forming cells (ECFC), which are mostly colony-forming units-erythroid (CFU-E). 125I-rSCF binds to a single class of cell surface receptors (23,000/ECFC) at 0 degrees C with a high-binding affinity (Kd = 17 pM). Saturation occurred at 0.5 nM (10 ng/ml) which produces a nearly maximum biological effect. One half of the radiolabelled rSCF was internalized by the cells after 30 min at 37 degrees C. No significant differences in the receptor number, dissociation constant, or internalization rate were found between normal and PV ECFC. Autoradiographic analysis of 125I-rSCF binding to normal BFU-E and ECFC showed that no differences were present in either the percentage of positive cells or the number of radioactive grains/cell between the normal and PV erythroid progenitors. The enhanced sensitivity of PV BFU-E and CFU-E to SCF does not appear to be related to changes in SCF receptor number, binding affinity or internalization and the hypersensitivity of PV erythroid progenitors to SCF must reside in a further internal cellular abnormality.

D'Ascenzo, M., R. Piacentini, et al. (2006). "Role of L-type Ca2+ channels in neural stem/progenitor cell differentiation." Eur J Neurosci **23**(4): 935-44.

Ca(2+) influx through voltage-gated Ca(2+) channels, especially the L-type (Ca(v)1), activates downstream signaling to the nucleus that affects gene expression and, consequently, cell fate. We

hypothesized that these Ca(2+) signals may also influence the neuronal differentiation of neural stem/progenitor cells (NSCs) derived from the brain cortex of postnatal mice. We first studied Ca(2+) transients induced by membrane depolarization in Fluo 4-AM-loaded NSCs using confocal microscopy. Undifferentiated cells (nestin(+)) exhibited no detectable Ca(2+) signals whereas, during 12 days of fetal bovine serum-induced differentiation, neurons (beta-III-tubulin(+)/MAP2(+)) displayed time-dependent increases in intracellular Ca(2+) transients, with DeltaF/F ratios ranging from 0.4 on day 3 to 3.3 on day 12. Patch-clamp experiments revealed similar correlation between NSC differentiation and macroscopic Ba(2+) current density. These currents were markedly reduced (-77%) by Ca(v)1 channel blockade with 5 microm nifedipine. To determine the influence of Ca(v)1-mediated Ca(2+) influx on NSC differentiation, cells were cultured in differentiative medium with either nifedipine (5 microm) or the L-channel activator Bay K 8644 (10 microm). The latter treatment significantly increased the percentage of beta-III-tubulin(+)/MAP2(+) cells whereas nifedipine produced opposite effects. Pretreatment with nifedipine also inhibited the functional maturation of neurons, which responded to membrane depolarization with weak Ca(2+) signals. Conversely, Bay K 8644 pretreatment significantly enhanced the percentage of responsive cells and the amplitudes of Ca(2+) transients. These data suggest that NSC differentiation is strongly correlated with the expression of voltage-gated Ca(2+) channels, especially the Ca(v)1, and that Ca(2+) influx through these channels plays a key role in promoting neuronal differentiation.

David, R., H. Theiss, et al. (2008). "Connexin 40 promoter-based enrichment of embryonic stem cell-derived cardiovascular progenitor cells." Cells Tissues Organs **188**(1-2): 62-9.

BACKGROUND: Pluripotent embryonic stem (ES) cells that can differentiate into functional cardiomyocytes as well as vascular cells in cell culture may open the door to cardiovascular cell transplantation. However, the percentage of ES cells in embryoid bodies (EBs) which spontaneously undergo cardiovascular differentiation is low (<10%), making strategies for their specific labeling and purification indispensable. METHODS: The human connexin 40 (Cx40) promoter was isolated and cloned in the vector pEGFP. The specificity of the construct was initially assessed in Xenopus embryos injected with Cx40-EGFP plasmid DNA. Stable Cx40-EGFP ES cell clones were differentiated and fluorescent cells were enriched manually as well as via fluorescence-activated cell sorting. Characterization of these cells was performed with respect to spontaneous beating as well as via RT-PCRs and immunofluorescent stainings. RESULTS: Cx40-EGFP reporter plasmid injection led to EGFP fluorescence specifically in the abdominal aorta of frog tadpoles. After crude manual enrichment of highly Cx40-EGFP-positive EBs, the appearance of cardiac and vascular structures was increased approximately 3-fold. Immunofluorescent stainings showed EGFP expression exclusively in vascular-like structures simultaneously expressing von Willebrand factor and in formerly beating areas expressing alpha-actinin. Cx40-EGFP-expressing EBs revealed significantly higher numbers of beating cardiomyocytes and vascular-like structures. Semiquantitative RT-PCRs confirmed an enhanced cardiovascular differentiation as shown for the cardiac markers Nkx2.5 and MLC2v, as well as the endothelial marker vascular endothelial cadherin. CONCLUSIONS: Our work shows the feasibility of specific labeling and purification of cardiovascular progenitor cells from differentiating EBs based on the Cx40 promoter. We provide proof of principle that the deleted CD4 (DeltaCD4) surface marker-based method for magnetic cell sorting developed by our group will be ideally suitable for transference to this promoter.

de Boer, F., A. M. Drager, et al. (2002). "Extensive early apoptosis in frozen-thawed CD34-positive stem cells decreases threshold doses for haematological recovery after autologous peripheral blood progenitor cell transplantation." Bone Marrow Transplant **29**(3): 249-55.

Stem cell doses necessary for engraftment after myelo-ablative therapy as defined for fresh transplants vary largely. Loss of CD34+ cell quality after cryopreservation might contribute to this variation. With a new early apoptosis assay including the vital stain Syto16, together with the permeability marker 7-AAD, CD34+ cell viability in leucapheresis samples of 49 lymphoma patients receiving a BEAM regimen was analysed. After freeze-thawing large numbers of non-viable, early apoptotic cells appeared, leading to only 42% viability compared to 72% using 7-AAD only. Based on this Syto16 staining in the frozen-thawed grafts, threshold numbers for adequate haematological recovery of 2.8-3.0 x 10(6) CD34+ cells/kg body weight determined for fresh grafts, now decreased to 1.2-1.3 x 10(6) CD34+ cells/kg. In whole blood transplantation of lymphoma patients (n = 45) receiving a BEAM-like regimen, low doses of CD34+ cells were sufficient for recovery (0.3-0.4 x 10(6)CD34+ cells/kg). In contrast to freeze-thawing of leucapheresis material, a high viability of CD34+ cells was preserved during storage for 3 days at 4 degrees C, leaving threshold doses for recovery unchanged. In conclusion, the Syto16 assay reveals the presence of

many more non-functional stem cells in frozen-thawed transplants than presumed thus far. This led to a factor 2.3-fold adjustment downward of viable CD34+ threshold doses for haematological recovery.

De Bruyn, C., A. Delforge, et al. (1994). "Modulation of human cord blood progenitor cell growth by recombinant human interleukin 3 (IL-3), IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF) in serum-supplemented and serum-free medium." Stem Cells **12**(6): 616-25.

We evaluated the growth of cord blood myeloid progenitors or colony forming units granulocyte-macrophage (CFU-GM) and their response to various recombinant growth factors or colony stimulating factors (CSFs): interleukin 3 (IL-3), IL-6, granulocyte-macrophage CSF (GM-CSF) and stem cell factor (SCF). Using classical stimulant (human placenta conditioned medium or HPCM), we observed a significantly higher day-14/day-7 CFU-GM ratio in CB than in bone marrow (BM). The association of IL-3, IL-6, GM-CSF and SCF induced significantly more CB day-14 CFU-GM than HPCM. This effect is significantly greater in CB than in bone marrow. Since fetal calf serum (FCS) is known to contain inhibitors, we have compared the ability of CSFs to induce CFU-GM formation in FCS-supplemented and FCS-free culture. In CB, using HPCM, we obtained significantly more CFU-GM in FCS-free medium than in FCS-supplemented medium. This difference was corrected by the addition of anti-transforming growth factor-beta (TGF-beta) neutralizing antibody. However, with the association of the four CSFs, no significant difference between FCS and FCS-free culture was observed. In conclusion: a) day-14/day-7 CFU-GM ratio was higher in CB than in BM indicating that CB CFU-GM are more primitive than BM CFU-GM; b) FCS can be successfully replaced by serum-free medium; c) FCS contains inhibitors of day-14 CFU-GM and among them TGF-beta; and d) the association IL-3, SCF, GM-CSF and IL-6 seems able to totally overcome the inhibitory effect of FCS.

de Kruijf, E. J., M. van Pel, et al. (2007). "Repeated hematopoietic stem and progenitor cell mobilization without depletion of the bone marrow stem and progenitor cell pool in mice after repeated administration of recombinant murine G-CSF." Hum Immunol **68**(5): 368-74.

Administration of recombinant-human G-CSF (rhG-CSF) is highly efficient in mobilizing hematopoietic stem and progenitor cells (HSC/HPC) from the bone marrow (BM) toward the peripheral blood. This study was designed to investigate whether repeated G-CSF-induced HSC/HPC mobilization in mice could lead to a depletion of the bone marrow HSC/HPC pool with subsequent loss of mobilizing capacity. To test this hypothesis Balb/c mice were treated with a maximum of 12 repeated 5-day cycles of either 10 microg rhG-CSF/day or 0.25 microg rmG-CSF/day. Repeated administration of rhG-CSF lead to strong inhibition of HSC/HPC mobilization toward the peripheral blood and spleen after >4 cycles because of the induction of anti-rhG-CSF antibodies. In contrast, after repeated administration of rmG-CSF, HSC/HPC mobilizing capacity remained intact for up to 12 cycles. The number of CFU-GM per femur did not significantly change for up to 12 cycles. We conclude that repeated administration of G-CSF does not lead to depletion of the bone marrow HSC/HPC pool.

De Propris, M. S., G. Meloni, et al. (2003). "Possibility of progenitor cell mobilization during the hematological recovery following peripheral blood stem cell autograft." Acta Haematol **109**(2): 57-63.

Twenty-four patients with hematological malignancies were studied during recovery following autografting in order to establish the proportion of patients that show CD34+ cell mobilization and the kinetics of mobilized CD34-positive cells. The patients showed a peak in peripheral blood (PB) CD34+ cells after a median of 14 days (range 12-20) following reinfusion. According to the number of circulating CD34+ cells, two groups could be clearly distinguished: 17 patients (group A) with <10 PB CD34+ cells/microl (median 1.2, range 0-5) and 7 patients (group B) with >10 CD34+ cells/microl (median 51, range 13-123). Compared to group A, patients of group B showed a faster hematological reconstitution of both polymorphonuclear leukocytes >500/microl (12 vs. 15 days) and platelets >50,000/microl (12 vs. 17 days). The expression of the beta1 integrin CD49d was similar in the two groups of patients, while a lower expression of the beta2 integrin CD11a and a greater expression of the L-selectin CD62L were observed in the PB CD34+ cells of group B patients. Both in the PB and in the BM, the number of CFU-GEMM, CFU- GM, CFU-E and BFU-E of group B was significantly greater than that of group A. However, when the clonogenic potential of a single CD34+ cell was evaluated, no major differences in the number of colonies produced per CD34+ cell were found between the two groups.

del Rosario, M. L. and K. I. Kao (1997). "Determination of the rate of reduction in platelet counts in recipients of hematopoietic stem and progenitor cell transplant: clinical implications for platelet transfusion therapy." Transfusion **37**(11-12): 1163-8.

BACKGROUND: To investigate how a delay between pretransfusion platelet count measurement and actual platelet transfusion affects the assessment of platelet transfusion responses, the rate of reduction in platelet counts was determined in 30 patients with relatively uncomplicated thrombocytopenia. STUDY DESIGN AND METHODS: Fifteen adult and 15 pediatric patients admitted for hematopoietic stem and progenitor cell transplantation were studied. Platelet counts before and after myeloablative conditioning and after prophylactic platelet transfusions were determined and studied as a function of time. The rates of reduction in platelet counts were determined by linear regression analysis. RESULTS: Platelet counts were reduced at linear rates after myeloablative conditioning or prophylactic platelet transfusion in all 30 patients. The average rates of reduction in platelet counts after myeloablation were 1261 +/- 583 and 1070 +/- 492 platelets per microL per hour (mean +/- SD) for adult and pediatric patients, respectively. The average rate of reduction after platelet transfusions during the thrombocytopenic phase was 740 +/- 280 and 820 +/- 288 platelets per microL per hour (mean +/- SD) for adult and pediatric patients, respectively. The rates of reduction in platelet counts between the two phases were significantly different in the two age groups (adult, $p < 0.0001$; pediatric, $p < 0.015$) and were proportionally correlated with initial platelet counts immediately before myeloablation and after prophylactic platelet transfusions. CONCLUSION: The rate of reduction in platelet count can have a significant impact on the evaluation of platelet transfusion responses when there is a delay between pretransfusion measurement of platelet count and the initiation of platelet transfusion. In addition, the rate of platelet reduction determined from this study can be used to confirm an accelerated rate of platelet consumption in thrombocytopenic patients.

Demirer, T., O. Ilhan, et al. (2001). "CD41+ and CD42+ hematopoietic progenitor cells may predict platelet engraftment after allogeneic peripheral blood stem cell transplantation." J Clin Apher **16**(2): 67-73.

The objective of this study was to quantify subpopulations of CD34+ cells such as CD41+ and CD42+ cells that might represent megakaryocyte (MK) precursors in peripheral blood stem cell (PBSC) collections of normal, recombinant human granulocyte-colony stimulating factor (rhG-CSF) primed donors and to determine whether there is a statistical association between the dose infused megakaryocytic precursors and the time course of the platelet recovery following an allogeneic PBSC transplantation. Twenty-six patients with various hematologic malignancies transplanted from their HLA identical siblings between July 1997 and December 1999 were used. All patients except one with severe aplastic anemia who had cyclophosphamide (CY) alone received busulfan-CY as preparative regimen and cyclosporine-methotrexate for GVHD prophylaxis. Normal healthy donors were given rhG-CSF 10 microg/kg/day subcutaneously twice daily and PBSCs were collected on days 5 and 6. The median number of infused CD34+, CD41+ and CD42+ cells were 6.61 x 10(6)/kg (range 1.47-21.41), 54.85 x 10(4)/kg (5.38-204.19), and 49.86 x 10(4)/kg (6.82-430.10), respectively. Median days of ANC 0.5 x 10(9)/L and platelet 20 x 10(9)/L were 11.5 (range 9-15) and 13 (8-33), respectively. In this study, the number of CD41+ and CD42+ cells infused much better correlated than the number of CD34+ cells infused with the time to platelet recovery of 20 x 10(9)/L in 26 patients receiving an allogeneic match sibling PBSC transplantation ($r = -0.727$ and $P < 0.001$ for CD41+ cells, $r = -0.806$ and $P < 0.001$ for CD42+ cells, $r = -0.336$ and $P > 0.05$ for CD34+ cells). There was an inverse correlation between the number of infused CD41+ and CD42+ cells and duration of platelet engraftment. Therefore, as the number of CD41+ and CD42+ cells increased, duration of platelet engraftment (time to reach platelet count of > or = 20 x 10(9)/L) shortened significantly. Based on this data we may conclude that flow cytometric measurement of CD41+ and CD42+ progenitor cells may provide an accurate indication of platelet reconstitutive capacity of the allogeneic PBSC transplant.

Demirkazik, A., A. Kessinger, et al. (2001). "Progenitor and lymphoma cells in blood stem cell harvests: impact on survival following transplantation." Bone Marrow Transplant **28**(2): 207-12.

This study evaluated whether cytokine-induced blood stem cell mobilization also mobilized lymphoma cells and whether lymphoma cell mobilization affected outcome post autologous blood stem cell transplant. Blood stem cell collections from 26 non-Hodgkin's lymphoma (NHL) patients harvested during steady-state (non-mobilized) and from 35 NHL patients harvested after cytokine administration (mobilized) were studied. The harvests were cultured and molecularly evaluated for clonal markers of the primary lymphoma. All patients underwent high-dose chemotherapy and autologous transplantation. Graft products from mobilized patients were more likely to contain lymphoma than graft products from non-mobilized patients (37% vs 19%) but this difference was not significant ($P = 0.16$). In a multivariate analysis, lymphoma contamination was not associated with patient age, gender, tumor grade, prior radiotherapy, duration of

prior chemotherapy, mononuclear cell count, or the number of aphereses performed to obtain the product. Heavily pre-treated patients were less likely to have lymphoma-contaminated harvests (P = 0.064). Lymphoma contamination was positively associated with the number of progenitor cells collected (P = 0.047). In multivariate analyses, the only significant independent predictor of lymphoma contamination was the number of mononuclear cells collected (P = 0.031). Lymphoma contamination of transplanted apheresis products had no apparent impact on event-free and overall survival.

Demirkazik, A., A. Kessinger, et al. (2002). "Effect of prior therapy and bone marrow metastases on progenitor cell content of blood stem cell harvests in breast cancer patients." Biol Blood Marrow Transplant **8**(5): 268-72.

This study was designed to examine the relationship of prior therapy, bone marrow metastases, mobilization, and blood progenitor/stem cell (BSC) collection in breast cancer patients. Cells were collected from 19 breast cancer patients during steady state (nonmobilized group) and from 69 breast cancer patients after cytokine administration (mobilized group). Characteristics of the patients were compared with the cells obtained. A significant inverse association was found between the number of chemotherapy regimens the patients had received prior to BSC collection and the mononuclear cell (MNC) count of the product per liter of blood processed (LBP) with apheresis (P = .0006) and the granulocyte monocyte/macrophage colony-forming cell (GM-CFC) numbers per LBP (P = .0002). This association was evident in both mobilized and nonmobilized patients. Similar results were seen in those 25 patients who had received prior radiation therapy (MNC/LBP, P = .0003; GM-CFC/LBP, P = .0004). Patients in both the mobilized and nonmobilized groups with marrow metastases at the time of collection also had significantly lower levels of MNC/LBP (P = .0039) and GM-CFC/LBP (P = .0001) than did those without marrow metastases. The findings suggest that prior administration of radiation therapy and/or chemotherapy and the presence of marrow metastases all negatively impacted the collection of mobilized and nonmobilized progenitor cells from breast cancer patients. The mechanisms of this impact are not understood.

Di Rosa, P., J. C. Villaescusa, et al. (2007). "The homeodomain transcription factor Prep1 (pKnox1) is required for hematopoietic stem and progenitor cell activity." Dev Biol **311**(2): 324-34.

Most of the hypomorphic Prep1(i/i) embryos (expressing 3-10% of the Prep1 protein), die between E17.5 and P0, with profound anemia, eye malformations and angiogenic anomalies [Ferretti, E., Villaescusa, J.C., Di Rosa, P., Fernandez-Diaz, L.-C., Longobardi, E., Mazzieri, R., Miccio, A., Micali, N., Selleri, L., Ferrari G., Blasi, F. (2006). Hypomorphic mutation of the TALE gene Prep1 (pKnox1) causes a major reduction of Pbx and Meis proteins and a pleiotropic embryonic phenotype. Mol. Cell. Biol. 26, 5650-5662]. We now report on the hematopoietic phenotype of these embryos. Prep1(i/i) fetal livers (FL) are hypoplastic, produce less common myeloid progenitors colonies (CFU-GEMM) in cytokine-supplemented methylcellulose and have an increased number of B-cells precursors that differentiate poorly. Prep1(i/i) FL is able to protect lethally irradiated mice only at high cell doses but the few protected mice show major anomalies in all hematopoietic lineages in both bone marrow (BM) and peripheral organs. Prep1(i/i) FL cells compete inefficiently with wild type bone marrow in competitive repopulation experiments, suggesting that the major defect lies in long-term repopulating hematopoietic stem cells (LTR-HSC). Indeed, wt embryonic expression of Prep1 in the aorta-gonad-mesonephros (AGM) region, fetal liver (FL), cKit(+)Sca1(+)Lin(-)AA4.1(+) (KSLA) cells and B-lymphocytes precursors agrees with the observed phenotype. We therefore conclude that Prep1 is required for a correct and complete hematopoiesis.

Donahue, R. E., K. Kuramoto, et al. (2005). "Large animal models for stem and progenitor cell analysis." Curr Protoc Immunol **Chapter 22**: Unit 22A 1.

Extrapolation of an understanding regarding hematopoiesis and, in particular, hematopoietic stem cells (HSCs) from rodent models or in vitro human cell models to applications in humans has proven very difficult. This is not surprising, given the differences between rodent and human hematopoietic physiology and the lack of true in vitro assays for HSCs. Therefore, translational preclinical development of genetic and cellular therapies is dependent on the utilization of practical and well-defined large animal models. This chapter will introduce the most commonly used model species, including macaques, baboons, dogs, cats, and sheep, and explain the particular advantages and limitations of each. Specific protocols for the support of macaques through ablative cell and gene therapy procedures will be included to introduce investigators to the types of resources and support required to maintain a large animal facility dedicated to high-intensity experimentation, and also to introduce investigators to the types of procedures that are possible.

Dontu, G., K. W. Jackson, et al. (2004). "Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells." Breast Cancer Res **6**(6): R605-15.

INTRODUCTION: Notch signaling has been implicated in the regulation of cell-fate decisions such as self-renewal of adult stem cells and differentiation of progenitor cells along a particular lineage. Moreover, depending on the cellular and developmental context, the Notch pathway acts as a regulator of cell survival and cell proliferation. Abnormal expression of Notch receptors has been found in different types of epithelial metaplastic lesions and neoplastic lesions, suggesting that Notch may act as a proto-oncogene. The vertebrate Notch1 and Notch4 homologs are involved in normal development of the mammary gland, and mutated forms of these genes are associated with development of mouse mammary tumors. METHODS: In order to determine the role of Notch signaling in mammary cell-fate determination, we have utilized a newly described in vitro system in which mammary stem/progenitor cells can be cultured in suspension as nonadherent 'mammospheres'. Notch signaling was activated using exogenous ligands, or was inhibited using previously characterized Notch signaling antagonists. RESULTS: Utilizing this system, we demonstrate that Notch signaling can act on mammary stem cells to promote self-renewal and on early progenitor cells to promote their proliferation, as demonstrated by a 10-fold increase in secondary mammosphere formation upon addition of a Notch-activating DSL peptide. In addition to acting on stem cells, Notch signaling is also able to act on multipotent progenitor cells, facilitating myoepithelial lineage-specific commitment and proliferation. Stimulation of this pathway also promotes branching morphogenesis in three-dimensional Matrigel cultures. These effects are completely inhibited by a Notch4 blocking antibody or a gamma secretase inhibitor that blocks Notch processing. In contrast to the effects of Notch signaling on mammary stem/progenitor cells, modulation of this pathway has no discernable effect on fully committed, differentiated, mammary epithelial cells. CONCLUSION: These studies suggest that Notch signaling plays a critical role in normal human mammary development by acting on both stem cells and progenitor cells, affecting self-renewal and lineage-specific differentiation. Based on these findings we propose that abnormal Notch signaling may contribute to mammary carcinogenesis by deregulating the self-renewal of normal mammary stem cells.

Douay, L., M. C. Giarratana, et al. (1995). "Characterization of late and early hematopoietic progenitor/stem cell sensitivity to mafosfamide." Bone Marrow Transplant **15**(5): 769-75.

The preservation of the hematopoietic value of the graft is essential in the context of bone marrow purging for autologous transplantation. In the present study we have investigated mafosfamide toxicity to transplantable hematopoietic stem cells using combinations of recombinant growth factors (GF) in semi-solid assays and by measurement of Long-Term Culture Initiating Cells (LTC-IC). Our data show that various subtypes of progenitor/stem cells were inhibited in a dose-dependent manner by mafosfamide. A hierarchy appeared clearly regarding sensitivity to the drug in the following order of increasing resistance: PCM CFU-GM (grown in presence of placenta-conditioned medium), 4R CFU-GM (grown in presence of GM-CSF + IL-3 + G-CSF + EPO), 5R CFU-GM (grown in presence GM-CSF + IL-3 + G-CSF + EPO + SCF) and LTC-IC with respective lethal dose 95 (LD95) levels of 40 micrograms, 55 micrograms, 90 micrograms and 140 micrograms/10(7) MNC (P = 0.05 to P = 0.018). Even the primitive stem cells treated with very high doses of mafosfamide (2 to 4 times the usually recommended dose) responded to a combination of SCF + GM-CSF + G-CSF + IL-3 in liquid marrow culture, suggesting that they were functionally spared by the treatment. We also observed a higher sensitivity of 5R CFU-GM and LTC-IC from patients with hematological malignancies, compared with normal donors, when marrow was treated with the dose chosen for clinical purging.(ABSTRACT TRUNCATED AT 250 WORDS)

Drachman, J. G. (2000). "Role of thrombopoietin in hematopoietic stem cell and progenitor regulation." Curr Opin Hematol **7**(3): 183-90.

Thrombopoietin performs an essential role during hematopoiesis by regulating the expansion and maturation of megakaryocytes. In keeping with this function, megakaryocytes, platelets, and their precursors all express the thrombopoietin receptor, Mpl, on their cell surface. However, Mpl is also expressed on primitive, pluripotent hematopoietic progenitors and plays an important role in the regulation of lineages other than megakaryocytes as well as primitive progenitors. Recently, the ability of thrombopoietin to maintain and expand repopulating stem cells has been demonstrated. Thus, thrombopoietin is unique among the hematopoietic cytokines because it is necessary both for terminal maturation and regulation of lineage-specific megakaryocytes and also for maintenance of the most primitive hematopoietic stem cells. Many new strategies are evolving to exploit the activity of thrombopoietin on primitive progenitors. This may

lead to faster hematopoietic recovery from marrow-suppressive therapy, effective methods of ex vivo expansion of hematopoietic stem cells, and retroviral transduction of stem cells to facilitate gene therapy.

Dreger, P., M. Kloss, et al. (1995). "Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts." Blood **86**(10): 3970-8.

Agents with stem cell-toxic potential are frequently used for salvage therapy of Hodgkin's disease (HD) and high-grade non-Hodgkin's lymphoma (NHL). Because many patients with relapsed or refractory lymphoma are candidates for autologous progenitor cell transplantation, possible toxic effects of salvage chemotherapy on progenitor cells must be taken into account. In a retrospective study, we have analyzed the influence of a salvage regimen containing the stem cell-toxic drugs BCNU and melphalan (Dexa-BEAM) on subsequently harvested bone marrow (BM)- and peripheral blood-derived progenitor cell grafts (PBPC) and compared it with other factors. Progenitor cells were collected from 96 patients with HD or high-grade NHL. Seventy-nine grafts were reinfused (35 PBPC and 44 BM) after high-dose chemotherapy. Compared with patients autografted with BM, hematopoietic recovery was significantly accelerated in recipients of PBPC. For PBPC, the number of Dexa-BEAM cycles (> or = v > 1) was the predominate prognostic factor affecting colony-forming unit-granulocyte-macrophage (CFU-GM) yield (66 v 6.8 x 10(4)/kg, P = .0001), CD34+ cell yield (6.6 v 1.6 x 10(6)/kg, P = .0001), neutrophil recovery to > 0.5 x 10(9)/L (9 v. 11 days, P = .0086), platelet recovery to > 20 x 10(9)/L (10 v 15.5 days, P = .0002), and platelet count on day +100 after transplantation (190 v 107 x 10(9)/L, P = .031) using univariate analysis. Previous radiotherapy was associated with significantly lower CFU-GM and CD34+ cell yields but had no influence on engraftment. Patient age, patient sex, disease activity, or chemotherapy other than Dexa-BEAM did not have any prognostic impact. Multivariate analysis confirmed that Dexa-BEAM chemotherapy was the overriding factor adversely influencing CFU-GM yield (P < .0001), CD34+ cell yield (P < .0001), and platelet engraftment (P < .0001). BM grafts were not significantly affected by previous Dexa-BEAM chemotherapy or any other variable tested. However, prognostic factors favoring the use of BM instead of PBPC were not identified using joint regression models involving interaction terms between the graft type (PBPC or BM) and the explanatory variables investigated. We conclude that, in contrast to previous radiotherapy or other chemotherapy, exposure to salvage regimens containing stem cell-toxic drugs, such as BCNU and melphalan, is a critical factor adversely affecting yields and performance of PBPC grafts. Marrow progenitor cells appear to be less sensitive to stem cell-toxic chemotherapy. PBPC should be harvested before repeated courses of salvage chemotherapy involving stem cell-toxic drugs to preserve the favorable repopulation kinetics of PBPC in comparison with BM.

Drize, N., J. Chertkov, et al. (1995). "Hematopoietic progenitor cell mobilization into the peripheral blood of mice using a combination of recombinant rat stem cell factor (rrSCF) and recombinant human granulocyte colony-stimulating factor (rhG-CSF)." Exp Hematol **23**(11): 1180-6.

A significant increase in the hematopoietic stem cell (HSC) concentration has been observed in the peripheral blood and spleen of mice treated with rhG-CSF alone or with a combination of rhG-CSF plus rrSCF. The longer the duration of cytokine treatment, the higher the stimulatory effect on stem cell mobilization. In addition, cytokine administration led to a reduced stem cell concentration in the bone marrow. The total amount of HSCs in the body did not change following cytokine administration. These data support the theory that stem cells are mobilized from the bone marrow into circulation, as opposed to expanding in blood and spleen. In sharp contrast to rhG-CSF alone, a combination of rhG-CSF plus rrSCF produced a strong increase in the self-maintenance ability of peripheral blood day 11 colony-forming units-spleen (CFU-S-11). After 10 days of cytokine treatment, long-term culture initiating cells (LTC-IC) were seen in the peripheral blood; in normal mice, the content of LTC-IC in the blood was below the detection level. The activation of stromal progenitors (cells capable of transferring the hematopoietic microenvironment) by cytokine treatment was observed here for the first time. The results suggest that a combination of rhG-CSF plus rrSCF is more effective than rhG-CSF alone in obtaining a large amount of transplantable HSCs.

Drouet, M., F. Herodin, et al. (2001). "Cell cycle activation of peripheral blood stem and progenitor cells expanded ex vivo with SCF, FLT-3 ligand, TPO, and IL-3 results in accelerated granulocyte recovery in a baboon model of autologous transplantation but G0/G1 and S/G2/M graft cell content does not correlate with transplantability." Stem Cells **19**(5): 436-42.

Ex vivo expansion is a new strategy for hematopoietic stem and progenitor cell transplantation based on cytokine-induced amplification to produce grafts of controlled maturity. If the cell cycle position

of CD34(+) cells has been reported to govern their engraftment potential, the respective role of stem and progenitor cells in short- and long-term hematopoietic recovery remains debated. Studies focused on long-term engraftment potential suggest impairment when using cultured grafts, but the capacity to sustain short-term recovery is still controverted. The aim of this study was: A) to evaluate the consequences of cell cycle activation on short and long-term engraftment capacity, and B) to determine if cell cycle status of grafts could predict hematopoietic recovery. We showed in a nonhuman primate model of autologous peripheral blood stem and progenitor cell transplantation that cell cycle activation of CD34(+) cells in the presence of stem cell factor + FLT3-ligand + thrombopoietin + interleukin 3 (six days of culture) which induced G1 and S/G2/M cell amplification (G0: 6.1% +/- 2.8%; G0/G1: 64.2% +/- 7.2%; S/G2/M: 30.4% +/- 7.3% respectively of expanded CD34(+) cells on average) resulted in the acceleration of short-term granulocyte recovery. By contrast, G0/G1 and S/G2/M cell content of expanded grafts did not correlate with short- or long-term engraftment.

Easterday, M. C., J. D. Dougherty, et al. (2003). "Neural progenitor genes. Germinal zone expression and analysis of genetic overlap in stem cell populations." Dev Biol **264**(2): 309-22.

The identification of the genes regulating neural progenitor cell (NPC) functions is of great importance to developmental neuroscience and neural repair. Previously, we combined genetic subtraction and microarray analysis to identify genes enriched in neural progenitor cultures. Here, we apply a strategy to further stratify the neural progenitor genes. In situ hybridization demonstrates expression in the central nervous system germinal zones of 54 clones so identified, making them highly relevant for study in brain and neural progenitor development. Using microarray analysis we find 73 genes enriched in three neural stem cell (NSC)-containing populations generated under different conditions. We use the custom microarray to identify 38 "stemness" genes, with enriched expression in the three NSC conditions and present in both embryonic stem cells and hematopoietic stem cells. However, comparison of expression profiles from these stem cell populations indicates that while there is shared gene expression, the amount of genetic overlap is no more than what would be expected by chance, indicating that different stem cells have largely different gene expression patterns. Taken together, these studies identify many genes not previously associated with neural progenitor cell biology and also provide a rational scheme for stratification of microarray data for functional analysis.

Era, T. and O. N. Witte (2000). "Regulated expression of P210 Bcr-Abl during embryonic stem cell differentiation stimulates multipotential progenitor expansion and myeloid cell fate." Proc Natl Acad Sci U S A **97**(4): 1737-42.

P210 Bcr-Abl is an activated tyrosine kinase oncogene encoded by the Philadelphia chromosome associated with human chronic myelogenous leukemia (CML). The disease represents a clonal disorder arising in the pluripotent hematopoietic stem cell. During the chronic phase, patients present with a dramatic expansion of myeloid cells and a mild anemia. Retroviral gene transfer and transgenic expression in rodents have demonstrated the ability of Bcr-Abl to induce various types of leukemia. However, study of human CML or rodent models has not determined the direct and immediate effects of Bcr-Abl on hematopoietic cells from those requiring secondary genetic or epigenetic changes selected during the pathogenic process. We utilized tetracycline-regulated expression of Bcr-Abl from a promoter engineered for robust expression in primitive stem cells through multilineage blood cell development in combination with the in vitro differentiation of embryonal stem cells into hematopoietic elements. Our results demonstrate that Bcr-Abl expression alone is sufficient to increase the number of multipotent and myeloid lineage committed progenitors in a dose-dependent manner while suppressing the development of committed erythroid progenitors. These effects are reversible upon extinguishing Bcr-Abl expression. These findings are consistent with Bcr-Abl being the sole genetic change needed for the establishment of the chronic phase of CML and provide a powerful system for the analysis of any genetic change that alters cell growth and lineage choices of the hematopoietic stem cell.

Erlich, S., S. R. Miranda, et al. (1999). "Fluorescence-based selection of gene-corrected hematopoietic stem and progenitor cells from acid sphingomyelinase-deficient mice: implications for Niemann-Pick disease gene therapy and the development of improved stem cell gene transfer procedures." Blood **93**(1): 80-6.

The general utility of a novel, fluorescence-based procedure for assessing gene transfer and expression has been demonstrated using hematopoietic stem and progenitor cells. Lineage-depleted hematopoietic cells were isolated from the bone marrow or fetal livers of acid sphingomyelinase-deficient mice, and retrovirally transduced with amphotropic or ecotropic vectors encoding a normal acid sphingomyelinase (ASM) cDNA. Anti-c-Kit antibodies were then used to label stem- and progenitor-enriched cell populations, and the Bodipy

fluorescence was analyzed in each group after incubation with a Bodipy-conjugated sphingomyelin. Only cells expressing the functional ASM (ie, transduced) could degrade the sphingomyelin, thereby reducing their Bodipy fluorescence as compared with nontransduced cells. The usefulness of this procedure for the in vitro assessment of gene transfer into hematopoietic stem cells was evaluated, as well as its ability to provide an enrichment of transduced stem cells in vivo. To show the value of this method for in vitro analysis, the effects of retroviral transduction using ecotropic versus amphotropic vectors, various growth factor combinations, and adult bone marrow versus fetal liver stem cells were assessed. The results of these studies confirmed the fact that ecotropic vectors were much more efficient at transducing murine stem cells than amphotropic vectors, and that among the three most commonly used growth factors (stem cell factor [SCF] and interleukins 3 and 6 [IL-3 and IL-6]), SCF had the most significant effect on the transduction of stem cells, whereas IL-6 had the most significant effect on progenitor cells. In addition, it was determined that fetal liver stem cells were only approximately twofold more "transducible" than stem cells from adult bone marrow. Transplantation of Bodipy-selected bone marrow cells into lethally irradiated mice showed that the number of spleen colony-forming units that were positive for the retroviral vector (as determined by polymerase chain reaction) was 76%, as compared with 32% in animals that were transplanted with cells that were nonselected. The methods described within this manuscript are particularly useful for evaluating hematopoietic stem cell gene transfer in vivo because the marker gene used in the procedure (ASM) encodes a naturally occurring mammalian enzyme that has no known adverse effects, and the fluorescent compound used for selection (Bodipy sphingomyelin) is removed from the cells before transplantation.

Ezoe, S., I. Matsumura, et al. (2004). "Cell cycle regulation in hematopoietic stem/progenitor cells." Cell Cycle **3**(3): 314-8.

Hematopoietic stem cells (HSCs) are characterized by pluripotentiality and a capacity for self-renewal. In order to both maintain a supply of mature blood cells and not to exhaust HSCs throughout the lifespan of the organism, most HSCs remain quiescent and only a limited number enter the cell cycle. In HSCs, the cell cycle is crucially regulated by external factors such as cytokines and interactions with stromal cells and the extracellular matrix (ECM) in the bone marrow (BM) microenvironment. In addition, intrinsic transcription factors expressed in HSCs, including c-Myb, GATA-2, HOX family proteins, and Bmi-1, also control their growth through their effect on gene transcription. In terms of the particular roles in regulation of the cell-cycle, p21WAF1 (p21) and p27KIP1 (p27) were shown to maintain the quiescence of HSCs and of progenitor cells, respectively, thereby governing their available pool sizes. Also, p16INK4A (p16) and p15INK4B (p15) are thought to act as tumor suppressors, since their inactivation and/or deletion are observable in various types of hematologic malignancies. These results make evident that appropriate cell cycle control, particularly at the early stage of stem/progenitor cells, is required for maintaining normal hematopoiesis.

Fabian, I., D. Douer, et al. (1985). "Human spleen cell generation of factors stimulating human pluripotent stem cell, erythroid, and myeloid progenitor cell growth." Blood **65**(4): 990-6.

Mitogen-stimulated murine spleen cells produce humoral substances capable of supporting murine hematopoiesis and pluripotent stem cell proliferation in vitro. Thus, we evaluated conditioned media generated by human spleen cells (SCM) in the presence or absence of mitogens for factors stimulatory for human pluripotent (CFU-GEMM), erythroid (BFU-E), and myeloid (CFU-GM) precursors. Two and one half percent to 10% SCM stimulated proliferation of all three types of precursor cells from nonadherent buoyant human marrow target cells. Mitogen-stimulated SCM augmented CFU-GM (175% to 225%), whereas CFU-GEMM and BFU-E growth was essentially unchanged. Cell separation procedures used to determine which cells provided these microenvironmental stimuli indicated that nonadherent mononuclear spleen cells provided the bulk of the CSF-GM, whereas adherent cells (95% nonspecific esterase + monocyte-macrophages) and nonadherent cells provided similar proportions of CSF-mix and erythroid burst-promoting activity (BPA). The nonadherent cells generating high levels of CSF-mix, BPA, and CSF-GM were predominantly Leu-1-negative, ie, non-T, cells. In the presence or absence of mitogens, SCM was a more potent source (1.3- to 3.8-fold) than peripheral leukocyte CM of the growth factors for the three progenitor cell types. Specific in situ cytochemical stains for analyzing morphology of myeloid colonies demonstrated that SCM stimulated the proliferation of the same types and proportions of colonies as human placental CM, suggesting that these CMs may contain similar CSF-GMs. These data show the contribution of spleen cell subsets to the generation of hematopoietic growth factors and the responsiveness of these cells to various mitogenic stimuli.

Facon, T., J. L. Harousseau, et al. (1999). "Stem cell factor in combination with filgrastim after chemotherapy improves peripheral blood progenitor cell yield and reduces apheresis requirements in multiple myeloma patients: a randomized, controlled trial." Blood **94**(4): 1218-25.

Stem cell factor (SCF) has been shown to synergize with filgrastim to mobilize CD34(+) cells into the peripheral blood. To determine if addition of SCF to chemotherapy and filgrastim reduces the number of leukaphereses required to achieve a target yield of 5 x 10(6) CD34(+) cells/kg, 102 patients with multiple myeloma were randomized to receive mobilization chemotherapy with cyclophosphamide (4 g/m(2)) and either SCF (20 micrograms/kg/d) combined with filgrastim (5 micrograms/kg/d) or filgrastim alone (5 micrograms/kg/d), administered daily until leukaphereses were completed. After collection, patients were treated with myeloablative therapy supported by autologous peripheral blood progenitor cell (PBPC) infusion and filgrastim (5 micrograms/kg/d). There was a significant difference between the treatment groups in the number of leukaphereses required to collect 5 x 10(6) CD34(+) cells/kg (median of 1 v 2 for SCF + filgrastim and filgrastim alone, respectively, P =.008). Patients receiving the combination of SCF plus filgrastim had a 3-fold greater chance of reaching 5 x 10(6) CD34(+) cells/kg in a single leukapheresis compared with patients mobilized with filgrastim alone. The median CD34(+) cell yield was significantly increased for the SCF group in the first leukapheresis (11.3 v 4.0 x 10(6)/kg, P =.003) and all leukaphereses (12.4 v 8.2 x 10(6)/kg, P =.007). Total colony-forming unit-granulocyte-macrophage (CFU-GM) and mononuclear cell counts were also significantly higher in the SCF group in the first leukapheresis and in all leukaphereses. As expected for patients mobilized to an optimal CD34(+) cell yield, the time to engraftment was similar between the 2 treatment groups. Cells mobilized with the combination of SCF plus filgrastim were thus considered effective and safe for achieving rapid engraftment. Treatment with SCF plus filgrastim was well tolerated, with mild to moderate injection site reactions being the most frequently reported adverse events. There were no serious allergic-like reactions to SCF. The addition of SCF to filgrastim after cyclophosphamide for PBPC mobilization resulted in a significant increase in CD34(+) cell yield and a concomitant reduction in the number of leukaphereses required to collect an optimal harvest of 5 x 10(6) CD34(+) cells/kg.

Fahlman, C., F. W. Jacobsen, et al. (1994). "Tumor necrosis factor-alpha (TNF-alpha) potently enhances in vitro macrophage production from primitive murine hematopoietic progenitor cells in combination with stem cell factor and interleukin-7: novel stimulatory role of p55 TNF receptors." Blood **84**(5): 1528-33.

Tumor necrosis factor-alpha (TNF-alpha) is a bifunctional regulator of hematopoiesis, and its cellular responses are mediated by two distinct cell surface receptors. TNF-alpha generally inhibits the growth of primitive murine hematopoietic progenitor cells (Lin-Scal+) in response to multiple cytokine combinations, and the p75 TNF receptor is essential in signaling such inhibition. In the present study we show the reverse phenomenon in that TNF-alpha on the same progenitor cell population in combination with stem cell factor (SCF) and interleukin-7 (IL-7) through the p55 TNF receptor can recruit additional progenitors to proliferate. In contrast, TGF-beta 1, another bifunctional regulator of hematopoietic progenitor cell growth, completely blocked SCF plus IL-7-induced proliferation. TNF-alpha increased the number of responding progenitors, as well as the size of the colonies formed. The synergistic effects of TNF-alpha were seen at the single cell level, suggesting that its effects are directly mediated. Finally, whereas SCF plus IL-7 promoted primarily granulopoiesis, the addition of TNF-alpha switched the differentiation toward the production of almost exclusively macrophages.

Farkas, L. M. and W. B. Huttner (2008). "The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development." Curr Opin Cell Biol **20**(6): 707-15.

The switch of neural stem and progenitor cells from proliferation to differentiation during development is a crucial determinant of brain size. This switch is intimately linked to the architecture of the two principal classes of neural stem and progenitor cells, the apical (neuroepithelial, radial glial) and basal (intermediate) progenitors, which in turn is crucial for their symmetric versus asymmetric divisions. Focusing on the developing rodent neocortex, we discuss here recent advances in understanding the cell biology of apical and basal progenitors, place key regulatory molecules into subcellular context, and highlight their roles in the control of proliferation versus differentiation.

Feng, Y., Y. L. Gao, et al. (2007). "Expression change of stem cell-derived neural stem/progenitor cell supporting factor gene in injured spinal cord of rats." Neurosci Bull **23**(3): 165-9.

OBJECTIVE: To explore the expression change of stem cell-derived neural stem/progenitor cell supporting factor (SDNSF) gene in the injuried spinal cord tissues of rats, and the relation between the

expressions of SDNSF and nestin. METHODS: The spinal cord contusion model of rat was established according to Allen's falling strike method. The expression of SDNSF was studied by RT-PCR and in situ hybridization (ISH), and the expression of nestin was detected by immunochemistry. RESULTS: RT-PCR revealed that SDNSF mRNA was upregulated on day 4 after injury, peaked on day 8-12, and decreased to the sham operation level on day 16. ISH revealed that SDNSF mRNA was mainly expressed in the gray matter cells, probably neurons, of spinal cord. The immunohistochemistry showed that accompanied with SDNSF mRNA upregulation, the nestin-positive cells showed erupted roots, migrated peripherad and proliferation on the 8-day slice. However, the distribution pattern of these new cells was different from that of SDNSF-positive cells. CONCLUSION: (1) SDNSF is expressed in the gray matter of spinal cord. The expression of SDNSF mRNA in the spinal cord varies with injured time. (2) The nestin-positive cells proliferate accompanied with spinal cord injury repair, but do not secrete SDNSF.

Ferrero, D., C. Cherasco, et al. (1999). "In vitro growth and quantification of early (CD33-/CD38-) myeloid progenitor cells: stem cell factor requirement and effects of previous chemotherapy." Haematologica **84**(5): 390-6.

BACKGROUND AND OBJECTIVE: All culture systems exploring the early (pre-CFU) hematopoietic compartment are generally complex, time-consuming and unsuitable for routine application. The aim of our study was to develop a stroma-free culture system to quantify early bone marrow (BM) myeloid progenitor cells. DESIGN AND METHODS: Low density, progenitor cell enriched BM cells underwent a double cytotoxic treatment with CD38 and CD33 monoclonal antibodies + rabbit complement, which depleted 99% of CFU-GM and BFU-E. Then they were cultured, both in agar and in limiting-dilution liquid culture, in the presence of 5637 cell line supernatant (containing GM-CSF, G-CSF and interleukin 1), stem cell factor (SCF) and interleukin 3 (IL3). RESULTS: The largest number (median 14.9 on 1x10(5) cells) and size (>50,000 cells) of myelomonocytic cell clones from CD33Eth /CD38Eth progenitors was reached after 3-4 weeks of liquid culture. SCF, but not IL3, was essential for that growth. The frequency of CD33-/CD38- progenitors grown in liquid culture was approximately three times greater than the LTC-IC frequency in the same cell suspension. An average 93% of CD33-/CD38- progenitors displayed HLA-DR antigens and 43% generated secondary CFU-GM. In the BM of 9/10 patients, previously exposed to chemotherapy, CD33-/CD38- progenitor frequency was quite low (median 0.9 on 1x10(5) cells), in spite of normal cellularity and morphology and sustained disease remission. INTERPRETATION AND CONCLUSIONS: CD33-/CD38- progenitors can be grown and quantified in a stroma-free culture system in a relatively short time. The test can reveal long-lasting, subclinical BM damage induced by chemotherapy and could also be valuable for estimating the amount of early myeloid progenitors for transplantation purposes.

Ferret-Bernard, S., P. Sai, et al. (2004). "In vitro induction of inhibitory macrophage differentiation by granulocyte-macrophage colony-stimulating factor, stem cell factor and interferon-gamma from lineage phenotypes-negative c-kit-positive murine hematopoietic progenitor cells." Immunol Lett **91**(2-3): 221-7.

CD11b+Gr-1+ inhibitory macrophages (iMacs) were implicated in profound depression of T cell functions sometimes observed during cyclophosphamide treatments and overwhelming infections, through a secretion of nitric oxide (NO). Myeloid origin and maturation stages of iMacs are still unknown. As tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) contributed crucially to the activation of inducible NO synthase (iNOS) gene transcription and to the differentiation of macrophages, we tested their roles in the induction of iMacs differentiation from bone marrow hematopoietic progenitor cells (HPC) of uncompromised mice. Lineage phenotypes-negative (lin)) c-kit+ cells of Balb/c mice were cultured 6 days with granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF, c-kit ligand) in presence or not of TNF-alpha or IFN-gamma. CD11b+Gr-1+ cells only derived in presence of [GM-CSF + SCF + TNF-alpha] or [GM-CSF + SCF + IFN-gamma] could express iNOS upon in vitro stimulation with [IFN-gamma + TNF-alpha] or [IFN-gamma + LPS] known to boost iNOS expression in murine macrophages. However, whereas [GM-CSF + SCF + TNF-alpha] induced only weakly iMacs generation and contributed also to the differentiation of CD11b+Gr-1-CD11c+ myeloid dendritic cells, [GM-CSF + SCF + IFN-gamma] induced exclusively and importantly iMacs differentiation. Moreover [GM-CSF + SCF + IFN-gamma]-generated iMacs were more mature than [GM-CSF + SCF + TNF-alpha]-derived iMacs since IFN-gamma increased more strongly CD11b+Gr-1+ cells expressing Ly-6C and generated lesser cells expressing MHC class II and CD86 molecules. Finally [GM-CSF + SCF + IFN-gamma]-generated CD11b+ cells showing a powerful suppressive activity on T cell proliferations, correlated with NO secretion. In conclusion, our study showed,

for the first time, that IFN-gamma induced very efficiently the differentiation of functional iMacs from lin- c-kit+ murine HPC in vitro, and indicated clearly that iMacs progenitors may be present in bone marrow of naive mice.

Fierabracci, A., M. A. Puglisi, et al. (2008). "Identification of an adult stem/progenitor cell-like population in the human thyroid." J Endocrinol **198**(3): 471-87.

There is evidence that tissue-specific stem cells reside in certain adult tissues. Their specific properties remain elusive, because they are rare and heterogeneous in parent tissues; furthermore, technical difficulties have been encountered in the identification and characterization of their progeny. The aim of this study was to isolate stem/progenitor cells from the human thyroid. We devised a method based on the enzymatic digestion of fresh surgical thyroid specimens, followed by culture of cells in the presence of epidermal growth factor and basic fibroblast growth factor. We also used markers that identify and characterize these cells. Spheroids with self-replicative potential were obtained from all thyroid specimens. The isolated population contained a subset of CD34+ CD45- cells and it was able, in differentiation conditions, to generate follicles with thyroid hormonal production. In support of the plasticity concept, we obtained evidence that, when most freshly isolated spheroids were co-cultured with a neuroblastoma cell line, they produced progeny expressing the neuronal marker beta-tubulin III. Spheroids were also able to undergo adipogenic differentiation when cultured in adipogenic medium. We conclude that a predominant functional type of stem/progenitor cell exists within the thyroid, with an intrinsic ability to generate thyroidal cells and the potential to produce non-thyroidal cells.

Filshie, R. J. (2002). "Cytokines in haemopoietic progenitor mobilisation for peripheral blood stem cell transplantation." Curr Pharm Des **8**(5): 379-94.

Haemopoietic progenitors mobilised into peripheral blood are now almost universally used in autologous haemopoietic stem cell transplantation in the treatment of a range of malignant and some nonmalignant disease. Although chemotherapy alone was initially used, all modern protocols now involve the use of cytokines, with or without chemotherapy. Important developments have included an in understanding of the importance of prior cancer therapy on progenitor yield, knowledge of the kinetics of mobilisation and development of necessary skills to collect and cryopreserve progenitors. More accurate measurement of haemopoietic progenitors and definitions of target cell yields for optimal haemopoietic recovery after high-dose therapy have also contributed to more predictable outcomes and provide a reference point for newer mobilisation approaches. Although G-CSF based regimens are usually successful, some patients either fail to mobilise sufficient progenitors or require an excessive number of collections. Clinical studies with the early acting cytokine, stem cell factor, in combination with G-CSF have demonstrated increased progenitor yields in a range of patients which may translate to clinical benefit in selected situations. In animal models and to a lesser extent in humans, other cytokines such as thrombopoietin and Flt-3 ligand or a number of engineered small molecules with single or dual agonist activity for cytokine receptors (IL-3, Flt-3L, TPO, G-CSF), have also been found to be promising mobilising agents. Further research into the relative importance of cell proliferation, cellular adhesion and the role of accessory cells and other signalling events is leading to an improved understanding of the underlying mechanisms of haemopoietic progenitor mobilisation. Administration of appropriate high-dose chemotherapy followed by re-infusion of haemopoietic progenitor cells capable of long-term reconstitution has long had a place in the treatment of a number of malignant (largely haematological) and non-malignant diseases. For many years these progenitor cells were obtained by direct aspiration of bone marrow under general anaesthetic, hence the term bone marrow transplantation. However, it has also been recognized that haemopoietic stem cells may be recovered from peripheral blood, albeit in low numbers, and also from umbilical cord blood. Further empirical observations showed that the number of haemopoietic progenitors circulating in the blood could be transiently augmented after chemotherapy and/or administration of one or more of a number of cytokines. Refinements to the clinical practice of progenitor mobilisation, collection and enumeration have proved very successful such that in many cases peripheral blood stem cells (PBSC) have largely replaced bone marrow as the preferred source.

Fischer, J. C., M. Frick, et al. (2005). "Superior mobilisation of haematopoietic progenitor cells with glycosylated G-CSF in male but not female unrelated stem cell donors." Br J Haematol **130**(5): 740-6.

Granulocyte colony-stimulating factor (G-CSF) effectively mobilises haematopoietic stem cells to the peripheral blood. It is unclear whether the mobilisation of stem cells with lenograstim (glycosylated G-CSF) or filgrastim (non-glycosylated G-CSF) leads to a higher cell number of collected engraft able progenitor cells. Thus, we investigated harvesting efficiency of the licensed G-CSF preparations in mobilising peripheral stem cells in a

randomised study. A total of 501 healthy unrelated donors, including 339 males and 162 females received either lenograstim (n = 261) or filgrastim (n = 240) at 10 microg/kg body weight (BW) per day. Aphaeresis was performed on day 5 and, if necessary, on day 6 of mobilisation. The number of CD34+ cells collected was 11.5% higher in the lenograstim group (7.19 x 10(6) vs. 6.44 x 10(6)/kg BW donor; P < 0.03). Univariate variance analysis revealed that this effect was caused by male donors: more progenitors cells per kg BW of the donor (7.73 x 10(6) vs. 6.88 x 10(6); P < 0.017) and of the recipient (10.1 x 10(6) vs. 8.88 x 10(6), P < 0.029) could be harvested. There was no significant difference in the percentage of donors in whom a second aphaeresis was required (9.6% vs. 11.6%). Lenograstim mobilises progenitor cells into the peripheral blood more effectively in males than filgrastim.

Fois, E., M. Desmartin, et al. (2007). "Recovery, viability and clinical toxicity of thawed and washed haematopoietic progenitor cells: analysis of 952 autologous peripheral blood stem cell transplantations." Bone Marrow Transplant **40**(9): 831-5.

Cryopreservation and thawing of haematopoietic stem cells are associated with cell loss and infusion-related toxicities. We analysed viability, total nucleated cell (TNC) and CD34+ cell recovery, and infusion-related toxicities of 952 thawed and washed products. Mean TNC and CD34+ viable cells recoveries were 55.9+/-18.6 and 98.0+/-36.5%, respectively. Mean cell viability was 68.25+/-18.9%. TNC recovery was correlated with viability but independent of the initial nucleated cell concentration. No difference in TNC recovery or viability was observed according to underlying diseases, except for myeloma, for which these variables were significantly lower (P<0.05). CD34+ cell recovery was not correlated with viability or CD34+ initial count and was similar for all diseases. Cryostorage duration was not associated with cell loss. Immediate adverse events occurred in 169 patients (19%) and were moderate (grade I or II) for the majority of patients. Clinical toxicity was associated with a higher infused cell number and the presence of clumps in infused bags. The washing procedure of cell products lead to a low rate of adverse events, but patients transplanted with high cell numbers or bags in which clumps were identified are predisposed to such complications.

Forraz, N., R. Pettengell, et al. (2004). "Characterization of a lineage-negative stem-progenitor cell population optimized for ex vivo expansion and enriched for LTC-IC." Stem Cells **22**(1): 100-8.

Current hematopoietic stem cell transplantation protocols rely heavily upon CD34+ cells to estimate hematopoietic stem and progenitor cell (HSPC) yield. We and others previously reported CD133+ cells to represent a more primitive cell population than their CD34+ counterparts. However, both CD34+ and CD133+ cells still encompass cells at various stages of maturation, possibly impairing long-term marrow engraftment. Recent studies demonstrated that cells lacking CD34 and hematopoietic lineage markers have the potential of reconstituting long-term in vivo hematopoiesis. We report here an optimized, rapid negative-isolation method that depletes umbilical cord blood (UCB) mononucleated cells (MNC) from cells expressing hematopoietic markers (CD45, glycophorin-A, CD38, CD7, CD33, CD56, CD16, CD3, and CD2) and isolates a discrete lineage-negative (Lin-) cell population (0.10% +/- 0.02% MNC, n=12). This primitive Lin- cell population encompassed CD34+/- and CD133+/- HSPC and was also enriched for surface markers involved in HSPC migration, adhesion, and homing to the bone marrow (CD164, CD162, and CXCR4). Moreover, our depletion method resulted in Lin- cells being highly enriched for long-term culture-initiating cells when compared with both CD133+ cells and MNC. Furthermore, over 8 weeks in liquid culture stimulated by a cytokine cocktail optimized for HSPC expansion, TPOFLK (thrombopoietin 10 ng/ml, Flt3 ligand 50 ng/ml, c-Kit ligand 20 ng/ml) Lin- cells underwent slow proliferation but maintained/expanded more primitive HSPC than CD133+ cells. Therefore, our Lin- stem cell offers a promising alternative to current HSPC selection methods. Additionally, this work provides an optimized and well-characterized cell population for expansion of UCB for a wider therapeutic potential, including adult stem cell transplantation.

Forsyth, N. R. and J. McWhir (2008). "Human embryonic stem cell telomere length impacts directly on clonal progenitor isolation frequency." Rejuvenation Res **11**(1): 5-17.

The pluripotentiality of human embryonic stem cells is expected to yield an abundance of clinically useful cell types. Using physiologic oxygen culture systems, we show that it is possible to isolate highly proliferative clonal progenitor cells from partially differentiated human embryonic stem cells. These progenitors have similar, though not identical, immunophenotypes with a resemblance to bone marrow-derived adherent stem cells. Through telomere length analysis of multiple early senescing clones, we were able to show that the starting telomere length of a human embryonic stem cell line impacts on the proliferative potential of clonally isolated

partially differentiated mortal progeny. Proliferative clones undergo growth arrest with telomere lengths consistent with telomere-driven replicative senescence. To bypass this phenomenon, we transduced progenitor cells with ectopic hTERT (the limiting catalytic component of telomerase). This enabled telomerase immortalization without affecting differentiation potential or immunophenotype. In summary we describe the derivation of clonal progenitor cells from human embryonic stem cells and the relevance of parental cell telomere length to the frequency of highly proliferative clone isolation.

Fortunel, N., J. Hatzfeld, et al. (2000). "Release from quiescence of primitive human hematopoietic stem/progenitor cells by blocking their cell-surface TGF-beta type II receptor in a short-term in vitro assay." Stem Cells **18**(2): 102-11.

Genetic alterations of the signaling cascade of transforming growth factor-beta (TGF-beta) are often associated with neoplastic transformation of primitive cells. This demonstrates the key role for this pleiotropic factor in the control of quiescence and cell proliferation in vivo. In the high proliferative potential-quiescent cell (HPP-Q) in vitro assay, the use of TGF-beta1 blocking antibodies (anti-TGF-beta1) allows the detection within two to three weeks of primitive hematopoietic cells called HPP-Q, which otherwise would not grow. However, the possibility of triggering cell proliferation by blocking the cell-surface TGF-beta receptors has not been investigated until now. We have tested here the efficiency of a blocking antibody against TGF-betaRII (anti-TGF-betaRII) on CD34(+)CD38(-) hematopoietic cells, a subpopulation enriched in primitive stem/progenitor cells, and compared its effect with that of anti-TGF-beta1. About twice as many HPP colony-forming cells were detected in the presence of anti-TGF-beta1 or anti-TGF-betaRII, compared to the control ($p < 0.02$). Moreover, anti-TGF-betaRII was as efficient as anti-TGF-beta1 for activating multipotent HPP-granulocyte erythroid macrophage megakaryocyte and HPP-Mix, bipotent HPP-granulocyte-macrophage (GM) and unipotent HPP-G, HPP-M and HPP-BFU-E. We therefore propose the use of anti-TGF-betaRII to release primitive cells from quiescence in the HPP-Q assay. This strategy could be extended to nonhematopoietic tissues, as TGF-beta1 may be a pleiotropic regulator of somatic stem cell quiescence.

Fortunel, N. O., J. A. Hatzfeld, et al. (2003). "Control of hematopoietic stem/progenitor cell fate by transforming growth factor-beta." Oncol Res **13**(6-10): 445-53.

A major obstacle to the use of adult somatic stem cells for cell therapy is our current inability to fully exploit stem cell self-renewal properties. The challenge is to obtain defined culture systems where cycling of primitive stem/progenitor cells is stimulated, while their differentiation and senescence are prevented. The cytokine transforming growth factor-beta1 (TGF-beta1) appears as a potential regulator of hematopoietic stem/ progenitor cell self-renewal, as it participates in the control of cell proliferation, survival/apoptosis, and cell immaturity/differentiation. TGF-beta1 acts via a complex regulatory network involving intracellular signaling molecules and cell surface receptors. According to the High Proliferative Potential-Quiescent (HPP-Q) cell working model that we introduced previously, TGF-beta1 maintains primitive hematopoietic stem/progenitor cells in a quiescent or slow cycling state, in part by downmodulating the cell surface expression of mitogenic cytokine receptors, thus preventing cells from responding rapidly to a mitogenic signal. We have established that this modulation concerns the tyrosine kinase receptors KIT and FLT3, and the IL-6 receptor (IL-6R), three important cytokine receptors controlling early human hematopoietic stem/progenitor cell development. In this article. we show a similar modulation by TGF-beta1 of a fourth receptor: the TPO receptor (MPL). As a consequence, TGF-beta1 decreased the cell cycle entry of stem/progenitor cells stimulated by the respective ligands of these receptors, the cytokines SF, FL, IL-6, and TPO, whereas neutralization of TGF-beta1 increased the cell responsiveness to these mitogenic cytokines. Other aspects of the function of TGF-beta1 in the regulation of early hematopoiesis (i.e., the control of stem/progenitor cell survival and immaturity) are reviewed in the discussion.

Foster, G. A. and B. M. Stringer (1999). "Genetic regulatory elements introduced into neural stem and progenitor cell populations." Brain Pathol **9**(3): 547-67.

The genetic manipulation of neural cells has advantage in both basic biology and medicine. Its utility has provided a clearer understanding of how the survival, connectivity, and chemical phenotype of neurones is regulated during, and after, embryogenesis. Much of this achievement has come from the recent generation by genetic means of reproducible and representative supplies of precursor cells which can then be analyzed in a variety of paradigms. Furthermore, advances made in the clinical use of transplantation for neurodegenerative disease have created a demand for an abundant, efficacious and safe supply of neural cells for grafting. This review describes how genetic methods, in juxtaposition to epigenetic means, have been used advantageously to achieve this goal. In particular, we

detail how gene transfer techniques have been developed to enable cell immortalization, manipulation of cell differentiation and commitment, and the controlled selection of cells for purification or safety purposes. In addition, it is now also possible to genetically modify antigen presentation on cell surfaces. Finally, there is detailed the transfer of therapeutic products to discrete parts of the central nervous system (CNS), using neural cells as elegant and sophisticated delivery vehicles. In conclusion, once the epigenetic and genetic controls over neural cell production, differentiation and death have been more fully determined, providing a mixture of hard-wired elements and more flexibly expressed characteristics becomes feasible. Optimization of the contributions and interactions of these two controlling systems should lead to improved cell supplies for neurotransplantation.

Fraser, J., I. Wulur, et al. (2007). "Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots." Cytotherapy **9**(5): 459-67.

BACKGROUND: Human adipose tissue has been shown to contain multipotent cells with properties similar to mesenchymal stromal cells. While there have been many studies of the biology of these cells, no study has yet evaluated issues associated with tissue harvest. METHODS: Adipose tissue was obtained from the subcutaneous space of the abdomen and hips of 10 donors using both syringe and pump-assisted liposuction. Tissue was digested with collagenase and then assayed for the presence of different stem and progenitor cell types using clonogenic culture assays, including fibroblast colony-forming unit (CFU-F) and alkaline phosphatase-positive colony-forming unit (CFU-AP). Paired analysis of samples obtained from the same individual was used to compare harvest method and site. RESULTS: Syringe suction provided significantly greater recovery of adipocytes and a non-significant trend towards improved recovery of cells in the adipocyte-depleted fraction. There was considerable donor-to-donor variation in stem cell recovery. However, paired analysis of tissue obtained from different subcutaneous sites in the same donor showed that tissue harvested from the hip yielded 2.3-fold more CFU-F/unit volume and a 7-fold higher frequency of CFU-AP than that obtained from the abdomen. These differences were statistically significant. DISCUSSION: Harvest site influences the stem and progenitor cell content of subcutaneous adipose tissue.

Froberg, M. K., U. C. Garg, et al. (1999). "Changes in serum osteocalcin and bone-specific alkaline phosphatase are associated with bone pain in donors receiving granulocyte-colony-stimulating factor for peripheral blood stem and progenitor cell collection." Transfusion **39**(4): 410-4.

BACKGROUND: Granulocyte-colony-stimulating factor (G-CSF) has been used to increase the number of CD34+ peripheral blood stem and progenitor cells collected by apheresis for use in autologous or allogeneic progenitor cell transplantation. The most frequent side effect of G-CSF treatment is bone pain, which occurs in over 80 percent of healthy progenitor cell donors. STUDY DESIGN AND METHODS: The possible mechanism of bone pain was investigated by measuring serum levels of osteocalcin (OC), bone-specific alkaline phosphatase (BAP), acid phosphatase (ACP), and tartrate-resistant acid phosphatase (TRAP) in seven healthy progenitor cell donors treated with human recombinant G-CSF administered subcutaneously for 5 consecutive days. RESULTS: All seven patients experienced bone pain during the treatment period. Serum levels of OC, BAP, ACP, and TRAP were measured in blood samples drawn on Days 0, 4, 5, 6, and 14. Levels of BAP were increased ($p<0.05$) over baseline on Days 4, 5, and 6, while those of OC decreased on Days 4, 5, and 6 ($p<0.05$). No significant changes occurred in ACP or TRAP levels. OC and BAP are considered markers of bone formation (osteoblast activity), and they correlate in many patients with metabolic bone disorders. The pattern of increased BAP and decreased OC has been reported in patients with osteolytic bone metastases. CONCLUSION: G-CSF treatment in healthy stem and progenitor cell donors may affect osteoblastic activity, and this activity may be associated with bone pain.

Fu, S. Q., C. N. Abboud, et al. (2002). "Impact of mobilized blood progenitor cell quality determined by the CFU-GM/CD34+ ratio on rapid engraftment after blood stem cell transplantation." Blood Cells Mol Dis **28**(3): 315-21.

To find a parameter to predict the quality of collected mobilized CD34+ blood as hemopoietic reconstituting cells, the ratio of CFU-GM to CD34+ cells was examined. One hundred six consecutive patients who underwent blood stem cell transplantation at the University of Rochester from 01/01/99 to 12/31/99 were examined retrospectively for the number of days to reach an absolute neutrophil count of 500 or 1000 cells/microl and an absolute platelet count of 20,000 or 50,000 cells/microl without transfusion support as measures of engraftment. Linear regression analyses were conducted to determine factors influencing engraftment. The number of CD34+ cells/kg and CFU-GM/kg correlated highly with the number of nucleated blood cells/kg. In this population, in which 90% of patients

received >2 x 10(6) CD34+ cells/kg, neither the number of CD34+ cells/kg nor the number of CFU-GM/kg correlated with the time to engraftment as judged by neutrophil or platelet levels. In contrast, the lower the ratio of CFU-GM to CD34+ cells, the more rapid the reconstitution of platelets to 20,000/microl (P = 0.03) and 50,000/microl (P = 0.02). Thus, a lower ratio of the CFU-GM/CD34+ appended to reflect a greater number of hematopoietic reconstituting cells in the blood cell collection. The CFU-GM/CD34+ ratio is an apparent predictor of earlier platelet engraftment, suggesting that the ratio reflects the engraftment potential of mobilized donor progenitor cells.

Fujii, S., H. Maeda, et al. (2008). "Investigating a clonal human periodontal ligament progenitor/stem cell line in vitro and in vivo." J Cell Physiol **215**(3): 743-9.

The lifespan of the tooth is influenced by the periodontal ligament (PDL), a specialized connective tissue that connects the cementum with the tooth socket bone. Generation of a cell line from PDL progenitor/stem cells would allow development of tissue engineering-based regenerative PDL therapy. However, little is known about the characteristics of PDL progenitor/stem cells because PDL tissue consists of a heterogeneous cell population and there are no pure PDL cell lines. Recently, we succeeded in immortalizing primary human PDL fibroblasts (HPLFs) by transfecting them with SV40 T-antigen and hTERT (Cell Tissue Res 2006; 324: 117-125). In this study, we isolated three clonal cell lines from these immortalized cells (lines 1-4, 1-11, and 1-24) that express RUNX-2, Col I, ALP, OPN, OCN, RANKL, OPG, scleraxis, periostin, Col XII, and alpha-SMA mRNA. Immunocytochemical analysis demonstrated that CD146 was expressed in cell lines 1-4 and 1-11 and that STRO-1 was expressed in lines 1-11 and 1-24. Lines 1-4 and 1-11 differentiated into osteoblastic cells and adipocytes when cultured in lineage-specific differentiation media. Four weeks after transplanting cell line 1-11 into immunodeficient mice with beta-tricalcium phosphate (beta-TCP), the transplant produced cementum/bone-like tissues around the beta-TCP. Eight weeks after transplantation, the 1-11 cell transplant formed PDL-like structures on the surface of the beta-TCP. These data suggest that cell line 1-11 was derived from a progenitor/stem cell present in the PDL and should be very useful for studying the biology and regeneration of human periodontium.

Fujii, T., Y. Zen, et al. (2008). "Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma--human and cell culture study." Hum Pathol **39**(8): 1185-96.

Cancer stem cells reportedly participate in the tumorigenesis of some neoplasms. Scirrhous hepatocellular carcinoma is a variant of hepatocellular carcinoma with abundant fibrous stroma. Herein, we clinicopathologically examined scirrhous (29 cases) and conventional (50 cases) hepatocellular carcinoma with reference to cancer stem cells. Scirrhous hepatocellular carcinoma was classifiable into 3 types based on small neoplastic cells at the periphery of tumor cell nests. Of 29 cases of scirrhous hepatocellular carcinoma, 21 contained small neoplastic cells. Immunohistochemically, those cells were positive for cytokeratin 7 and ATP-binding cassette transporter G2. In 11 cases, those small tumor cells were also positive for cytokeratin 19, neural cell adhesion molecule, and epithelial cell adhesion molecule (type 1), whereas 10 cases did not show such additional expression (type 2). The remaining 8 tumors did not contain small tumor cells with stem cell features (type 3). In the central parts of tumor nests, carcinoma cells got hepatocellular markers and lost expression of neural cell adhesion molecule, and epithelial cell adhesion molecule, suggesting hepatocellular maturation. Transforming growth factor beta1, a fibrogenic cytokine, was also detected in those small tumor cells. Culture cells extracted as "side population" from hepatocellular carcinoma cell lines (HuH7 and PLC5) expressed more intensely cytokeratins 7 and 19, neural cell adhesion molecule, epithelial cell adhesion molecule, and transforming growth factor beta1 than did non-side population cells. Small tumor cells with stem cell features in scirrhous hepatocellular carcinoma may correspond to side population of culture cells and might be involved in fibrogenesis of scirrhous hepatocellular carcinoma.

Fujimori, Y., K. Izumi, et al. (2009). "Isolation of small-sized human epidermal progenitor/stem cells by Gravity Assisted Cell Sorting (GACS)." J Dermatol Sci **56**(3): 181-7.

BACKGROUND: Small diameter characterizes epidermal progenitor/stem cells. We have developed Gravity Assisted Cell Sorting (GACS) to simply enrich small-sized epidermal progenitor/stem cells. OBJECTIVE: The cells sorted by GACS were characterized by fluorescence-activated cell sorting analysis, and cultured for up to 7 weeks. The cultured cells were then used for reconstruction of skin equivalent. METHODS: GACS was performed on primary cultures (primary cell) and passage 6-7 cultures (cultured cell) of keratinocytes. A keratinocyte suspension was sized into two groups: cells trapped by a 20 microm filter (trapped cells), and cells flowing through both a 20 and 11 microm filter (non-trapped cells). RESULTS: In the primary cell groups, viability of the trapped cells was 62.5+/-7.2%

compared to 77.0+/-3.7% for the non-trapped cells. In the cultured cell groups, viability of the trapped cells was 64.3+/-14.9%, compared to the non-trapped cells (93.1+/-2.0%). Flow cytometric analysis showed better discrimination by cell size between trapped and non-trapped cells in culture than in the primary cell suspension. Non-trapped cells contained a larger number of cells with high levels of alpha6 integrin and low levels of CD71 (alpha6 integrin(bri)CD71(dim)), indicating an enriched progenitor/stem cell population. The difference in these markers between the non-trapped and trapped cells was seen in both the primary and cultured cell groups although this difference was more distinct in cultured cells. Culture of both groups showed that cultures originating from the trapped cells senesced after approximately 15 days while the non-trapped keratinocytes grew for up to 40 days. Manufacture of an epidermis/dermal device (artificial skin) showed that non-trapped cells formed a significantly thicker epithelial layer than the trapped cells, demonstrating the enhanced regenerative capability of the smaller diameter, alpha6 integrin(bri)CD71(dim) cells separated by GACS. CONCLUSION: These results indicate that GACS is simple and useful technique to enrich for epidermal progenitor/stem cell populations, and is more efficient when used on cells in culture.

Fujita, A., M. Kobayashi, et al. (1996). "Synergistic response of cord blood myeloid progenitor cells to the combined administration of human granulocyte colony-stimulating factor and human stem cell factor in vitro." Pediatr Res **40**(3): 388-92.

We compared the in vitro response of myeloid progenitor cells [colony-forming units of culture (CFU-C)] that were prepared from human umbilical cord blood to the administration of the combination of granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) versus that of CFU-C obtained from normal human bone marrow. Progenitors were purified according to CD34 expression; the number and size of colonies were evaluated by culture in agar or methylcellulose, respectively. In the presence of G-CSF alone, the mean number of colonies was significantly greater in the bone marrow culture versus that of cord blood. SCF alone bad little effect on colony formation, but in the presence of optimal or suboptimal concentrations of G-CSF, SCF significantly increased colony formation from both cell sources. Its effect on cord blood significantly exceeded that on bone marrow. SCF used in combination with G-CSF significantly increased the size of the colonies with cord blood CFU-C; this effect was less marked with bone marrow CFU-C. The percentage of cells that expressed c-Kit, the SCF receptor, did not appear to differ between the two sources of CFU-C. Results indicate that cord blood CFU-C showed a greater response to SCF in combination with G-CSF than did bone marrow CFU-C.

Fukuchi, Y., H. Nakajima, et al. (2004). "Human placenta-derived cells have mesenchymal stem/progenitor cell potential." Stem Cells **22**(5): 649-58.

Mesenchymal stem/progenitor cells (MSCs) are widely distributed in a variety of tissues in the adult human body (e.g., bone marrow [BM], kidney, lung, and liver). These cells are also present in the fetal environment (e.g., blood, liver, BM, and kidney). However, MSCs are a rare population in these tissues. Here we tried to identify cells with MSC-like potency in human placenta. We isolated adherent cells from trypsin-digested term placentas and established two clones by limiting dilution. We examined these cells for morphology, surface markers, gene expression patterns, and differentiation potential and found that they expressed several stem cell markers, hematopoietic/ endothelial cell-related genes, and organ-specific genes, as determined by reverse transcription-polymerase chain reaction and fluorescence-activated cell sorter analysis. They also showed osteogenic and adipogenic differentiation potentials under appropriate conditions. We suggest that placenta-derived cells have multilineage differentiation potential similar to MSCs in terms of morphology, cell-surface antigen expression, and gene expression patterns. The placenta may prove to be a useful source of MSCs.

Fukuda, S. and L. M. Pelus (2002). "Elevation of Survivin levels by hematopoietic growth factors occurs in quiescent CD34+ hematopoietic stem and progenitor cells before cell cycle entry." Cell Cycle **1**(5): 322-6.

Survivin is a member of the inhibitor of apoptosis protein (IAP) family that is overexpressed during G(2)/M phase in most cancer cells. In contrast, we previously reported that Survivin is expressed throughout the cell cycle in normal CD34(+) hematopoietic stem and progenitor cells stimulated by the combination of Thrombopoietin (Tpo), Stem Cell Factor (SCF) and Flt3 ligand (FL). In order to address whether Survivin expression is specifically up-regulated by hematopoietic growth factors before cell cycle entry, we isolated quiescent CD34(+) cells and investigated Survivin expression in response to growth factor stimulation. Survivin is up-regulated in CD34(+) cells with 2N DNA content following growth factor addition, suggesting it becomes elevated during G(0)/G(1). Survivin is barely detectable in freshly isolated umbilical cord blood (UCB) Ki-

67(negative) and Cyclin D(negative) CD34(+) cells, however incubation with Tpo, SCF and FL for 20 hrs results in up-regulation without entry of cells into cell cycle. Culture of G(0) CD34(+) cells isolated based on Hoechst 33342/PyroninY staining with Tpo, SCF and FL for 48 hrs, results in significantly elevated Survivin mRNA and protein levels. Moreover, labeling of fresh G(0) CD34(+) cells with 5-(and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) before culture with growth factors for up to 72 hrs, revealed that Survivin expression was elevated in CFSE(bright) G(0) CD34(+) cells, indicating that up-regulation occurred before entry into G1. These results suggest that up-regulation of Survivin expression in CD34(+) cells is an early event in cell cycle entry that is regulated by hematopoietic growth factors and does not simply reflect cell cycle progression and cell division.

Gabrilove, J. L., K. White, et al. (1994). "Stem cell factor and basic fibroblast growth factor are synergistic in augmenting committed myeloid progenitor cell growth." Blood **83**(4): 907-10.

Stem cell factor (SCF) and basic fibroblast growth factor (bFGF) are hematopoietic cytokines produced by bone marrow stromal cells. It is known that, although SCF and bFGF have limited clonogenic activity on their own, they can augment colony-stimulating factor (CSF)-mediated progenitor cell growth. Because these factors are both sequestered by stromal cells, we examined their interaction on progenitor cell growth in conjunction with granulocyte-macrophage-CSF (GM-CSF). In this study, we show that clonogenic growth derived from low-density bone marrow cells stimulated by GM-CSF is significantly augmented ($P < .001$) in the presence of maximal (100 ng/mL) concentrations of SCF in combination with 100 ng/mL of bFGF. When CD34+ cells are used, the synergistic effect of bFGF and SCF for GM-CSF-mediated progenitor cell growth is further increased, resulting in as much as a sevenfold increase in detectable colony-forming units granulocyte-macrophage ($P < .001$). These data suggest that the synergistic activity of bFGF and SCF is mediated directly on hematopoietic precursors. These observations suggest that bFGF and SCF, concentrated locally on stromal cell surfaces, might interact in concert with other hematopoietic cytokines to regulate stem cell proliferation and differentiation in hematopoietic niches in the bone marrow.

Gastens, M. H., K. Goltry, et al. (2007). "Good manufacturing practice-compliant expansion of marrow-derived stem and progenitor cells for cell therapy." Cell Transplant **16**(7): 685-96.

Ex vivo expansion is being used to increase the number of stem and progenitor cells for autologous cell therapy. Initiation of pivotal clinical trials testing the efficacy of these cells for tissue repair has been hampered by the challenge of assuring safe and high-quality cell production. A strategy is described here for clinical-scale expansion of bone marrow (BM)-derived stem cells within a mixed cell population in a completely closed process from cell collection through postculture processing using sterile connectable devices. Human BM mononuclear cells (BMMNC) were isolated, cultured for 12 days, and washed postharvest using either standard open procedures in laminar flow hoods or using automated closed systems. Conditions for these studies were similar to long-term BM cultures in which hematopoietic and stromal components are cultured together. Expansion of marrow-derived stem and progenitor cells was then assessed. Cell yield, number of colony forming units (CFU), phenotype, stability, and multilineage differentiation capacity were compared from the single pass perfusion bioreactor and standard flask cultures. Purification of BMMNC using a closed Ficoll gradient process led to depletion of 98% erythrocytes and 87% granulocytes, compared to 100% and 70%, respectively, for manual processing. After closed system culture, mesenchymal progenitors, measured as CD105+CD166+CD14-CD45- and fibroblastic CFU, expanded 317- and 364-fold, respectively, while CD34+ hematopoietic progenitors were depleted 10-fold compared to starting BMMNC. Cultured cells exhibited multilineage differentiation by displaying adipogenic, osteogenic, and endothelial characteristics in vitro. No significant difference was observed between manual and bioreactor cultures. Automated culture and washing of the cell product resulted in 181 x 10(6) total cells that were viable and contained fibroblastic CFU for at least 24 h of storage. A combination of closed, automated technologies enabled production of good manufacturing practice (GMP)-compliant cell therapeutics, ready for use within a clinical setting, with minimal risk of microbial contamination.

Gaudron, S., C. Grillon, et al. (1999). "In vitro effect of acetyl-N-Ser-Asp-Lys-Pro (AcSDKP) analogs resistant to angiotensin I-converting enzyme on hematopoietic stem cell and progenitor cell proliferation." Stem Cells **17**(2): 100-6.

The tetrapeptide Acetyl-N-Ser-Asp-Lys-Pro (AcSDKP), an inhibitor of hematopoietic stem cell proliferation, is known to reduce in vivo the damage resulting from treatment with chemotherapeutic agents or ionizing radiation on the stem cell compartment. Recently, AcSDKP has been shown to be a physiological substrate of the N-active site of

angiotensin I-converting enzyme (ACE). Four analogs of the tetrapeptide expressing a high stability towards ACE degradation in vitro have been synthesized in order to provide new molecules likely to improve the myeloprotection displayed by AcSDKP. These analogs are three pseudopeptides with a modified peptidic bond, Ac-Serpsi(CH2-NH)Asp-Lys-Pro, Ac-Ser-Asppsi(CH2-NH)Lys-Pro, Ac-Ser-Asp-Lyspsi(CH2-N)Pro, and one C-terminus modified peptide (AcSDKP-NH2). We report here that these analogs reduce in vitro the proportion of murine colony-forming units-granulocyte/macrophage in S-phase and inhibit the entry into cycle of high proliferative potential colony-forming cells. The efficacy of AcSDKP analogs in preventing in vitro primitive hematopoietic stem cells from entering into cycle suggests that these molecules could be new candidates for the powerful inhibition of hematopoietic stem and progenitor cell proliferation in vivo.

Georgantas, R. W., 3rd, R. Hildreth, et al. (2007). "CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control." Proc Natl Acad Sci U S A **104**(8): 2750-5.

MicroRNAs (miRNAs) are a recently identified class of epigenetic elements consisting of small noncoding RNAs that bind to the 3' untranslated region of mRNAs and down-regulate their translation to protein. miRNAs play critical roles in many different cellular processes including metabolism, apoptosis, differentiation, and development. We found 33 miRNAs expressed in CD34+ hematopoietic stem-progenitor cells (HSPCs) from normal human bone marrow and mobilized human peripheral blood stem cell harvests. We then combined these data with human HSPC mRNA expression data and with miRNA-mRNA target predictions, into a previously undescribed miRNA:mRNA interaction database called the Transcriptome Interaction Database. The in silico predictions from the Transcriptome Interaction Database pointed to miRNA control of hematopoietic differentiation through translational control of mRNAs critical to hematopoiesis. From these predictions, we formulated a model for miRNA control of stages of hematopoiesis in which many of the genes specifying hematopoietic differentiation are expressed by HSPCs, but are held in check by miRNAs until differentiation occurs. We validated miRNA control of several of these target mRNAs by demonstrating that their translation in fact is decreased by miRNAs. Finally, we chose miRNA-155 for functional characterization in hematopoiesis, because we predicted that it would control both myelopoiesis and erythropoiesis. As predicted, miRNA-155 transduction greatly reduced both myeloid and erythroid colony formation of normal human HSPCs.

Gersdorff Korsgaard, M. P., P. Christophersen, et al. (2001). "Identification of a novel voltage-gated Na+ channel rNa(v)1.5a in the rat hippocampal progenitor stem cell line HiB5." Pflugers Arch **443**(1): 18-30.

A conditionally immortalised cell line, HiB5, derived from embryonic hippocampal precursor cells expressed a voltage-gated Na+ channel with electrophysiological characteristics shifted to more negative voltages and a lower sensitivity to tetrodotoxin [concentration required for half-maximal inhibition (IC50) 0.9 microM] compared with endogenous neuronal Na+ channels. The channel activation and steady-state inactivation occurred at very negative potentials with the threshold for activation at -55 mV and half-maximal inactivation at -78.7 mV. The channel was blocked by lamotrigine and sipatrigine voltage and state dependently, with potencies 5-20 times higher (IC50 12 and 1.8 microM at -80 mV respectively) than the corresponding block of endogenous Na+ channels from neurones and cloned rNa(v)1.2a (rBIIA) alpha-subunits. Both compounds slowed the channel's recovery from inactivation. Whereas lamotrigine and sipatrigine had similar effects on the fast inactivated state, the effect of sipatrigine on the slow inactivation state was more pronounced, rendering this compound overall the more effective. The molecular subtype mainly expressed by HiB5 cells was identified using RT-PCR and was a novel splice variant of rNa(v)1.5 (rNa(v)1.5a). It differs from the known rNa(v)1.5 version in that it lacks 53 amino acids in the intracellular loop between domains II and III. rNa(v)1.5a was also detected in mRNA derived from rat whole brain.

Ghiaur, G., A. Lee, et al. (2006). "Inhibition of RhoA GTPase activity enhances hematopoietic stem and progenitor cell proliferation and engraftment." Blood **108**(6): 2087-94.

Ras-related Rho GTPases regulate actin cytoskeletal organization, adhesion, gene transcription, and cell-cycle progression. The Rac subfamily of Rho GTPases and Cdc42 has been shown to play essential roles in hematopoietic stem cell (HSC) engraftment and mobilization. Here, we study the role of RhoA, a related Rho GTPase, in HSC functions. Using retrovirus-mediated gene transfer of a dominant-negative (DN) mutant of RhoA (RhoAN19), we demonstrate that down-regulation of RhoA activity resulted in increased HSC engraftment and self-renewal as measured by competitive repopulation and serial transplantation assays. However, overexpression of RhoAN19 resulted in

decreased migration toward SDF-1alpha and alpha(4)beta(1)- and alpha(5)beta(2)-integrin-mediated adhesion of hematopoietic progenitor cells in vitro. Low RhoA activity was associated with higher proliferation rate of hematopoietic progenitor cells and increased cells in active phases of cell cycle, most likely via decreasing p21Cip/Waf expression and increasing cyclin D1 levels. Thus, reducing RhoA activity by optimizing the balance between adhesion/migration and proliferation/self-renewal results in a net increase in HSC engraftment. This mechanism could provide a novel therapeutic target to enhance HSC therapies.

Gibbons, A. E., P. Price, et al. (1995). "Analysis of hematopoietic stem and progenitor cell populations in cytomegalovirus-infected mice." Blood **86**(2): 473-81.

We have studied the effects of murine cytomegalovirus (MCMV) infection on bone marrow stem and progenitor cell populations to find an explanation for the defects in hematopoiesis that accompany CMV infections in patients. Sublethal MCMV infection of BALB/c mice resulted in a 5- to 10-fold decrease in the numbers of myeloid (colony-forming unit-granulocyte-macrophage [CFU-GM]) and erythroid (burst-forming unit-erythroid [BFU-E]) progenitor cells in the marrow, but not in primitive myeloerythroid progenitor cell (colony-forming unit-spleen [CFU-S]) numbers. In contrast, we observed a 10- to 20-fold reduction in CFU-S as well as CFU-GM and BFU-E in lethally infected mice. Depletion of marrow CFU-GM was less severe in C57BL/10 and C3H/HeJ mice, which are more resistant to the effects of MCMV infection. Treatment of bone marrow cells with MCMV preparations in vitro did not reduce the numbers of CFU-GM, although up to 10% of the cells were productively infected. This finding suggests that CFU-GM were not susceptible to lytic MCMV infection in vitro and are probably not eliminated by lytic infection in vivo. Increases in the frequencies of Sca-1+Lin- marrow cells, a population that includes cells with the characteristics of pluripotential stem cells, were observed in MCMV-infected BALB/c, C57BL/10, and DBA/2J mice. Increases in the frequencies of c-kit+Lin- marrow cells were only seen in DBA/2J mice. MCMV infection did not impair the function of pluripotential stem cells because transplantation of marrow from MCMV-infected donors into irradiated recipient mice resulted in successful reconstitution of the T, B, and myeloid cell lineages.

Giebel, B. (2008). "Cell polarity and asymmetric cell division within human hematopoietic stem and progenitor cells." Cells Tissues Organs **188**(1-2): 116-26.

Like other somatic stem cells, hematopoietic stem cells (HSC) contain the capacity to self-renew and to give rise to committed progenitor cells that are able to replenish all hematopoietic cell types. To keep a constant level of HSC, the decision whether their progeny maintain the stem cell fate or become committed to differentiation needs to be highly controlled. In this context it became evident that HSC niches fulfill important functions in keeping the level of HSC more or less constant. Before discovering such niches, it was widely assumed that HSC divide asymmetrically to give birth to a daughter cell maintaining the stem cell fate and to another one which is committed to differentiation. Here, I summarize some of the experimental data being compatible with the model of asymmetric cell division and review some of our latest findings, which demonstrate the occurrence of asymmetric cell divisions within the HSC and hematopoietic progenitor cell compartment. Since cell polarity is an essential prerequisite for asymmetrically dividing as well as for migrating cells, I will also discuss some aspects of cell polarity of primitive hematopoietic cells.

Giebel, B. and J. Beckmann (2007). "Asymmetric cell divisions of human hematopoietic stem and progenitor cells meet endosomes." Cell Cycle **6**(18): 2201-4.

Hematopoietic stem cells (HSC) are undifferentiated cells, which self-renew over a long period of time and give rise to committed hematopoietic progenitor cells (HPC) containing the capability to replenish the whole blood system. Since both uncontrolled expansion as well as loss of HSC would be fatal, the decision of self-renewal versus differentiation needs to be tightly controlled. There is good evidence that both HSC niches as well as asymmetric cell divisions are involved in controlling whether HSC self-renew or become committed to differentiate. In this context, we recently identified four proteins which frequently segregate asymmetrically in dividing HSC/HPC. Remarkably, three of these proteins, the tetraspanins CD53 and CD63, and the transferrin receptor are endosome-associated proteins. Here, we highlight these observations in conjunction with recent findings in model organisms which show that components of the endosomal machinery are involved in cell-fate specification processes.

Giebel, B. and I. Bruns (2008). "Self-renewal versus differentiation in hematopoietic stem and progenitor cells: a focus on asymmetric cell divisions." Curr Stem Cell Res Ther **3**(1): 9-16.

Like other somatic stem cells, hematopoietic stem cells (HSC) have the ability to either self-renew

or to differentiate. They are essentially required for the hematopoietic homeostasis. In this context HSC do not only need to replenish peripheral blood cells of all lineages, but also have to keep their pool relatively constant. Since disruption of the underlying control mechanisms can lead to degeneration or expansion of the HSC-pool as it occurs after irradiation or in leukemia, it is an important concern to unveil mechanisms that govern the decision of self-renewal versus differentiation in HSC-biology. There is good evidence that certain extrinsic cues provided in a special environment, the HSC-niches, essentially take part in regulating the HSC-pool in vivo and might also be involved in leukemogenesis. Apart from that, asymmetric cell divisions seem to be another control instance in hematopoietic homeostasis. It has been shown that siblings of primitive hematopoietic cells often adopt different cell fates, and very recently we identified four proteins that segregate asymmetrically in a proportion of dividing primitive hematopoietic cells. Whether asymmetric cell division participates in leukemogenesis, remains to be investigated. However, on the example of neural stem cells of the Drosophila larvae, the neuroblasts, asymmetrically segregating molecules have been identified, i.e. the tumor suppressor protein Brat and the transcription factor Prospero, that are required to suppress self-renewal in one of the arising daughter cells and whose loss of function results in tumor formation. These findings provide an attractive model of how defects in the process of asymmetric cell divisions might transform normal HSC/HPC into leukemic cells.

Ginsburg, H., T. Jehuda-Cohen, et al. (1986). "Does stem cell self renewal and progenitor cell commitment operate through an effector-memory cell mechanism?" Immunol Lett **13**(3): 107-19.

We propose a model for stem cell self renewal and transition into commitment towards a variety of cell lineages. In this model the production of both "effector cells" (as represented by the mature cells in the different cell lineages) and of progenitor "memory" lymphocytes, takes place concomitantly. The experimental evidence supporting this model is as follows: Pure lymphocytic suspensions (PLS) are established and persist in culture when nude mouse-spleen and lymph-node cells are maintained on X-irradiated fibroblast monolayers in the presence of the S-phase cytotoxic agent cytosine arabinoside (Ara-C). From these PLS the following colony types can be initiated by the corresponding inducing (stimulating) factors (CSF): histiocytes (tissue macrophages) - CSF-1; granulocytes-macrophages (GM) - CSF-GM; mast cells - MMSF; granular-NK mucus secreting cells - IL-2; and multilineage colonies - IL-3. Mitotically active blast cells (formed by transformation of lymphocytes), condense into motile small cells when the stimulatory factor is removed. These "memory" lymphocytes are committed as they carry the receptors for the specific CSF; they respond by retransformation into blast cells. A dramatic increase in mast-cell colony forming cells is found in bone marrow, spleen and lymph-nodes of mice infected with Schistosoma mansoni. By maintaining PLS with both Ara-C and each of the CSFs and then titrating the incidence of CFC in the residual PLS, we find that each one of the CSFs acts on an independent set of cells in the PLS to produce the corresponding colony type. Finally, the concept suggests that the various blast cells carrying the receptors, undergo condensation into memory lymphocytes when dissociated from the environment prevailed by the corresponding CSF. In this way pluripotential blast-cells condense into motile lymphocytes which are committed to pluripotentiality.

Glaspy, J. A., E. J. Shpall, et al. (1997). "Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients." Blood **90**(8): 2939-51.

The safety and optimal dose and schedule of stem cell factor (SCF) administered in combination with filgrastim for the mobilization of peripheral blood progenitor cells (PBPCs) was determined in 215 patients with high-risk breast cancer. Patients received either filgrastim alone (10 microg/kg/d for 7 days) or the combination of 10 microg/kg/d filgrastim and 5 to 30 microg/kg/d SCF for either 7, 10, or 13 days. SCF patients were premedicated with antiallergy prophylaxis. Leukapheresis was performed on the final 3 days of cytokine therapy and, after high-dose chemotherapy and infusion of PBPCs, patients received 10 microg/kg/d filgrastim until absolute neutrophil count recovery. The median number of CD34+ cells collected was greater for patients receiving the combination of filgrastim and SCF, at doses greater than 10 microg/kg/d, than for those receiving filgrastim alone (7.7 v 3.2 x 10(6)/kg, $P < .05$). There were significantly ($P < .05$) more CD34+ cells harvested for the 20 microg/kg/d SCF (median, 7.9 x 10(6)/kg) and 25 microg/kg/d SCF (median, 13.6 x 10(6)/kg) 7-day combination groups than for the filgrastim alone patients (median, 3.2 x 10(6)/kg). The duration of administration of SCF and filgrastim (7, 10, or 13 days) did not significantly affect CD34+ cell yield. Treatment groups mobilized with filgrastim alone or with the cytokine combination had similar hematopoietic engraftment and overall survival after PBPC infusion. In conclusion, the results of this study indicate that SCF therapy enhances CD34+ cell yield and is associated with manageable levels of toxicity when combined with filgrastim for PBPC mobilization. The combination of 20 microg/kg/d SCF

and 10 microg/kg/d filgrastim with daily apheresis beginning on day 5 was selected as the optimal dose and schedule for the mobilization of PBPCs.

Goldschneider, I., D. Metcalf, et al. (1980). "Analysis of rat hemopoietic cells on the fluorescence-activated cell sorter. I. Isolation of pluripotent hemopoietic stem cells and granulocyte-macrophage progenitor cells." J Exp Med **152**(2): 419-37.

A scheme is presented whereby pluripotent hemopoietic stem cells (PHSC) from rat bone marrow can be enriched 320-fold with the aid of the fluorescence- activated cell sorter. This scheme is based on the observations that PHSC are strongly positive for Thy-1 antigen (upper 10th percentile); have light- scattering properties (size distribution) between those of bone marrow lymphocytes and myeloid progenitor cells; and are relatively resistant to cortisone. It is estimated that PHSC may constitute 80 percent of the cells isolated according to these parameters. Candidate PHSC are described at the light and electron microscopic levels. At least two populations of accessory cells appear to influence the number and/or the nature of the hemopoietic colonies that form in the in vivo spleen colony-forming unit assay. Putative amplifier cells are strongly Thy-1(+) and cortisone sensitive; putative suppressor cells are weakly Thy-1(+) and cortisone resistant. Three subsets of granulocyte (G) -macrophage (M) progenitor cells (in vitro colony-forming cells [CFC]) are identified on the basis of relative fluorescence intensity for Thy-1 antigen: G-CFC are strongly Thy-l(+); M-CFC are weakly Thy-l(+); and cells that produce mixed G and M CFC have intermediate levels of Thy-1. GM-cluster-forming cells and mature G and M are Thy-1(-). The results suggest that G-CFC are bipotential cells that give rise to G and M-CFC; and that the latter produce mature M through a cluster-forming cell intermediate. Thy-1 antigen is also demonstrated on members of the eosinophil, megakaryocyte, erythrocyte, and lymphocyte cell series in rat bone marrow. In each instance, the relative concentration of Thy-1 antigen is inversely related to the state of cellular differentiation.

Gonzalez-Barca, E., A. Fernandez de Sevilla, et al. (2000). "Autologous stem cell transplantation (ASCT) with immunologically purged progenitor cells in patients with advanced stage follicular lymphoma after early partial or complete remission: toxicity, follow-up of minimal residual disease and survival." Bone Marrow Transplant **26**(10): 1051-6.

The role of autologous stem cell transplant (ASCT) in indolent lymphomas is a controversial issue. From 1994 to 1999, we performed ASCT with immunologically purged progenitor cells in 15 patients with advanced stage follicular lymphoma (FL) after early partial or complete remission. Results of the purging strategy and follow-up of minimal residual disease after transplant were analyzed with PCR amplification of bcl-2/IgH rearrangement for the t(14;18) translocation. A comparison of transplanted patients with a group of controls was carried out to evaluate differences in progression-free survival and overall survival. Eighty percent of patients received one chemotherapy regimen before ASCT and were in first remission. All the patients received cyclophosphamide plus hyperfractionated total body irradiation as the conditioning regimen. Nine patients were transplanted with bone marrow (BM) and six with peripheral blood progenitor cells (PBPC). Engraftment was delayed in one patient transplanted with BM. Two patients died during the transplant procedure. Ten of 12 evaluable patients were PCR positive in the BM for bcl-2 rearrangement at diagnosis. Six of them (60%) were still positive after chemotherapy, and one patient was transplanted with a positive hematopoietic product after purging. With a median follow-up of 27 months, six of eight evaluable patients still remain PCR negative in the BM. With a median follow-up of 4.7 years from diagnosis, progression-free survival was 83% (95% CI: 63-100). The risk of disease progression of non-transplanted patients was 19.2 times higher than that of transplanted patients (P = 0.01), but no differences were found in overall survival. Regarding patients in first remission, the risk of relapse was 12.6 times higher in non-transplanted than in transplanted patients (P = 0.04). This procedure seems to offer a good chance to achieve a PCR-negative state and prolonged freedom from recurrence. According to these results, prospective randomized trials are warranted.

Gonzalez-Requejo, A., L. Madero, et al. (1998). "Progenitor cell subsets and engraftment kinetics in children undergoing autologous peripheral blood stem cell transplantation." Br J Haematol **101**(1): 104-10.

The main objective of the present study was to determine the role of CD34+ cell subsets in the haemopoietic recovery of children undergoing peripheral blood stem cell transplantation. For this purpose, 38 leukaphereses from 33 children with malignancies mobilized with G-CSF were analysed. Using dual-colour flow cytometry, different subpopulations of CD34+ cells were quantified and the number of each reinfused subsets correlated with haemopoietic resurgence. Multivariate analysis showed that the number of CD34+CD38- cells and CD34+CD38+ cells correlated better with time to neutrophil and platelet recovery, respectively, than the total number of CD34+ cells. Threshold values for

rapid haemopoietic recovery, determined by the receiver operating characteristic analysis, were found to be 0.5 X 10(6) CD34+CD38- cells for neutrophil engraftment, and 2.0x10(6) CD34+CD38+ cells for platelet recovery. It is suggested that the analysis of CD34+ cell subsets could increase understanding of the repopulation capacity of a given leukapheresis product in peripheral blood stem cell transplantation procedures in children. In particular, this procedure could be extremely useful when low numbers of CD34+ cells are collected.

Gordon, M. Y., N. Levicar, et al. (2006). "Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor." Stem Cells **24**(7): 1822-30.

A phase I study was performed to determine the safety and tolerability of injecting autologous CD34(+) cells into five patients with liver insufficiency. The study was based on the hypothesis that the CD34(+) cell population in granulocyte colony-stimulating factor (G-CSF)-mobilized blood contains a subpopulation of cells with the potential for regenerating damaged tissue. We separated a candidate CD34(+) stem cell population from the majority of the CD34(+) cells (99%) by adherence to tissue culture plastic. The adherent and nonadherent CD34(+) cells were distinct in morphology, immunophenotype, and gene expression profile. Reverse transcription-polymerase chain reaction-based gene expression analysis indicated that the adherent CD34(+) cells had the potential to express determinants consistent with liver, pancreas, heart, muscle, and nerve cell differentiation as well as hematopoiesis. Overall, the characteristics of the adherent CD34(+) cells identify them as a separate putative stem/progenitor cell population. In culture, they produced a population of cells exhibiting diverse morphologies and expressing genes corresponding to multiple tissue types. Encouraged by this evidence that the CD34(+) cell population contains cells with the potential to form hepatocyte-like cells, we gave G-CSF to five patients with liver insufficiency to mobilize their stem cells for collection by leukapheresis. Between 1 x 10(6) and 2 x 10(8) CD34(+) cells were injected into the portal vein (three patients) or hepatic artery (two patients). No complications or specific side effects related to the procedure were observed. Three of the five patients showed improvement in serum bilirubin and four of five in serum albumin. These observations warrant further clinical trials.

Gordon, M. Y., S. B. Marley, et al. (2000). "Contact-mediated inhibition of human haematopoietic progenitor cell proliferation may be conferred by stem cell antigen, CD34." Hematol J **1**(2): 77-86.

INTRODUCTION: The function of CD34, a transmembrane sialomucin expressed by human haematopoietic progenitor cells, is poorly understood. Its structure suggests it may act as a cell adhesion and signalling molecule. MATERIALS AND METHODS: KGIa cells and primary CD34-positive marrow cells were tested for their ability to aggregate in the presence of the anti-CD34 antibody QBEND10; CFU-GM colonies were grown using standard methods and tested for their content of colony-forming cells by replating; 'haematons' were isolated from marrow by filtration; the phosphorylation of CD34 was investigated by immunoprecipitation and Western blotting DISCUSSION: CD34-positive cells in human bone marrow, like KG1a cells, aggregate when incubated with QBEND10. Staining aggregates with anti-CD34-FITC revealed that aggregation involved co-localisation of CD34 at intercellular binding sites. We examined myeloid colonies (CFU-GM) grown from normal human bone marrow cells, and multicellular aggregates ('haematons') separated from freshly aspirated marrow by filtration, and found CD34-positive cells bound together with co-localisation of the CD34 at the binding sites. This finding shows that CD34-positive cell-cell adhesion occurs physiologically in vitro and in vivo. QBEND10-induced aggregation of KG1a and CD34-positive cells was enhanced by staurosporine (a protein kinase C inhibitor) and inhibited by genistein (a protein tyrosine kinase inhibitor). Moreover, aggregated cells had increased phosphorylation of tyrosine on CD34 and translocation of protein kinase C (PKC) to the cytoplasm, compared with non-aggregated cells. We used the ability of primary colonies to produce secondary colonies on replating as a functional parameter and found that the replating ability of the colonies was increased by treatment with genistein (P=0.003). In addition, the ability of individual samples of primary CD34-positive cells to undergo QBEND10-induced aggregation and the ability of CD34-positive cell-derived colonies to produce secondary clones on replating were inversely related (r=0.86). CONCLUSION: Our results suggest that homotypic aggregation of haematopoietic progenitor cells may be an important mechanism for preventing inappropriate proliferation in vivo. Thus, regulation of expression of the CD34 molecule may play an important role in maintaining the normal level of haematopoietic activity by contact-mediated inhibition of progenitor cell proliferation.

Goussetis, E., M. Theodosaki, et al. (2003). "Kinetics of quiescent cord blood stem/progenitor cells with

high proliferative potential in stem-cell expansion culture." Cytotherapy **5**(6): 500-8.

BACKGROUND: The most primitive engrafting hematopoietic stem cell (HSC) resides mainly in a tumor growth factor-beta (TGF-beta)-dependent quiescent phase of the cell cycle. In this study, ex vivo expansion of UC blood (UCB) HSCs has been investigated, with the aim of showing whether quiescent HSCs can be recovered from expansion culture. METHODS: AC133(+) stem/progenitor cells from six full term-pregnancies UCB-samples were immunomagnetically selected, followed by ex vivo expansion culture in the presence of thrombopoietin (TPO), c-kit ligand (KL), flt-3 ligand (FL) and IL-6. Quiescent HSCs were detected by a clonogenic assay that allows the detection of multipotent and committed single- lineage quiescent stem/progenitor cells, named mHPP-Q and cHPP-Q, respectively, by means of a TGF-beta blocking Ab. RESULTS: Expansion culture of fresh selected AC133(+) cells for 1 week caused maintenance rather than expansion of mHPP-Q cells and a 1-fold increase in cHPP-Q cells. A further week culture initiated with 7-day expanded AC133(+) cells resulted in an additional 1.5-fold expansion of cHPP-Q while no mHPP-Q cells could be detected. Amplification of cHPP-Q cells in long-term expansion cultures initiated with 14-day expanded AC133(+) cells was observed for at least a further 4 weeks. DISCUSSION: A small proportion of HPP-Q cells recovered from 7-day expansion cultures retain their multilineage potential: longer culturing of these cells results in the loss of multilineage potential while they maintain quiescent behavior and high proliferative potential.

Gouti, M. and A. Gavalas (2008). "Hoxb1 controls cell fate specification and proliferative capacity of neural stem and progenitor cells." Stem Cells **26**(8): 1985-97.

The directed differentiation of embryonic stem cells (ESCs) into neural stem cells (NSCs) of specific identities and the identification of endogenous pathways that may mediate expansion of NSCs are fundamental goals for the treatment of degenerative disorders and trauma of the nervous system. We report that timely induction of a Hoxb1 transgene in ESC-derived NSCs resulted in the specification of NSCs toward a hindbrain-specific identity through the activation of a rhombomere 4-specific genetic program and the repression of anterior neural identity. This change was accompanied by changes in signaling pathways that pattern the dorsoventral (DV) axis of the nervous system and concomitant changes in the expression of DV neural progenitor markers. Furthermore, Hoxb1 mediated the maintenance and expansion of posterior neural progenitor cells. Hoxb1(+) cells kept proliferating upon mitogen withdrawal and became transiently amplifying progenitors instead of terminally differentiating. This was partially attributed to Hoxb1-dependent activation of the Notch signaling pathway and Notch-dependent STAT3 phosphorylation at Ser 727, thus linking Hox gene function with maintenance of active Notch signaling and the JAK/STAT pathway. Thus, timely expression of specific Hox genes could be used to establish NSCs and neural progenitors of distinct posterior identities. ESC-derived NSCs have a mixed DV identity that is subject to regulation by Hox genes. Finally, these findings set the stage for the elucidation of molecular pathways involved in the expansion of posterior NSCs and neural progenitors. Disclosure of potential conflicts of interest is found at the end of this article.

Grassinger, J., D. N. Haylock, et al. (2009). "Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins." Blood **114**(1): 49-59.

Osteopontin (OPN), a multifunctional acidic glycoprotein, expressed by osteoblasts within the endosteal region of the bone marrow (BM) suppresses the proliferation of hemopoietic stem and progenitor cells and also regulates their lodgment within the BM after transplantation. Herein we demonstrate that OPN cleavage fragments are the most abundant forms of this protein within the BM. Studies aimed to determine how hemopoietic stem cells (HSCs) interact with OPN revealed for the first time that murine and human HSCs express alpha(9)beta(1) integrin. The N-terminal thrombin cleavage fragment of OPN through its binding to the alpha(9)beta(1) and alpha(4)beta(1) integrins plays a key role in the attraction, retention, regulation, and release of hemopoietic stem and progenitor cells to, in, and from their BM niche. Thrombin-cleaved OPN (trOPN) acts as a chemoattractant for stem and progenitor cells, mediating their migration in a manner that involves interaction with alpha(9)beta(1) and alpha(4)beta(1) integrins. In addition, in the absence of OPN, there is an increased number of white blood cells and, specifically, stem and progenitor cells in the peripheral circulation.

Gratama, J. W., J. Kraan, et al. (2003). "Validation of the single-platform ISHAGE method for CD34(+) hematopoietic stem and progenitor cell enumeration in an international multicenter study." Cytotherapy **5**(1): 55-65.

BACKGROUND: Flow cytometric enumeration of CD34+ hematopoietic sterm and progenitor cells (HPC) is the reference point for

undertaking apheresis and evaluation of adequacy for PBSC engraftment. An external quality assurance (EQA) scheme for CD34+ HPC enumeration has been operational in Belgium, Netherlands and Luxemburg (Benelux) since 1995. Within this group, a multicenter survey was held to validate the state-of-the-art methodology, i.e., multiparametric definition of HPC based on light scatter, expression of CD34 and CD45, and counting beads (i.e., 'single platform ISHAGE' method). METHODS: 'Real-time' EQA was used to monitor the application of the single-platform ISHAGE method by 36 participants. Three send-outs of stabilized blood with CD34+ cell counts 35-60 cells/microl were distributed to 36 participants, who were required to assay the samples on three occasions using the standard assay and their local techniques. These results were compared with thosed obtained by 111-116 UK NEQAS participants testing the same specimens. RESULTS: Using the single platform ISHAGE methods, between-laboratory coefficients of variations (CVs) as low as 10% were achieved. Intra-laboratory CVs were < 5% for approximately 50% of the participants. Local single-platform techniques yielded between-laboratory CVs as low as 9% in both Benelux and UK NEQAS cohorts. In contrast, the lowest between-laboratory CVs using dual-platform techniques were 17% (Benelux) and 21% (UK NEQAS), respectively. CONCLUSION: The single-platform ISHAGE method for CD34+ cell enumeration has been validated by an international group of 36 laboratories. The observed varation between laboratories allows a meaningful comparison of CD34+ cell enumeration.

Gratama, J. W., A. Orfao, et al. (1998). "Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis." Cytometry **34**(3): 128-42.

The need for a rapid and reliable marker for the engraftment potential of hematopoietic stem and progenitor cell (HPC) transplants has led to the development of flow cytometric assays to quantitate such cells on the basis of their expression of CD34. The variability associated with enumeration of low-frequency cells (i.e., as low as 0.1% or 5 cells/microl) is exceedingly large, but recent developments have improved the accuracy and precision of the assay. Here, we review and compare the major techniques. Based on the current state of the art, we recommend 1) bright fluorochrome conjugates of class II or III monoclonal antibodies (mAbs) that detect all glycoforms of CD34, 2) use of a vital nucleic acid dye to exclude platelets, unlysed red cells, and debris or use of 7-amino actinomycin D to exclude dead cells during data acquisition, 3) counterstaining with CD45 mAb to be included in the definition of HPC, 4) during list mode data analysis, Boolean gating to resolve the CD34+ HPCs from irrelevant cell populations on the basis of the low levels of CD45 expression and low sideward light-scatter signals of HPCs, 5) inclusion of CD34dim and CD34bright populations in the CD34+ cell count, 6) omission of the negative control staining, and 7) for apheresis products, enumeration of at least 100 CD34+ cells to ensure a 10% precision. Unresolved technical questions are 1) the replacement of conventional dual-platform by single-platform assay formats, i.e., derivation of absolute CD34+ cell counts from a single flow cytometric assessment instead of from combined flow cytometer (percent CD34+) and hematology analyzer (absolute leukocyte count) data, 2) the cross-calibration of the available single-platform assays, and 3) the optimal method for sample preparation. An important clinical question to be addressed is the definition of the precise phenotypes and required numbers of HPCs responsible for short- and long-term recovery to optimize HPC transplant strategies.

Guardiola, P., V. Runde, et al. (2002). "Retrospective comparison of bone marrow and granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for allogeneic stem cell transplantation using HLA identical sibling donors in myelodysplastic syndromes." Blood **99**(12): 4370-8.

In this multicenter retrospective study, the outcomes of 234 patients with myelodysplastic syndrome (MDS) who underwent transplantation between 1995 and 1999 from HLA-identical siblings were analyzed according to the hematopoietic stem cell source used, that is, bone marrow (BM, n = 132) or granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells (PBPCs, n = 102). There were 69 cases of refractory anemia (RA), 86 RA with excess blasts (RAEB), 75 RAEB in transformation (RAEB-t), and 4 unclassified MDS at diagnosis. The International Prognostic Scoring System was intermediate-2 or high in 104 of the 158 available scores. Multivariate analyses focused on transplantation-related mortality (TRM), 2-year treatment failure incidence, and survival. Use of PBPCs reduced the median duration of neutropenia and thrombocytopenia by 4 and 12 days, respectively. The incidence of acute GVHD was similar whatever the graft type used. Chronic GVHD was more likely to have occurred with PBPCs (odds ratio [OR], 1.62; 95% confidence interval [CI], 0.87-3.02). Two-year TRM was significantly reduced with PBPCs (relative risk [RR], 0.33; 95% CI, 0.15-0.73; P <.007), except for patients who had either RA or high-risk cytogenetics. The 2-year treatment failure incidence was significantly decreased with PBPCs, from 38% to

13% (RR, 0.22; 95% CI, 0.10-0.48; P <.001). Estimate of the 2-year event-free survival was 50% with PBPCs versus 39% with BM. In multivariate analysis, the outcome was significantly improved with PBPCs (RR, 0.27; 95% CI, 0.13-0.52; P <.001), except for patients with either RA or high-risk cytogenetics. In conclusion, PBPCs might be preferred for allogeneic transplantation in MDS patients at high risk for relapse on the basis of morphologic criteria because the use of this hematopoietic stem cell was associated with lower treatment failure incidence and improved survival.

Gudmundsson, K. O., L. Thorsteinsson, et al. (2007). "Gene expression analysis of hematopoietic progenitor cells identifies Dlg7 as a potential stem cell gene." Stem Cells **25**(6): 1498-506.

Inducible hematopoietic stem/progenitor cell lines represent a model for studying genes involved in self-renewal and differentiation. Here, gene expression was studied in the inducible human CD34+ acute myelogenous leukemia cell line KG1 using oligonucleotide arrays and suppression subtractive cloning. Using this approach, we identified Dlg7, the homolog of the Drosophila Dlg1 tumor suppressor gene, as downregulated at the early stages of KG1 differentiation. Similarly, Dlg7 was expressed in normal purified umbilical cord blood CD34+CD38- progenitors but not in the more committed CD34+CD38+ population. Dlg7 expression was not detected in differentiated cells obtained from hematopoietic colonies, nor was expression detected in purified T-cells, B-cells, and monocytes. When analyzed in different types of stem cells, Dlg7 expression was detected in purified human bone marrow-derived CD133+ progenitor cells, human mesenchymal stem cells, and mouse embryonic stem (ES) cells. Overexpression of Dlg7 in mouse ES cells increased their growth rate and reduced the number of EBs emerging upon differentiation. In addition, the EBs were significantly smaller, indicating an inhibition in differentiation. This inhibition was further supported by higher expression of Bmp4, Oct4, Rex1, and Nanog in EBs overexpressing Dlg7 and lower expression of Brachyury. Finally, the Dlg7 protein was detected in liver and colon carcinoma tumors but not in normal adjacent tissues, suggesting a role for the gene in carcinogenesis. In conclusion, our results suggest that Dlg7 has a role in stem cell survival, in maintaining stem cell properties, and in carcinogenesis. Disclosure of potential conflicts of interest is found at the end of this article.

Guo, Y., B. Graham-Evans, et al. (2006). "Murine embryonic stem cells secrete cytokines/growth modulators that enhance cell survival/anti-apoptosis and stimulate colony formation of murine hematopoietic progenitor cells." Stem Cells **24**(4): 850-6.

Stromal cell-derived factor (SDF)-1/CXCL12, released by murine embryonic stem (ES) cells, enhances survival, chemotaxis, and hematopoietic differentiation of murine ES cells. Conditioned medium (CM) from murine ES cells growing in the presence of leukemia inhibitory factor (LIF) was generated while the ES cells were in an undifferentiated Oct-4 expressing state. ES cell-CM enhanced survival of normal murine bone marrow myeloid progenitors (CFU-GM) subjected to delayed growth factor addition in vitro and decreased apoptosis of murine bone marrow c-kit(+)lin- cells. ES CM contained interleukin (IL)-1alpha, IL-10, IL-11, macrophage-colony stimulating factor (CSF), oncostatin M, stem cell factor, vascular endothelial growth factor, as well as a number of chemokines and other proteins, some of which are known to enhance survival/anti-apoptosis of progenitors. Irradiation of ES cells enhanced release of some proteins and decreased release of others. IL-6, FGF-9, and TNF-alpha, not detected prior to irradiation was found after ES cells were irradiated. ES cell CM also stimulated CFU-GM colony formation. Thus, undifferentiated murine ES cells growing in the presence of LIF produce/release a number of biologically active interleukins, CSFs, chemokines, and other growth modulatory proteins, results which may be of physiological and/or practical significance.

Hacein-Bey, S., G. D. Basile, et al. (1998). "gammac gene transfer in the presence of stem cell factor, FLT-3L, interleukin-7 (IL-7), IL-1, and IL-15 cytokines restores T-cell differentiation from gammac(-) X-linked severe combined immunodeficiency hematopoietic progenitor cells in murine fetal thymic organ cultures." Blood **92**(11): 4090-7.

X-linked severe combined immunodeficiency (SCID-Xl) is a rare human inherited disorder in which early T and natural killer (NK) lymphocyte development is blocked. The genetic disorder results from mutations in the common gammac chain that participates in several cytokine receptors including the interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15 receptors. We have shown in a previous report that gammac gene transfer into SCID-Xl bone marrow (BM) cells restores efficient NK cell differentiation. In this study, we have focused on the introduction of the gammac gene into SCID-Xl hematopoietic stem cells with the goal of obtaining differentiation into mature T cells. For this purpose, we used the in vitro hybrid fetal thymic organ culture (FTOC) system in which a combination of cytokines consisting of stem cell factor (SCF), Flt-3L, IL-7, IL-1, and IL-15 is added

concomitantly. In this culture system, CD34(+) marrow cells from two SCID-Xl patients were able to mature into double positive CD4(+) CD8(+) cells and to a lesser degree into CD4(+) TCRbeta+ single positive cells after retroviral-mediated gammac gene transfer. In addition, examination of the output cell population at the TCR DJbeta1 locus exhibited multiple rearrangements. These results indicate that restoration of the gammac/JAK/STAT signaling pathway during the early developmental stages of thymocytes can correct the T-cell differentiation block in SCID-Xl hematopoietic progenitor cells and therefore establishes a basis for further clinical gammac gene transfer studies.

Han, L., A. T. Wierenga, et al. (2009). "Single-cell STAT5 signal transduction profiling in normal and leukemic stem and progenitor cell populations reveals highly distinct cytokine responses." PLoS One **4**(11): e7989.

BACKGROUND: Signal Transducer and Activator of Transcription 5 (STAT5) plays critical roles in normal and leukemic hematopoiesis. However, the manner in which STAT5 responds to early-acting and lineage-restricted cytokines, particularly in leukemic stem/progenitor cells, is largely unknown. METHODOLOGY/PRINCIPAL FINDINGS: We optimized a multiparametric flow cytometry protocol to analyze STAT5 phosphorylation upon cytokine stimulation in stem and progenitor cell compartments at a single-cell level. In normal cord blood (CB) cells, STAT5 phosphorylation was efficiently induced by TPO, IL-3 and GM-CSF within CD34(+)CD38(-) hematopoietic stem cells (HSCs). EPO- and SCF-induced STAT5 phosphorylation was largely restricted to the megakaryocyte-erythroid progenitor (MEP) compartment, while G-CSF as well IL-3 and GM-CSF were most efficient in inducing STAT5 phosphorylation in the myeloid progenitor compartments. Strikingly, mobilized adult peripheral blood (PB) CD34(+) cells responded much less efficiently to cytokine-induced STAT5 activation, with the exception of TPO. In leukemic stem and progenitor cells, highly distinct cytokine responses were observed, differing significantly from their normal counterparts. These responses could not be predicted by the expression level of cytokine receptors. Also, heterogeneity existed in cytokine requirements for long-term expansion of AML CD34(+) cells on stroma. CONCLUSIONS/SIGNIFICANCE: In conclusion, our optimized multiparametric flow cytometry protocols allow the analysis of signal transduction at the single cell level in normal and leukemic stem and progenitor cells. Our study demonstrates highly distinctive cytokine responses in STAT5 phosphorylation in both normal and leukemic stem/progenitor cells.

Han, M. (1992). "Synergistic effects of murine stem cell factor in combination with a variety of cytokines on the expansion of murine hematopoietic progenitor cells in short-term suspension cultures." Hokkaido Igaku Zasshi **67**(5): 674-83.

In the present study, it is represented that the ability of murine stem cell factor (SCF) to expand hematopoietic progenitor cells in short-term suspension culture when used alone or with IL-1 beta, IL-3, IL-6, M-CSF and IL1 beta Plus IL-3. SCF alone had a limited effect on the expansion of early primitive hematopoietic progenitor cells (CFU-HPP: high proliferative potential colony forming unit, and CFU-S: colony forming unit in spleen) even at a high concentration, but expanded mature hematopoietic progenitor cells (CFU-GM: colony forming unit-granulocyte/macrophage, and BFU-E: burst forming unit-erythroid) markedly at low concentrations. When SCF was used in combination with other cytokines, the expansion of primitive hematopoietic progenitor cells was significantly increased; namely, CFU-HPP were expanded approximately 2 to 5-fold compared with SCF alone. A marked expansion of hematopoietic progenitor cells was observed in a combination of SCF plus IL-1 beta plus IL-3. In this setting, CFU-S was increased 2.2-fold compared with the number of CFU-S in fresh bone marrow and CFU-HPP were increased 8.5-fold compared with the number of primary CFU-HPP. These results suggest that these factors may be utilized in experiments of murine bone marrow transplantation (BMT) and also in human BMT. Namely, the adequate number of hematopoiesic progenitor cells and stem cells required for the successful engraftment can be obtained from small volume of peripheral or bone marrow blood by this procedure, thus obtiating the donor's burden.

Haridass, D., Q. Yuan, et al. (2009). "Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice." Am J Pathol **175**(4): 1483-92.

Fetal liver progenitor cell suspensions (FLPC) and hepatic precursor cells derived from embryonic stem cells (ES-HPC) represent a potential source for liver cell therapy. However, the relative capacity of these cell types to engraft and repopulate a recipient liver compared with adult hepatocytes (HC) has not been comprehensively assessed. We transplanted mouse and human HC, FLPC, and ES-HPC into a new immunodeficient mouse strain (Alb-

uPA(tg(+/-))Rag2(-/-)gamma(c)(-/-) mice) and estimated the percentages of HC after 3 months. Adult mouse HC repopulated approximately half of the liver mass (46.6 +/- 8.0%, 1 x 10(6) transplanted cells), whereas mouse FLPC derived from day 13.5 and 11.5 post conception embryos generated only 12.1 +/- 3.0% and 5.1 +/- 1.1%, respectively, of the recipient liver and smaller cell clusters. Adult human HC and FLPC generated overall less liver tissue than mouse cells and repopulated 10.0 +/- 3.9% and 2.7 +/- 1.1% of the recipient livers, respectively. Mouse and human ES-HPC did not generate HC clusters in our animal model. We conclude that, in contrast to expectations, adult HC of human and mouse origin generate liver tissue more efficiently than cells derived from fetal tissue or embryonic stem cells in a highly immunodeficient Alb-uPA transgenic mouse model system. These results have important implications in the context of selecting the optimal strategy for human liver cell therapies.

Hart, C., D. Drewel, et al. (2004). "Expression and function of homing-essential molecules and enhanced in vivo homing ability of human peripheral blood-derived hematopoietic progenitor cells after stimulation with stem cell factor." Stem Cells **22**(4): 580-9.

Hematopoietic stem cell (HSC) homing from blood to bone marrow is a multistep process involving rolling, extravasation, migration, and finally adhesion in the correct microenvironment. With view to the hematopoietic recovery after clinical stem cell transplantation, we investigated the effect of stem cell factor (SCF) on the expression and the adhesive function of the alpha4beta1 and alpha5beta1 integrins very-late antigen (VLA)-4 and VLA-5 on peripheral blood-derived hematopoietic progenitor cells. After SCF stimulation, the expression of VLA-4 and VLA-5 on CD34+/c-kit+ cells obtained from healthy donors increased from 54% to 90% and from 3% to 82%, respectively. For patient-derived cells, the increase was 67% to 90% and 12% to 46%. The proportion of mononuclear cells adhering to the fibronectin fragment CH296 increased by stimulation with SCF from 14% to 23%. Accordingly, functional studies showed an approximate 30% increase of adherent long-term culture-initiating cell. The improvement of the homing abilities of SCF-stimulated HSC was confirmed by transplantation into sublethally irradiated nonobese diabetic-scid/scid mice. Six weeks after the transplantation, in eight of eight animals receiving human HSC with the addition of SCF, a profound multilineage hematopoietic engraftment was detected, whereas in the control group receiving only HSC, none of eight animals engrafted. Our data provide the first in vivo evidence that stimulation with cytokines improves the homing ability of transplanted human hematopoietic progenitor cells.

Hartung, G., L. Uharek, et al. (1998). "Superior antileukemic activity of murine peripheral blood progenitor cell (PBPC) grafts mobilized by G-CSF and stem cell factor (SCF) as compared to G-CSF alone." Bone Marrow Transplant **21 Suppl 3**: S16-20.

We have established a murine model to compare the antileukemic effect of PBPC grafts obtained after treatment with SCF + G-CSF and G-CSF alone. C57/BL6, DBA and Balb/c mice were splenectomized and injected with optimal doses of rhG-CSF (250 microg/kg/day s.c.) or rrSCF (100 microg/kg/day s.c.) or with a combination thereof. On day 5, we determined the hematopoietic potential (number of CD34+ cells, CFUs, total CFC, CFU-gm), the proportion of lymphoid (T, NK and B cells) and myeloid components and graft-versus-leukemia activity after allogeneic and syngeneic PBPCT and BMT in Balb/c mice bearing a B-lymphoblastic leukemia cell line (A20). The absolute number of progenitor cells increased two-fold after administering a combination of G-CSF and SCF as compared to G-CSF alone (1500 vs 940 CD34+ cells/microl; 190 vs 70 total CFC/microl; 150 vs 50 CFU-gm/microl and 6600 vs 3000 CFUs/ml). Although no differences could be detected in the cellular composition, especially in the number of T cells, PBPC grafts mobilized by the combination of G-CSF + SCF demonstrated significantly higher antileukemic activity compared to G-CSF alone (94% vs 71% freedom from leukemia, $P < 0.05$). Because the incidence of lethal GVHD was similar in both groups, improved GVL activity resulted in superior overall survival. Our data suggest that the higher number of progenitor cells can be harvested after G-CSF + SCF and that grafts mobilized by G-CSF + SCF exert significantly enhanced antileukemic activity compared to those harvested after treatment with G-CSF alone.

Hartung, G., M. Zeis, et al. (2003). "Enhanced antileukemic activity of allogeneic peripheral blood progenitor cell transplants following donor treatment with the combination of granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) in a murine transplantation model." Bone Marrow Transplant **32**(1): 49-56.

Allogeneic peripheral blood progenitor cells (PBPCs) have mostly been mobilized by granulocyte colony-stimulating factor (G-CSF). There is neither clinical nor experimental data available addressing the question if other hematopoietic growth factors or combinations thereof might influence engraftment, graft-versus-host disease (GvHD), and graft-versus-leukemia (GvL) effects after allogeneic peripheral

blood progenitor cell transplantation (PBPCT). We used a murine model to investigate these parameters after transplantation of PBPCs mobilized with G-CSF and SCF either alone or in combination. Treatment of splenectomized DBA and Balb/c mice with 250 microg/kg/day G-CSF for 5 days resulted in an increase of CFU-gm from 0 to 53/microl. The highest progenitor cell numbers (147/microl) were observed after treatment with 100 microg/kg/day SCF administered in conjunction with G-SCF. No differences were detected with regard to the number of T cells (CD3+), T cell subsets (CD4+, CD8+), B cells (CD19+) and NK cells (NK1.1+) in PBPC grafts mobilized by G-CSF plus SCF compared to those mobilized with G-CSF alone. The antileukemic activity of syngeneic and MHC-identical allogeneic PBPC grafts was investigated in lethally irradiated Balb/c mice bearing the B-lymphatic leukemia cell line A20. In this model, PBPCs mobilized by G-CSF plus SCF exerted a significantly higher antileukemic activity compared to grafts mobilized by G-CSF alone (94 vs 71% freedom from leukemia at day 100, $P<0.05$). The antileukemic effect was lowest after BMT (38% freedom from leukemia). Since significant differences in the incidence of lethal GvHD were not observed, improved GVL-activity resulted in superior overall survival. Our data demonstrate that the utilization of specific hematopoietic growth factors not only improve the yield of hematopoietic progenitor cells but can also significantly enhance the immunotherapeutic potential of allografts.

Haslauer, M., K. Baltensperger, et al. (1999). "Erythropoietin- and stem cell factor-induced DNA synthesis in normal human erythroid progenitor cells requires activation of protein kinase Calpha and is strongly inhibited by thrombin." Blood **94**(1): 114-26.

Proliferation, differentiation, and survival of erythroid progenitor cells are mainly regulated by stem cell factor (SCF) and erythropoietin (Epo). Using normal human progenitors, we analyzed the role of Ca2+-sensitive protein kinase C (PKC) subtypes and of G-protein-coupled receptor ligands on growth factor-dependent DNA synthesis. We show that stimulation of DNA synthesis by the two growth factors requires activation of PKCalpha. Inhibitors of Ca2+-activated PKC subtypes blocked the growth factor-induced 3H-thymidine incorporation. SCF and Epo caused no significant translocation of PKCalpha into the membrane, but treatment of intact cells with either of the two cytokines resulted in enhanced activity of immunoprecipitated cytosolic PKCalpha. Stimulation of PKC with the phorbol ester PMA mimicked the cytokine effect on DNA synthesis. Epo-, SCF-, and PMA-induced thymidine incorporation was potently inhibited by thrombin (half-maximal inhibition with 0.1 U/mL). This effect was mediated via the G-protein-coupled thrombin receptor and the Rho guanosine triphosphatase. Adenosine diphosphate caused a modest Ca2+-dependent stimulation of DNA synthesis in the absence of cytokines and specifically enhanced the effect of SCF. Cyclic 3', 5'-adenosine monophosphate exerted a selective inhibitory effect on Epo-stimulated thymidine incorporation. Our results define PKCalpha as major intermediate effector of cytokine signaling and suggest a role for thrombin in controlling erythroid progenitor proliferation.

Hattori, K., B. Heissig, et al. (2001). "Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells." Blood **97**(11): 3354-60.

The chemokine, stromal cell-derived factor-1 (SDF1), is produced in the bone marrow and has been shown to modulate the homing of stem cells to this site by mediating chemokinesis and chemotaxis. Therefore, it was hypothesized that elevation of SDF1 level in the peripheral circulation would result in mobilization of primitive hematopoietic stem and progenitor cells. SDF1 plasma level was increased by intravenous injection of an adenoviral vector expressing SDF1alpha (AdSDF1) into severe combined immunodeficient mice. This resulted in a 10-fold increase in leukocyte count, a 3-fold increase in platelets, and mobilization of progenitors, including colony-forming units-granulocyte-macrophage to the peripheral circulation. In addition, AdSDF1 induced mobilization of cells with stem cell potential, including colony-forming units in spleen and long-term reconstituting cells. These data demonstrate that overexpression of SDF1 in the peripheral circulation results in the mobilization of hematopoietic cells with repopulating capacity, progenitor cells, and precursor cells. These studies lay the foundation for using SDF1 to induce mobilization of hematopoietic stem and progenitor cells in in vivo studies. (Blood. 2001;97:3354-3360)

Hayashi, R., M. Yamato, et al. (2008). "Enrichment of corneal epithelial stem/progenitor cells using cell surface markers, integrin alpha6 and CD71." Biochem Biophys Res Commun **367**(2): 256-63.

Corneal epithelial stem cells are believed to reside in the basal layer of the limbal epithelium, but no definitive cell surface markers have been identified. For keratinocytes, stem/progenitor cells are known to be enriched by cell surface markers, integrin alpha(6) and CD71, as a minor subpopulation which shows high integrin alpha(6) and low CD71 expressions (alpha(6)(bri)/CD71(dim)). In the present study, we investigated the possibility that corneal epithelial stem cells can be enriched by integrin

alpha(6) and CD71. The alpha(6)(bri)/CD71(dim) cells were separated by fluorescence-activated cell sorting, as a minor subpopulation of the limbal epithelial cells. They were enriched for relatively small cells, showing a higher clonogenic capacity and expression of stem cell markers, but a lower expression of differentiation markers, compared to other cell populations. The cells were localized immunohistochemically in the basal region of the limbal epithelium. These results indicate that the alpha(6)(bri)/CD71(dim) subpopulation enriched corneal epithelial stem cells.

Hayashi, R., M. Yamato, et al. (2007). "N-Cadherin is expressed by putative stem/progenitor cells and melanocytes in the human limbal epithelial stem cell niche." Stem Cells **25**(2): 289-96.

Corneal epithelial stem cells are known to be localized to the basal layer of the limbal epithelium, providing a model system for epithelial stem cell biology; however, the mechanisms regarding the maintenance of these stem cells in their specialized niche remain poorly understood. N-cadherin is a member of the classic cadherin family and has previously been demonstrated to be expressed by hematopoietic stem cells. In the present study, we demonstrate that N-cadherin is expressed by putative stem/progenitor cells, as well as melanocytes, in the human limbal epithelial stem cell niche. In addition, we demonstrate that upon in vitro culture using 3T3 feeder layers, loss of N-cadherin expression occurs with cell proliferation. These results indicate that N-cadherin may be a critical cell-to-cell adhesion molecule between corneal epithelial stem/progenitor cells and their corresponding niche cells in the limbal epithelium.

Heng, B. C., H. K. Haider, et al. (2005). "Combining transfusion of stem/progenitor cells into the peripheral circulation with localized transplantation in situ at the site of tissue/organ damage: a possible strategy to optimize the efficacy of stem cell transplantation therapy." Med Hypotheses **65**(3): 494-7.

Several studies have demonstrated the efficacy of localized in situ transplantation of stem/progenitor cells for tissue/organ regeneration. However, the possible limitations of such an approach have largely been overlooked. This is contrary to the intrinsic physiological process of tissue/organ regeneration in vivo, which is thought to involve the mobilization of stem/progenitor cells resident within the tissue/organ itself, as well as from ectopic sites, in particular the bone marrow. Signaling pathways and other molecular processes within stem/progenitor cells transplanted in situ may not be primed to achieve optimal tissue/organ regeneration, and may even be confused by the sudden rapid transition in the cellular microenvironment encountered during transplantation. To overcome these putative limitations, a possible strategy may be to combine transfusion of stem/progenitor cells into the peripheral circulation with localized transplantation in situ at the site of tissue/organ damage. This could better replicate the natural physiological process of tissue/organ repair in vivo. Possible synergistic interactions between the transplanted stem/progenitor cells in situ with migratory transfused cells from the peripheral circulation may further enhance tissue/organ regeneration. The transfused stem/progenitor cells may be induced to home in on a damaged tissue/organ, via the controlled release of specific cytokines or chemokines (i.e., SDF-1) emanating from that particular tissue/organ. There are a number of possible ways to achieve this. For example, the transplanted cells may be delivered on tissue-engineered scaffolds that are designed for the controlled release of specific homing factors such as SDF-1. Another alternative may be to stimulate or genetically modulate the transplanted cells to copiously secrete homing factors such as SDF-1, to encourage the migration and homing of transfused cells within the peripheral circulation. At the same time, it may also be advantageous to pre-stimulate the transfused cells to strongly express surface receptors specific to homing factors such as SDF-1, in particular CXCR-4. More rigorous investigations should be carried out on the possible strategy of combining in situ transplantation of stem/progenitor cells with transfusion into the peripheral circulation, together with induced homing of the transfused cells to the site of organ/tissue damage. This may possibly result in better efficacy for some, but not all models of tissue/organ regeneration.

Henriksson, H., M. Thornemo, et al. (2009). "Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species." Spine (Phila Pa 1976) **34**(21): 2278-87.

STUDY DESIGN: Descriptive experimental study in 4 different mammals. OBJECTIVE: To investigate cell proliferation/regeneration and localize stem cells/progenitor cells within the intervertebral disc (IVD). SUMMARY OF BACKGROUND DATA: Disc degeneration (DD) is believed to play a major role in patients with chronic lumbar pain. Lately, biologic treatment options for DD have gained increasing interest. Normal regeneration processes within the IVD and have previously been sparsely described and therefore it is of great interest to increase the knowledge about these processes. Methods. Detection of cell proliferations zones and

label-retaining cells were done by in vivo 5-bromo-2-deoxyuridine (BrdU) labeling in 18 rabbits, killed after 4, 6, 10, 14, 28, or 56 days. Results were visualized with immunohistochemistry and fluorescence/confocal microscopy. Localization of progenitor cell were further investigated by immunohistochemistry using antibodies towards Notch1, Delta4, Jagged1, C-KIT, KI67, and Stro-1 in normal IVD from rabbits (n = 3), rats (n = 2), minipigs (n = 2), and in human degenerated IVD (n = 4). Further, flowcytometry analysis using progenitor markers were performed on additional human IVD cells (n = 3). RESULTS: BrdU positive cells were found in comparable numbers at early and late time points in most regions of the anulus fibrosus (AF) and nucleus pulposus demonstrating slow ongoing cell proliferation. In the AF border to ligament zone (AFo) and the perichondrium region (P) a stem cell niche-like pattern was determined (a high number of BrdU positive cells at early time points vs. only a few label retaining cells at later time points). In normal and DD tissue from the 4 investigated species progenitor cell markers were detected. Conclusion. The IVD is a tissue with ongoing slow cell proliferation both in the AF and the nucleus pulposus. The stem cell niche pattern detected in AFo and P can be suggested to play a role for IVD morphology and function. These findings may be of importance for the development of biologic treatment strategies.

Herbert, K. E., J. P. Levesque, et al. (2008). "The use of experimental murine models to assess novel agents of hematopoietic stem and progenitor cell mobilization." Biol Blood Marrow Transplant **14**(6): 603-21.

The recent explosion in the understanding of the cellular and molecular mechanisms underlying hematopoietic stem and progenitor cell (HSPC) mobilization has facilitated development of novel therapeutic agents, targeted at improving mobilization kinetics as well as HSPC yield. With the development of new agents comes the challenge of choosing efficient and relevant preclinical studies for the testing of the HSPC mobilization efficacy of these agents. This article reviews the use of the mouse as a convenient small animal model of HSPC mobilization and transplantation, and outlines the range of murine assays that can be applied to assess novel HSPC mobilizing agents. Techniques to demonstrate murine HSPC mobilization are discussed, as well as the role of murine assays to confirm human HSPC mobilization, and techniques to investigate the biologic phenotype of HSPC mobilized by these novel agents. Technical aspects regarding mobilization regimens and control arms, and choice of experimental animals are also discussed.

Hicks, C., R. Wong, et al. (2007). "Viable CD34+/CD133+ blood progenitor cell dose as a predictor of haematopoietic engraftment in multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation." Ann Hematol **86**(8): 591-8.

Both CD34 (cluster of differentiation 34) and the more recently described CD133 are markers of primitive stem cells with haematopoietic repopulating ability. Most transplanting centres use a minimum number of CD34+ cells as the requirement for a transplant and consider this a predictor of haematopoietic engraftment. However, transplanted CD34+ cell dose does not always give a close correlation with time to engraftment nor explain delayed engraftment in some patients. We have retrospectively evaluated the potential of measuring viable CD133+ cell numbers in the autograft as an alternative predictor of haematological engraftment after autologous stem-cell transplantation in a cohort of patients with multiple myeloma (MM). We found an average 32% loss of viability of CD34+ cells in the post-thaw sample compared with the fresh sample. Of the original estimated CD34+ cell numbers transplanted per kg, 43% of the thawed samples were double positive for CD34+/CD133+. In this patient group, the CD34+/CD133+ subset gave the closest statistical correlation with time to neutrophil engraftment ($p < 0.05$), particularly for patients given above median (1.8 x 10(6)/kg) dose of the double-positive cells. The CD34+/CD133+ population was the only parameter to give a significant correlation with white cell engraftment in this patient cohort ($p < 0.05$). There was no significant correlation between CD34+, viable CD34+ or viable CD34+/CD133+ cells/kilogram with platelet engraftment. Determination of viable CD34+/CD133+ progenitor cell dose in the autograft may be a useful tool to predict neutrophil recovery after autologous transplantation than conventional assessment of CD34+ numbers. These results warrant further investigation of the role of CD133 in haematopoietic engraftment.

Hildrestrand, G. A., D. B. Diep, et al. (2007). "The capacity to remove 8-oxoG is enhanced in newborn neural stem/progenitor cells and decreases in juvenile mice and upon cell differentiation." DNA Repair (Amst) **6**(6): 723-32.

In mammalian cells, 8-oxoguanine DNA glycosylase-1 (OGG1) is the main DNA glycosylase for the removal of 8-oxoguanine (8-oxoG). 8-oxoG, one of the most common products of the oxidative attack of DNA, is a premutagenic lesion that accumulates spontaneously at high frequencies in the

genome. In this study, Ogg1 mRNA expression was detected throughout embryonic development in mice. In situ hybridization showed that in the neonatal brain, Ogg1 expression was detected in a distinct layer of cells in the medial wall of the lateral ventricle, which may correspond to ependymal cells, and in some scattered cells in the subventricular zone (SVZ), a brain region rich in neural stem/progenitor cells. Using neurospheres as a model for the study of neural stem/progenitor cells, we found that both the expression and activity of Ogg1 were high in neurospheres derived from newborn mice and decreased in adults and upon induction of cell differentiation. Furthermore, Ogg1 was shown to be the major DNA glycosylase initiating 8-oxoG repair in neurospheres. Our results strongly indicate that enhanced DNA repair capacity is an important mechanism by which neural stem/progenitor cells maintain their genome.

Hiraoka, A., T. Nagasawa, et al. (1998). "A human myeloid cell line producing stem cell growth factor, KPB-M15, secretes another growth factor active on murine hematopoietic progenitor cells." Acta Haematol **100**(4): 174-80.

Human stem cell growth factor (SCGF) produced by a myeloid cell line, KPB-M15, exhibits species-specific hematopoietic activities. However, KPB-M15-conditioned medium induced colony formation of mouse bone marrow cells. KPB-M15-derived colony-stimulating activity (CSA) was purified through Butyl-Toyopearl 650c and Cu2+ chelating-Sepharose 6B chromatography. TSK-G3000SW gel filtration of the purified preparation presented 3 distinct peaks around Vo, 150 kD and 85 kD. Gel fractions extracted from SDS-PAGE had macrophage colony-stimulating factor (M-CSF)-specific amino acid sequences. PCR, Northern hybridization and ELISA demonstrated that KPB-M15 cells secreted a significant amount of M-CSF and IL-6. Anti-M-CSF but not anti-IL-6 antibody abrogated CSA in KPB-M15-CM. IL-6 hardly synergized with M-CSF to enhance colony formation. Collectively, M-CSF is a sole CSA for murine hematopoietic progenitor cells in KPB-M15-CM. This is the first report of a human myeloid cell line, KPB-M15, constitutively producing M-CSF in addition to SCGF and IL-6. It can be useful in investigating the mechanism of production of M-CSF.

Hiraoka, A., K. Yano Ki, et al. (2001). "Stem cell growth factor: in situ hybridization analysis on the gene expression, molecular characterization and in vitro proliferative activity of a recombinant preparation on primitive hematopoietic progenitor cells." Hematol J **2**(5): 307-15.

INTRODUCTION: In situ hybridization of whole mouse fetuses and their tibias with a stem cell growth factor (SCGF) antisense probe demonstrated specific expression of SCGF mRNA around skeletal tissues, particularly in bone marrow cells, proliferating chondrocytes, the perichondrium and periosteum, but little expression in resting or hypertrophic chondrocytes. METHODS: Recombinant human (rh) SCGF-alpha was purified from a conditioned medium of SCGF-alpha gene-transfected CHO cells. The molecular mass of rhSCGF-alpha, 45 kDa, was shifted down to 40 kDa by digestion with endo-O-glycosidase and sialidase, suggesting O-glycosylation of rhSCGF-alpha with sialic acids. RESULTS: For human bone marrow CD34+Lin- cells, rhSCGF-alpha alone did not stimulate colony-formation, but small cluster-formation (10.3 +/- 2.5/1 x 10(3) CD34+Lin- cells). It promoted growth of erythroid and granulocyte/macrophage (GM) colonies in the primary culture with erythropoietin and GM colony-stimulating factor (CSF) or G-CSF, respectively, and further supported GM progenitor cells in a short-term liquid culture. In contrast, rhSCGF-alpha suppressed stem cell factor (SCF)-stimulated erythroid bursts, indicating some competitive interaction between SCGF and SCF. rhSCGF-alpha was synergistic with interleukin-3 and the flt3 ligand to enhance GM colony-growth, but not synergistic with those inducing ex vivo expansion of GM progenitor cells. CONCLUSION: SCGF is selectively produced by osseous and hematopoietic stromal cells, and can mediate their proliferative activity on primitive hematopoietic progenitor cells.

Hirohata, S., T. Yanagida, et al. (2002). "Bone marrow CD34+ progenitor cells stimulated with stem cell factor and GM-CSF have the capacity to activate IgD- B cells through direct cellular interaction." J Leukoc Biol **71**(6): 987-95.

Recent studies have suggested the involvement of bone marrow in the pathogenesis of rheumatoid arthritis (RA), in which proliferation of monocyte-lineage cells (MLC) as well as local B cell activation in the synovium play an important role. Here, we show that bone marrow-derived MLC have the capacity to activate human peripheral blood IgD- B cells. Bone marrow CD34+ cells from RA patients that had been stimulated with stem cell factor and GM-CSF for 3-4 weeks (>90% CD14+ HLA-DR+ cells, <0.5% CD19+ B cells, and <0.5% CD3+ T cells; MLC) induced the production of IgG much more effectively than that of IgM by highly purified B cells from healthy donors in the presence of IL-2 and IL-10. CD34+ cells from cord blood or from bone marrow of osteoarthritis patients also displayed the capacity to induce IgG production. The induction of IgG

production by the bone marrow-derived MLC was markedly decreased when they were separated from B cells by a membrane filter. The bone marrow-derived MLC interacted preferentially with IgD- B cells to induce IgG production. These results indicate that upon stimulation with stem cell factor and GM-CSF, CD34+ progenitor cells differentiate into MLC that activate preferentially IgD- B cells through direct cellular interactions to produce IgG. Therefore, the data suggest that the accelerated recruitment of MLC from the bone marrow to the synovium might play a role in the local B cell activation in RA.

Hiroyama, T., K. Miharada, et al. (2008). "Establishment of mouse embryonic stem cell-derived erythroid progenitor cell lines able to produce functional red blood cells." PLoS One **3**(2): e1544.

BACKGROUND: The supply of transfusable red blood cells (RBCs) is not sufficient in many countries. If erythroid cell lines able to produce transfusable RBCs in vitro were established, they would be valuable resources. However, such cell lines have not been established. To evaluate the feasibility of establishing useful erythroid cell lines, we attempted to establish such cell lines from mouse embryonic stem (ES) cells. METHODOLOGY/PRINCIPAL FINDINGS: We developed a robust method to obtain differentiated cell lines following the induction of hematopoietic differentiation of mouse ES cells and established five independent hematopoietic cell lines using the method. Three of these lines exhibited characteristics of erythroid cells. Although their precise characteristics varied, each of these lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. Following transplantation of these erythroid cells into mice suffering from acute anemia, the cells proliferated transiently, subsequently differentiated into functional RBCs, and significantly ameliorated the acute anemia. In addition, we did not observe formation of any tumors following transplantation of these cells. CONCLUSION/SIGNIFICANCE: To the best of our knowledge, this is the first report to show the feasibility of establishing erythroid cell lines able to produce mature RBCs. Considering the number of human ES cell lines that have been established so far, the intensive testing of a number of these lines for erythroid potential may allow the establishment of human erythroid cell lines similar to the mouse erythroid cell lines described here. In addition, our results strongly suggest the possibility of establishing useful cell lines committed to specific lineages other than hematopoietic progenitors from human ES cells.

Ho, Y. C., H. P. Lee, et al. (2006). "Baculovirus transduction of human mesenchymal stem cell-derived progenitor cells: variation of transgene expression with cellular differentiation states." Gene Ther **13**(20): 1471-9.

We have previously demonstrated that baculovirus can efficiently transduce human mesenchymal stem cells (MSCs). In this study, we further demonstrated, for the first time, that baculovirus can transduce adipogenic, chondrogenic and osteogenic progenitors originating from MSCs. The transduction efficiency (21-90%), transgene expression level and duration (7-41 days) varied widely with the differentiation lineages and stages of the progenitors, as determined by flow cytometry. The variation stemmed from differential transgene transcription (as revealed by real-time reverse transcription-polymerase chain reaction), rather than from variability in virus entry or cell cycle (as determined by quantitative real-time PCR and flow cytometry). Nonetheless, the baculovirus-transduced cells remained capable of differentiating into adipogenic, osteogenic and chondrogenic pathways. The susceptibility to baculovirus transduction was higher for adipogenic and osteogenic progenitors, but was lower for chondrogenic progenitors. In particular, the duration of transgene expression was prolonged in the transduced adipogenic and osteogenic progenitors (as opposed to the MSCs), implicating the possibility of extending transgene expression via a proper transduction strategy design. Taken together, baculovirus may be an attractive alternative to genetically modify adipogenic and osteogenic progenitors in the ex vivo setting for cell therapy or tissue engineering.

Hoffmann, M., H. H. Chang, et al. (2008). "Noise-driven stem cell and progenitor population dynamics." PLoS One **3**(8): e2922.

BACKGROUND: The balance between maintenance of the stem cell state and terminal differentiation is influenced by the cellular environment. The switching between these states has long been understood as a transition between attractor states of a molecular network. Herein, stochastic fluctuations are either suppressed or can trigger the transition, but they do not actually determine the attractor states. METHODOLOGY/PRINCIPAL FINDINGS: We present a novel mathematical concept in which stem cell and progenitor population dynamics are described as a probabilistic process that arises from cell proliferation and small fluctuations in the state of differentiation. These state fluctuations reflect random transitions between different activation patterns of the underlying regulatory network. Importantly, the associated noise amplitudes are state-

dependent and set by the environment. Their variability determines the attractor states, and thus actually governs population dynamics. This model quantitatively reproduces the observed dynamics of differentiation and dedifferentiation in promyelocytic precursor cells. CONCLUSIONS/SIGNIFICANCE: Consequently, state-specific noise modulation by external signals can be instrumental in controlling stem cell and progenitor population dynamics. We propose follow-up experiments for quantifying the imprinting influence of the environment on cellular noise regulation.

Horn, P. A., B. M. Thomasson, et al. (2003). "Distinct hematopoietic stem/progenitor cell populations are responsible for repopulating NOD/SCID mice compared with nonhuman primates." Blood **102**(13): 4329-35.

The nonobese diabetic/severe combined immune-deficient (NOD/SCID) mouse xenotransplantation assay is the most commonly used surrogate assay for the study of human candidate stem cells. In contrast to large animal and human studies, however, it is limited by the short life span of the recipients, the limited proliferative demand placed on the transplanted cells, and the inability to support differentiation into all hematopoietic lineages. In the present study, we directly compared hematopoietic repopulation in NOD/SCID mice with autologous reconstitution in the baboon, a well-established preclinical large animal model for stem cell transplantation. Baboon CD34-enriched marrow cells were retrovirally marked and infused into the irradiated baboon and the NOD/SCID mice. Although the percentage of gene-marked cells was high and remained stable in NOD/SCID mice up to 12 weeks and in those that underwent secondary transplantation, we observed a considerable decline and overall a significantly (10-fold) lower percentage of gene-marked cells in the baboons. In addition, clonal integration site analysis revealed common proviral vector integrants in NOD/SCID repopulating cells and in the baboon at 6 weeks but not at 6 months after transplantation. These results suggest that distinct hematopoietic stem/progenitor cells are responsible for hematopoietic reconstitution in NOD/SCID mice compared with nonhuman primates.

Hoshi, N., T. Kusakabe, et al. (2007). "Side population cells in the mouse thyroid exhibit stem/progenitor cell-like characteristics." Endocrinology **148**(9): 4251-8.

Side population (SP) cells are characterized by their ability to efflux the vital dye Hoechst 33342 (Sigma-Aldrich, St. Louis, MO) due to expression of the ATP binding cassette (ABC)-dependent transporter ABCG2, and are highly enriched for stem/progenitor cell activity. In this study we identified SP cells in murine thyroid, which are composed of two populations of cells: CD45(-)/c-kit(-)/Sca1(+) and CD45(-)/c-kit(-)/Sca1(-) cells. Quantitative RT-PCR analysis revealed that SP cells highly express ABCG2 and the stem cell marker genes encoding nucleostemin and Oct4, whereas the expression of genes encoding the thyroid differentiation markers, thyroid peroxidase, thyroglobulin (TG), and TSH receptor, and two transcription factors, thyroid transcription factor 1 (TITF1) and paired PAX8, critical for thyroid specific gene expression, are low in SP cells as compared with the main population cells. In situ hybridization and double immunofluorescence demonstrated that cells expressing Abcg2 gene reside in the interfollicular space of the thyroid gland. Approximately half and a small percentage of the ABCG2-positive cells were also positive for vimentin and calcitonin, respectively. After 9 wk under three-dimensional thyroid primary culture conditions, main population cells formed an epithelial arrangement and follicle-like structures that are immunoreactive for TITF1 and TG. In contrast, SP cells demonstrated very few morphological changes without any epithelial or follicle-like structure and negative immunostaining for TITF1 and TG. These results demonstrate that thyroid possesses SP cells that may represent stem/progenitor cells.

Huang, H. M., J. C. Li, et al. (1999). "Optimal proliferation of a hematopoietic progenitor cell line requires either costimulation with stem cell factor or increase of receptor expression that can be replaced by overexpression of Bcl-2." Blood **93**(8): 2569-77.

In vitro proliferation of hematopoietic stem cells requires costimulation by multiple regulatory factors whereas expansion of lineage-committed progenitor cells generated by stem cells usually requires only a single factor. The distinct requirement of factors for proliferation coincides with the differential temporal expression of the subunits of cytokine receptors during early stem cell differentiation. In this study, we explored the underlying mechanism of the requirement of costimulation in a hematopoietic progenitor cell line TF-1. We found that granulocyte-macrophage colony-stimulating factor (GM-CSF) optimally activated proliferation of TF-1 cells regardless of the presence or absence of stem cell factor (SCF). However, interleukin-5 (IL-5) alone sustained survival of TF-1 cells and required costimulation of SCF for optimal proliferation. The synergistic effect of SCF was partly due to its anti-apoptosis activity. Overexpression of the IL-5 receptor alpha subunit (IL5Ralpha) in TF-1 cells by genetic selection or retroviral infection also

resumed optimal proliferation due to correction of the defect in apoptosis suppression. Exogenous expression of an oncogenic anti-apoptosis protein, Bcl-2, conferred on TF-1 cells an IL-5-dependent phenotype. In summary, our data suggested SCF costimulation is only necessary when the expression level of IL5Ralpha is low and apoptosis suppression is defective in the signal transduction of IL-5. Expression of Bcl-2 proteins released the growth restriction of the progenitor cells and may be implicated in leukemia formation.

Huang, X. J., D. H. Liu, et al. (2006). "Prophylactic infusion of donor granulocyte colony stimulating factor mobilized peripheral blood progenitor cells after allogeneic hematological stem cell transplantation in patients with high-risk leukemia." Leukemia **20**(2): 365-8.

Humpe, A., J. Riggert, et al. (1997). "Comparison of CD34+ cell numbers and colony growth before and after cryopreservation of peripheral blood progenitor and stem cell harvests: influence of prior chemotherapy." Transfusion **37**(10): 1050-7.

BACKGROUND: Quantitative determination of hematopoietic progenitor cells is a major issue in peripheral blood progenitor and stem cell collection and transfusion, although the extent is still an object of discussion. STUDY DESIGN AND METHODS: In 116 leukapheresis collections from 42 patients, immunophenotyping for CD34+ cells, evaluation of in vitro proliferative capacity by a colony-forming unit-granulocyte-macrophage (CFU-GM) assay, and viability assessment by trypan blue exclusion were performed before and after storage in liquid nitrogen at -196 degrees C. RESULTS: Before storage, the median number of CD34+ cells was 1.46 x 10(6) (range, 0.01-54.05 x 10(6)) per kg of body weight (BW). There was no significant difference between precryopreservation and postcryopreservation numbers. The median number of CFU-GM was 2.25 x 10(5) (range, 0.02-157.49 x 10(5)) per kg of BW before cryopreservation and significantly ($p < 0.001$) lower, 0.83 x 10(5) (range, 0-220.36 x 10(5)) per kg of BW, after cryopreservation. The correlation coefficient of prestorage and poststorage values was 0.92. The median ratio of poststorage and prestorage values was 42.3 percent (0-304.8%). Male patients who underwent intense chemotherapy (> 5 cycles) showed a significantly lower ratio of postcryopreservation and precryopreservation CFU-GM values than other patients ($p = 0.0047$). A strong linear correlation was determined between the number of CD34+ cells per kg of BW and the number of CFU-GM per kg of BW before and after cryopreservation. A viability below 50 percent predicted a high loss of in vitro proliferative capacity, while a viability above 50 percent did not correlate with a high ratio of CFU-GM from after and before cryopreservation. CONCLUSION: A good correlation between the variables used for characterization of peripheral blood progenitor cells--the number of CD34+ cells and the number of CFU-GM--was observed. Viability assessment by trypan blue exclusion does not seem to be a substitute for assays evaluating in vitro proliferative capacity.

Hwang-Verslues, W. W., W. H. Kuo, et al. (2009). "Multiple lineages of human breast cancer stem/progenitor cells identified by profiling with stem cell markers." PLoS One **4**(12): e8377.

Heterogeneity of cancer stem/progenitor cells that give rise to different forms of cancer has been well demonstrated for leukemia. However, this fundamental concept has yet to be established for solid tumors including breast cancer. In this communication, we analyzed solid tumor cancer stem cell markers in human breast cancer cell lines and primary specimens using flow cytometry. The stem/progenitor cell properties of different marker expressing-cell populations were further assessed by in vitro soft agar colony formation assay and the ability to form tumors in NOD/SCID mice. We found that the expression of stem cell markers varied greatly among breast cancer cell lines. In MDA-MB-231 cells, PROCR and ESA, instead of the widely used breast cancer stem cell markers CD44(+)/CD24(-/low) and ALDH, could be used to highly enrich cancer stem/progenitor cell populations which exhibited the ability to self renew and divide asymmetrically. Furthermore, the PROCR(+)/ESA(+) cells expressed epithelial-mesenchymal transition markers. PROCR could also be used to enrich cells with colony forming ability from MB-361 cells. Moreover, consistent with the marker profiling using cell lines, the expression of stem cell markers differed greatly among primary tumors. There was an association between metastasis status and a high prevalence of certain markers including CD44(+)/CD24(-/low), ESA(+), CD133(+), CXCR4(+) and PROCR(+) in primary tumor cells. Taken together, these results suggest that similar to leukemia, several stem/progenitor cell-like subpopulations can exist in breast cancer.

Ichihara, M., T. Hotta, et al. (1994). "Effects of stem cell factor (SCF) on human marrow neutrophil, neutrophil/macrophage mixed, macrophage and eosinophil progenitor cell growth." Int J Hematol **59**(2): 81-9.

The effects of stem cell factor (SCF) on the subpopulations of granulocyte/macrophage colony-forming units (CFU-GM) were examined.

Hematopoietic progenitor cells were enriched from normal adult bone marrow specimens by immunomagnetic beads using an anti-CD34 antibody and lineage marker antibodies for positive selection and negative selection, respectively. SCF enabled neutrophil and neutrophil/macrophage mixed progenitors to respond to granulocyte/macrophage colony-stimulating factor (GM-CSF) or interleukin 3 (IL-3) and to develop the colony and further cluster formation. The neutrophil colonies stimulated by GM-CSF or IL-3 consisted mainly of immature cells, while the colonies stimulated by granulocyte colony-stimulating factor (G-CSF) consisted of mature neutrophils irrespective of the addition of SCF. In macrophage and eosinophil lineages, SCF augmented the colony formation in the presence of GM-CSF or IL-3, whereas the enhancement of total progenitor cell growth (colonies plus clusters) was not so marked as compared with the neutrophil lineage. Time-course observation revealed that SCF could stimulate macrophage and eosinophil progenitors to form colonies rapidly. These findings indicate that SCF acts on late myeloid progenitor cells in manners different from the lineages of commitment.

Ikegami, T., M. Nakamura, et al. (2005). "Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury." Eur J Neurosci **22**(12): 3036-46.

We previously reported that the transplantation of neural stem/progenitor cells (NSPCs) can contribute to the repair of injured spinal cord in adult rats and monkeys. In some cases, however, most of the transplanted cells adhered to the cavity wall and failed to migrate and integrate into the host spinal cord. In this study we focused on chondroitin sulfate proteoglycan (CSPG), a known constituent of glial scars that is strongly expressed after spinal cord injury (SCI), as a putative inhibitor of NSPC migration in vivo. We hypothesized that the digestion of CSPG by chondroitinase ABC (C-ABC) might promote the migration of transplanted cells and neurite outgrowth after SCI. An in vitro study revealed that the migration of NSPC-derived cells was inhibited by CSPG and that this inhibitory effect was attenuated by C-ABC pre-treatment. Consistently, an in vivo study of C-ABC treatment combined with NSPC transplantation into injured spinal cord revealed that C-ABC pre-treatment promoted the migration of the transplanted cells, whereas CSPG-immunopositive scar tissue around the lesion cavity prevented their migration into the host spinal cord in the absence of C-ABC pre-treatment. Furthermore, this combined treatment significantly induced the outgrowth of a greater number of growth-associated protein-43-positive fibers at the lesion epicentre, compared with NSPC transplantation alone. These findings suggested that the application of C-ABC enhanced the benefits of NSPC transplantation for SCI by reducing the inhibitory effects of the glial scar, indicating that this combined treatment may be a promising strategy for the regeneration of injured spinal cord.

Imamura, M., X. Zhu, et al. (1996). "In vitro expansion of murine hematopoietic progenitor cells by leukemia inhibitory factor, stem cell factor, and interleukin-1 beta." Exp Hematol **24**(11): 1280-8.

Murine hematopoietic progenitor cells were markedly expanded in the presence of leukemia inhibitory factor (LIF), interleukin (IL)-1 beta and/or stem cell factor (SCF), although SCF+IL-1 beta +IL-3, LIF+SCF+IL-3, and SCF+IL-1 beta showed an appreciable effect on the in vitro expansion of hematopoietic progenitor cells as well. In the presence of LIF+SCF+IL-1 beta, highly proliferative potential colony-forming units (CFU-HPP) and colony forming units of mixed lineages (CFU-Mix) were more efficiently expanded than colony forming units granulocytes/macrophage (CFU-GM) and burst-forming units of erythroid lineage (BFU-E) compared with the colony formations of freshly obtained bone marrow cells. The cell yield on day 5 in the presence of LIF+SCF+IL-1 beta was comparable to that in SCF+IL-1 beta and SCF+IL-1 beta +IL-3. Nevertheless, colony formations were marked in LIF+SCF+IL-1 beta, thus suggesting that this combination can generate hematopoietic progenitor cells that possess greater potential for CFU-HPP, CFU-Mix, CFU-GM, and BFU-E colony formations. Hematopoietic cells expanded in the presence of LIF+SCF+IL-1 beta showed increased mRNA expressions of IL-1 beta, IL-3, IL-6, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage (GM)-CSF, interferon gamma (IFN-gamma), and LIF compared with those of nontreated hematopoietic cells. Furthermore, LIF+SCF+IL-1 beta induced increased IL-3 and GM-CSF mRNA expression in hematopoietic cells but induced decreased macrophage inflammatory protein 1 alpha (MIP1 alpha) mRNA expression as compared with SCF+IL-1 beta +IL-3. These results suggest that the balance between stimulatory and inhibitory cytokines plays an important role in in vitro expansion of hematopoietic progenitor cells.

Irons, R. D. and W. S. Stillman (1996). "The effects of benzene and other leukaemogenic agents on haematopoietic stem and progenitor cell differentiation." Eur J Haematol Suppl **60**: 119-24.

A characteristic shared by a diverse group of myelotoxic compounds and leukaemogens is the ability to act synergistically with granulocyte-macrophage colony stimulating factor (GM-CSF) in increasing clonogenic response. Pretreatment of murine or human bone marrow cells with the benzene metabolite, hydroquinone, but not phenol, catechol or trans, trans-muconaldehyde, results in a selective enhancement of GM-CSF but not an interleukin-3 (IL-3)-mediated clonogenic response. Clonal enhancement is preserved and magnified in enriched populations of CD34+ cells (> 95% purity), suggesting an intrinsic effect on haematopoietic progenitor cell (HPC) recruitment rather than a secondary effect involving accessory cytokines. Clonogenic enhancement of murine HPCs is not accompanied by alterations in GM-CSF receptor expression or ligand affinity and appears to be mediated via a p53-independent mechanism. These observations suggest that hydroquinone treatment alters recruitment and differentiation in a primitive subpopulation of CD34+ cells and are consistent with a role for altered stem cell differentiation in the development of chemically induced myelodysplasias.

Ito, C. Y., C. Y. Li, et al. (2003). "Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A-null mice." Blood **101**(2): 517-23.

Despite its wide use as a marker for hematopoietic stem cells (HSCs), the function of stem cell antigen-1 (Sca-1) (also known as lymphocyte activation protein-6A [Ly-6A]) in hematopoiesis remains poorly defined. We have previously established that Sca-1(-/-) T cells develop normally, although they are hyperresponsive to antigen. Here, we report detailed analysis of hematopoiesis in Sca-1-deficient animals. The differentiation potential of Sca-1-null bone marrow was determined from examination of the most mature precursors (culture colony-forming units [CFU-Cs]) to less committed progenitors (spleen CFUs [CFU-Ss]) to long-term repopulating HSCs. Sca-1-null mice are mildly thrombocytopenic with a concomitant decrease in megakaryocytes and their precursors. Bone marrow cells derived from Sca-1(-/-) mice also have decreased multipotential granulocyte, erythroid, macrophage, and megakaryocyte CFU (GEMM-CFU) and CFU-S progenitor activity. Competitive repopulation assays demonstrated that Sca-1(-/-) HSCs are at a competitive disadvantage compared with wild-type HSCs. To further analyze the potential of Sca-1(-/-) HSCs, serial transplantations were performed. While secondary repopulations using wild-type bone marrow completely repopulated Sca-1(-/-) mice, Sca-1(-/-) bone marrow failed to rescue one third of lethally irradiated wild-type mice receiving secondary bone marrow transplants from irradiation-induced anemia and contributed poorly to the surviving transplant recipients. These data strongly suggest that Sca-1 is required for regulating HSC self-renewal and the development of committed progenitor cells, megakaryocytes, and platelets. Thus, our studies conclusively demonstrate that Sca-1, in addition to being a marker of HSCs, regulates the developmental program of HSCs and specific progenitor populations.

Ito, M., K. Kizawa, et al. (2004). "Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen." Differentiation **72**(9-10): 548-57.

The lowermost portion of the resting (telogen) follicle consists of the bulge and secondary hair germ. We previously showed that the progeny of stem cells in the bulge form the lower follicle and hair, but the relationship of the bulge cells with the secondary hair germ cells, which are also involved in the generation of the new hair at the onset of the hair growth cycle (anagen), remains unclear. Here we address whether secondary hair germ cells are derived directly from epithelial stem cells in the adjacent bulge or whether they arise from cells within the lower follicle that survive the degenerative phase of the hair cycle (catagen). We use 5-bromo-2'-deoxyuridine to label bulge cells at anagen onset, and demonstrate that the lowermost portion of the bulge collapses around the hair and forms the secondary hair germ during late catagen. During the first six days of anagen onset bulge cells proliferate and self-renew. Bulge cell proliferation at this time also generates cells that form the future secondary germ. As bulge cells form the secondary germ cells at the end of catagen, they lose expression of a biochemical marker, S100A6. Remarkably, however, following injury of bulge cells by hair depilation, progenitor cells in the secondary hair germ repopulate the bulge and re-express bulge cell markers. These findings support the notion that keratinocytes can "dedifferentiate" to a stem cell state in response to wounding, perhaps related to signals from the stem cell niche. Finally, we also present evidence that quiescent bulge cells undergo apoptosis during follicle remodeling in catagen, indicating that a subpopulation of bulge cells is not permanent.

Jacobsen, F. W., T. Stokke, et al. (1995). "Transforming growth factor-beta potently inhibits the viability-promoting activity of stem cell factor and other cytokines and induces apoptosis of primitive murine hematopoietic progenitor cells." Blood **86**(8): 2957-66.

In contrast with the extensively characterized effects of transforming growth factor-beta (TGF-beta) on proliferation and differentiation of hematopoietic progenitors, little is known about the effects of TGF-beta on viability of normal hematopoietic progenitors. In the present report, we demonstrate that TGF-beta potently counteracts hematopoietic growth factor (HGF)-induced survival of individually cultured primitive Lin-Sca-1+ bone marrow progenitors. Specifically, 74% of single Lin-Sca-1+ cells cultured for 40 hours in the presence of stem cell factor (SCF) survived, whereas only 16% survived in the presence of SCF plus TGF-beta. Similarly, the enhanced survival of primitive hematopoietic progenitors in response to granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-1, IL-6, or IL-11 was also potently opposed by TGF-beta. Furthermore, it is demonstrated that neutralization of endogenous TGF-beta present in the cultures enhances survival of Lin-Sca-1+ progenitors in the absence, as well as in the presence, of HGFs such as SCF and IL-6. The reduced HGF-induced survival of primitive hematopoietic progenitors in the presence of TGF-beta was associated with increased apoptosis, as detected by an in situ terminal deoxynucleotidyl transferase (TdT) assay. After 16 hours of incubation in the absence of HGFs, 61% +/- 6% of the hematopoietic progenitors had DNA strand breaks characteristic of apoptosis. The presence of SCF reduced the frequency of apoptic cells to 27% +/- 5%, whereas 55% +/- 3% of the cells had signs of apoptosis in the presence of SCF plus TGF-beta.

Jadhav, A. P., S. H. Cho, et al. (2006). "Notch activity permits retinal cells to progress through multiple progenitor states and acquire a stem cell property." Proc Natl Acad Sci U S A **103**(50): 18998-9003.

Signaling through the Notch pathway regulates multiple aspects of development. The vertebrate retina allows an investigation of the basis for these various effects, because the major cell types of the retina arise from a common progenitor that expresses Notch1. The Notch pathway was constitutively activated in distinct populations of retinal cells during development. Prolonged Notch activity in progenitor cells maintained cells in the progenitor state without perturbing temporal identity, promoting early progenitor characteristics early in development and late progenitor characteristics later in development. Eventually, constitutive Notch activation led these cells to acquire characteristics of glial and stem cells. In contrast, reactivating the Notch pathway in newly postmitotic retinal cells promoted mature glial cell formation in a subset of cells. These data suggest that prolonged Notch activity does not disrupt the normal progression of progenitor temporal states, and that down-regulating or overcoming Notch activity is required for proper formation of both neuronal and glial cell fates.

Ju, X. D., S. Q. Lou, et al. (2004). "Effect of hydroxyurea and etoposide on transduction of human bone marrow mesenchymal stem and progenitor cell by adeno-associated virus vectors." Acta Pharmacol Sin **25**(2): 196-202.

AIM: To study the effect of hydroxyurea and etoposide on transduction of human marrow mesenchymal and progenitor stem cells by adeno-associated virus (AAV). METHODS: Isolated human bone marrow mesenchymal stem and progenitor cells (hMSCs) were cultured in DMEM containing 10% FBS or 5% FBS and dexamethasone 1 micromol/L respectively. After being treated with hydroxyurea and etoposide, hMSCs were transduced by AAV-LUC. After two days luciferase activity (relative light unites per second or RLU/s) were tested, which indirectly reflected the relative transduction efficiency of different groups, and virus DNA was isolated by Hirt extraction for Southern hybridization. RESULTS: Transduction luciferase activity and transduction efficiency in cultures treated with hydroxyurea and etoposide were significantly higher than that in control cultures. Dividing cells had about 20-fold higher transduction efficiency compared with control cells. Transduction efficiency in stationary cells was about 50 times higher than that in control cells. Southern analysis showed that hydroxyurea and etoposide enhanced second-strand DNA synthesis by rAAV. CONCLUSION: Hydroxyurea and etoposide could increase transduction efficiency of hMSCs by AAV vectors, and stationary cells were more sensitive to these drugs than dividing cells.

Jung, C. G., H. Hida, et al. (2004). "Pleiotrophin mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derived nestin-positive cells." Faseb J **18**(11): 1237-9.

Neural stem cells are promising candidates for donor cells in neural transplantation. However, the mechanism by which neural stem cells differentiate into neurons is not well understood. In the present study, a serial analysis of gene expression (SAGE) was carried out to generate a gene file of neural stem (progenitor) cells from the mouse ventral mesencephalon. Among the 15,815 tags investigated, the mRNA of the housekeeping genes (elongation factor 1-alpha, ATPase subunit 6, GAPDH, actin), laminin receptor 1, HSP 70, pleiotrophin, and nestin were highly expressed. Because pleiotrophin (PTN) exhibits mitogenic and trophic effects on neural

development and exhibits trophic effects on survival of dopaminergic (DAergic) neurons, we investigated the role of PTN in neurogenesis, especially to DAergic neurons. Here, we show that PTN increased the production of tyrosine hydroxylase (TH)-positive neurons from embryonic stem (ES) cell-derived nestin-positive cells. The expression of Nurr1 mRNA was enhanced by PTN. L-dopa in the culture medium was increased by PTN. This effect was as strong as with sonic hedgehog. Data suggest that PTN mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon, and PTN promotes the production of DAergic neurons from ES cell-derived nestin-positive cells.

Juttner, C. A., L. B. To, et al. (1987). "Successful peripheral blood stem-cell autograft with a near-critical dose of myeloid progenitor cells in acute non-lymphoblastic leukaemia in relapse." Med J Aust **147**(6): 292-3.

Rapid, complete and sustained haemopoietic reconstitution was achieved in a 69-year-old man with acute non-lymphoblastic leukaemia in relapse who received an autograft of peripheral blood cells that were collected during very early remission. The patient received 1.7 X 10(8) nucleated cells/kg bodyweight containing 63 X 10(4) myeloid progenitor cells (CFU-GM)/kg bodyweight. Trilineage engraftment was evident in the bone marrow seven days after the graft. Normal neutrophil and platelet counts were attained by day 17, on which day the patient was discharged from hospital. He remained in complete remission three months after the graft with normal blood counts and bone-marrow cellularity. The rapid and sustained haemopoietic activity in this patient, in conjunction with our previous experience of four other patients who received autografts with peripheral blood stem cells, supports the concept we have proposed that a minimum CFU-GM dose of 50 X 10(4)/kg bodyweight produces complete and sustained engraftment. The rapid recovery minimizes aplasia-related risks and suggests that such autografting can be carried out safely in first remission even in older patients. This technique should be considered as a new therapeutic option for patients with acute non-lymphoblastic leukaemia.

Kakinuma, S., H. Nakauchi, et al. (2009). "Hepatic stem/progenitor cells and stem-cell transplantation for the treatment of liver disease." J Gastroenterol **44**(3): 167-72.

Allogeneic liver transplantation is still the only effective treatment available to patients with liver failure. However, because there is a serious shortage of liver donors, an alternative therapeutic approach is needed. Transplantation of mature hepatocytes has been evaluated in clinical trials, but the long-term efficacy remains unclear and the paucity of donor cells limits this strategy. Stem-cell transplantation is a more promising alternative approach. Several studies have provided information about the mechanism underlying the proliferation and differentiation of hepatic stem/progenitor cells. Moreover, in experimental models of liver disease, transplantation of hepatic stem/progenitor cells or hepatocyte-like cells derived from multipotent stem cells led to donor cell-mediated repopulation of the liver and improved survival rates. However, before stem-cell transplantation can be applied in the clinic to treat liver failure in humans, it will be necessary to overcome several difficulties associated with the technique.

Kakinuma, S., H. Ohta, et al. (2009). "Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver." J Hepatol **51**(1): 127-38.

BACKGROUND/AIMS: Hepatic stem/progenitor cells possess active proliferative ability and the capacity for differentiation into hepatic and cholangiocytic lineages. Our group and others have shown that a prospectively defined population in mid-gestational fetal liver contains hepatic stem/progenitor cells. However, the phenotypes of such cells are incompletely elucidated. We analyzed the profile of cell-surface molecules on primary hepatic stem/progenitor cells. METHODS: Expression of cell surface molecules on primary hepatic stem/progenitor cells in mouse mid-gestational fetal liver was analyzed using flow cytometric multicolor analyses and colony-formation assays. The potential of the cells for liver repopulation was examined by transplantation assay. RESULTS: We found that CD13 (aminopeptidase N) was detected on the cells of the previously reported (Dlk/Pref-1(+)) hepatic stem/progenitor fraction. Colony-formation assays revealed that the CD13(+) fraction, compared with the Dlk(+) fraction, of non-hematopoietic cells in fetal liver was enriched in hepatic stem/progenitor cells. Transplantation assay showed the former fraction exhibited repopulating potential in regenerating liver. Moreover, flow cytometric analysis for over 90 antigens demonstrated enrichment of hepatic stem/progenitor cells using several positive selection markers, including (hitherto unknown) CD13, CD73, CD106, and CD133. CONCLUSIONS: Our data indicated that CD13 is a positive selection marker for hepatic stem/progenitor cells in mid-gestational fetal liver.

Kanemura, Y., H. Mori, et al. (2005). "In vitro screening of exogenous factors for human neural stem/progenitor cell proliferation using measurement

of total ATP content in viable cells." Cell Transplant **14**(9): 673-82.

One of the newest and most promising methods for treating intractable neuronal diseases and injures is the transplantation of ex vivo-expanded human neural stem/progenitor cells (NSPCs). Human NSPCs are selectively expanded as free-floating neurospheres in serum-free culture medium containing fibroblast growth factor 2 (FGF2) and/or epidermal growth factor (EGF); however, the culture conditions still need to be optimized for performance and cost before the method is used clinically. Here, to improve the NSPC culture method for clinical use, we used an ATP assay to screen the effects of various reagents on human NSPC proliferation. Human NSPCs responded to EGF, FGF2, and leukemia inhibitory factor (LIF) in a dose-dependent manner, and the minimum concentrations eliciting maximum effects were 10 ng/ml EGF, 10 ng/ ml FGF2, and 5 ng/ml LIF. EGF and LIF were stable in culture medium without NSPCs, although FGF2 was degraded. In the presence of human NSPCs, however, FGF2 and LIF were both degraded very rapidly, to below the estimated minimum concentration on day 3, but EGF remained above the minimum concentration for 5 days. Adding supplemental doses of each growth factor during the incubation promoted human NSPC proliferation. Among other supplements, insulin and transferrin promoted human NSPC growth, but progesterone, putrescine, selenite, D-glucose, and lactate were not effective and were cytotoxic at higher concentrations. Supplementing with conditioned medium from human NSPCs significantly increased human NSPC proliferation, but using a high percentage of the medium had a negative effect. These findings suggest that human NSPC culture is regulated by a balance in the culture medium between decreasing growth factor levels and increasing positive or negative factors derived from the NSPCs. Thus, in designing culture conditions for human NSPCs, it is useful to take the individual properties of each factor into consideration.

Kasbia, G., F. Al-Gahtani, et al. (2008). "Reduced hemoglobin on day of peripheral blood progenitor cell collection is associated with low graft content of vascular progenitors and increased toxicity after autologous hematopoietic stem cell transplantation." Transfusion **48**(11): 2421-8.

BACKGROUND: Tissue damage after hematopoietic stem cell transplantation (HSCT) occurs as a result of high-dose chemotherapy and radiation. The aim was to determine the importance of pretransplant anemia on toxicity and red blood cell (RBC) transfusion requirements after autologous HSCT. STUDY DESIGN AND METHODS: A total of 350 patients undergoing autologous HSCT were included in the analysis. Patient factors and pretransplant laboratory values of possible relevance were assessed in multivariate regression analysis. RESULTS: Reduced hemoglobin (Hb) on the first day of peripheral blood progenitor cell (PBPC) collection was significantly associated with increased organ toxicity after HSCT, as measured by the Seattle criteria. Lower Hb levels at baseline before transplantation, but not at PBPC collection, were significantly associated with increased RBC transfusion requirements. In a second cohort of 28 patients, higher Hb levels on the day of PBPC collection were significantly associated with increased levels of endothelial-like vascular progenitor cells in PBPC grafts. CONCLUSION: Our observations suggest that higher Hb levels on the day of PBPC collection may be a marker of reduced toxicity associated with HSCT and increased vascular progenitors in PBPC collections. Further, baseline anemia before transplant may reflect an unfavorable hematopoietic microenvironment that leads to increased RBC transfusion requirements.

Kasenda, B., S. H. Kassmer, et al. (2008). "The stromal cell-derived factor-1alpha dependent migration of human cord blood CD34 haematopoietic stem and progenitor cells switches from protein kinase C (PKC)-alpha dependence to PKC-alpha independence upon prolonged culture in the presence of Flt3-ligand and interleukin-6." Br J Haematol **142**(5): 831-5.

Addition of the inflammatory cytokine interleukin (IL)-6 to the culture medium of human cord blood haematopoietic stem and progenitor cells (HSPCs) has been shown to lead to an altered stromal cell-derived factor-1alpha-dependent migratory phenotype. This study investigated whether this effect was attributed to a differential engagement of protein kinase C (PKC) isotypes. The migratory activity of both Flt3-ligand and Flt3-ligand/IL-6 cultured cord blood HSPCs was PKC-alpha dependent on day 1, but PKC-alpha independent after 5 d of cultivation. PKC-alpha expression was not down-regulated in cells cultured for 5 d indicating a switch of signalling molecules directing cell migration.

Kashiwakura, I., O. Inanami, et al. (2003). "Protective effects of thrombopoietin and stem cell factor on X-irradiated CD34+ megakaryocytic progenitor cells from human placental and umbilical cord blood." Radiat Res **160**(2): 210-6.

In previous studies we characterized the radiosensitivity of CFU-megakaryocytes from human placental and umbilical cord blood and the effects of various early-acting cytokines. We found that the maximal clonal growth of CFU-megakaryocytes in

vitro and maximal protection against X-ray damage were supported by a combination of thrombopoietin and stem cell factor. However, the mechanism by which the two cytokines exert a synergistic effect remained unclear, so we extended these studies to investigate the radioprotective action of synergistic thrombopoietin and stem cell factor on the survival of X-irradiated CD34(+) CFU-megakaryocytes. A combination of thrombopoietin and stem cell factor led to activation of mitogen-activated protein kinase and extracellular signal-regulated protein kinase and to suppression of caspase 3 in X-irradiated CD34(+) cells. When PD98059 and various synthetic substrates-specific inhibitors of these proteins-were used, the combination had less effect on the clonal growth of X-irradiated CD34(+) CFU-megakaryocytes. However, the addition of wortmannin, a specific inhibitor of the phosphatidylinositol-3 kinase pathway, did not alter the synergistic action of thrombopoietin plus stem cell factor. We suggest that part of this synergistic effect can be explained by activation of mitogen-activated protein kinase and extracellular signal-regulated protein kinase and by suppression of the caspase cascade.

Kashofer, K., E. K. Siapati, et al. (2006). "In vivo formation of unstable heterokaryons after liver damage and hematopoietic stem cell/progenitor transplantation." Stem Cells **24**(4): 1104-12.

Following reports of lineage plasticity in human hematopoietic stem cells (HSCs), we investigated the potential of human cord blood HSC-enriched cells to create hepatocytes in hosts after inducing liver damage. Carbon tetrachloride induces severe liver damage and subsequent repair via mitosis of resident hepatocytes. It additionally leads to a threefold increase in homing of human mononuclear cells to bone marrow and liver and subsequently to a substantial enhancement of bone marrow engraftment. Eight weeks after liver damage and infusion of an enhanced green fluorescent protein (eGFP) lentivirus-transduced human HSC-enriched cell population, we observed eGFP-positive cells with clear hepatocyte morphology in the livers of animals. These eGFP-positive cells co-expressed human albumin, and reverse-transcription polymerase chain reaction (PCR) analysis demonstrated the presence of human albumin and alpha-anti-trypsin mRNA. However, two antibodies against human mitochondria and human nuclei failed to mark eGFP-positive hepatocyte-like cells but did give clear staining of donor-derived hematopoietic cells. Subsequent fluorescent in situ hybridization (FISH) analysis revealed the presence of mouse Y chromosome in eGFP-positive hepatocyte-like cells. To resolve this discrepancy, we performed single-cell PCR analysis of microdissected eGFP-positive hepatocyte-like cells and found that they contained mostly mouse and little human genomic material. FISH analysis highlighting the centromeres of all human chromosomes revealed only few human chromosomes in these cells. From these results, we conclude that similar to their murine counterparts, human hematopoietic cells have the potential to fuse with resident host hepatocytes. Because no selective pressure is applied to retain the human genomic material, it is gradually lost over time, leading to a variable phenotype of the chimeric cells and making their detection difficult.

Kattman, S. J., E. D. Adler, et al. (2007). "Specification of multipotential cardiovascular progenitor cells during embryonic stem cell differentiation and embryonic development." Trends Cardiovasc Med **17**(7): 240-6.

The fully formed heart is composed of diverse cell lineages including myocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts that derive from distinct subsets of mesoderm during embryonic development. Findings from lineage tracing studies indicate that cardiomyocytes develop from cells that express fetal liver kinase-1, suggesting that the cardiac lineages may arise from a progenitor cell with vascular cardiomyocyte potential. Recent studies using the embryonic stem cell model have led to the identification of a fetal liver kinase-1(+) progenitor cell that displays both vascular and cardiomyocyte potential. A comparable progenitor was also isolated from the early mouse embryo. Identification and isolation of these cardiovascular progenitor cells establishes a new model of heart development that will provide insights into the mechanisms regulating cardiovascular lineage diversification. These progenitor cells may also represent a novel cell population for models of congenital heart disease and cell replacement therapy.

Kaushansky, K., N. Fox, et al. (2002). "Lineage-specific growth factors can compensate for stem and progenitor cell deficiencies at the postprogenitor cell level: an analysis of doubly TPO- and G-CSF receptor-deficient mice." Blood **99**(10): 3573-8.

Multiple lines of evidence indicate that thrombopoietin (TPO) substantially impacts the number of hematopoietic stem cells and progenitors of all myeloid lineages. Nevertheless, tpo knock-out mice (T(-)) display thrombocytopenia only; blood erythroid and neutrophil levels are normal despite 60% to 85% reductions in stem and progenitor cells. The compensatory mechanism(s) for these deficiencies remains uncertain; lineage-specific cytokines such as erythropoietin or granulocyte

colony-stimulating factor (G-CSF) have been postulated but never proven to be responsible. To directly test whether G-CSF can compensate for the myeloid progenitor cell reduction in the T(-) model of hematopoietic deficiency, T(-) and G-CSF-receptor knock-out (GR(-)) mice were crossed, and F1 animals bred to obtain doubly nullizygous mice (T(-)GR(-)). This experiment also allowed us to test the hypothesis that G-CSF contributes to the residual platelet production in T(-) mice. We found that T(-)GR(-) F2 mice displayed similar blood platelet levels as that seen in T(-) mice, indicating that G-CSF does not account for the residual megakaryopoiesis in T(-) mice. However, we also noted excessive perinatal mortality of T(-)GR(-) animals, caused by infection due to a profound and significant decrease in marrow and peripheral blood neutrophils, far greater than that seen in either T(-) or GR(-) mice. These data indicate that in the additional absence of GR, T(-) mice cannot compensate for their 62% reduction in myeloid progenitors and become profoundly neutropenic, supporting the hypothesis that G-CSF can compensate for the myeloid effects of TPO deficiency by expanding the pool of cells between the granulocyte-macrophage colony-forming unit and mature neutrophil stages of granulopoiesis.

Kawano, Y., Y. Takaue, et al. (1993). "Effects of progenitor cell dose and preleukapheresis use of human recombinant granulocyte colony-stimulating factor on the recovery of hematopoiesis after blood stem cell autografting in children." Exp Hematol **21**(1): 103-8.

The effects of progenitor cell dose on the recovery kinetics of hematopoiesis were assessed in 34 children who underwent marrow-ablative chemotherapy with peripheral blood stem cell (PBSC) autografting. One patient was not engrafted, probably because of a low dose of reinfused cells. In 25 patients whose PBSCs were harvested after intensive chemotherapy without recombinant granulocyte colony-stimulating factor (rG-CSF), we found a significant correlation between the colony-forming unit for granulocyte-macrophage (CFU-GM) infused per kilogram of the patient's body weight and the time to achieve an absolute granulocyte count (AGC) of > 0.5 x 10(9)/L ($r = -0.817$, $p < 0.001$) or a platelet count of > 50 x 10(9)/L ($r = -0.703$, $p < 0.001$). No association was seen between the number of burst-forming units for erythroid (BFU-E) infused and the speed of hematopoietic recovery. In the other 8 patients with low progenitor yields (< 1 x 10(5) CFU-GM/kg), in vivo induction of PBSC was attempted by administering rG-CSF (250 to 1000 micrograms/m2/day). Seven-day administration of rG-CSF before each leukapheresis increased the committed (CFU-GM: 37.7-fold; BFU-E: 45.6-fold) as well as multipotent (CFU-mix: 6.8-fold) progenitors. These collected cells were cryopreserved and then reinfused. Using a model for simulating hematopoietic recovery kinetics, we suggest that cells induced by rG-CSF could speed the recovery of granulocyte or platelet counts after PBSC autografting.

Ke, X., L. Jia, et al. (2002). "Effects of novel human chemokine-like factor 1 (CKLF1) on bone marrow hematopoietic stem cell/progenitor cell in vitro." Zhonghua Xue Ye Xue Za Zhi **23**(6): 301-3.

OBJECTIVE: To explore the effects of a novel human chemokine-like factor 1 (CKLF1) on stem cell/progenitor cells. METHODS: Human bone marrow mononuclear cells were separated by Ficoll (1.077 g/ml), and cultured in suspension and semisolid colony culture. The effects of CKLF1 on CFU-GM and CFU-Mix were observed. RESULTS: The number of CFU-GM was significantly increased in 10 groups by addition of CKLF1 plasmid supernatant. The mean value was 234.81 +/- 98.71/1 x 10(5) cells, 2.42 fold of control group ($P < 0.05$). The mean value of CFU-Mix in groups of negative control, CKLF1, PHA, GM-CSF and G-CSF were 82.00 +/- 20.25, 105.76 +/- 36.70, 146.63 +/- 27.09, 143.33 +/- 60.23 per 1 x 10(5) cells, respectively, no statistical differences were seen between control and CKLF1 groups. The CD(34)(+) cells were detected by FACS. The average percentage in the groups of normal control, CKLF1, PHA and GM-CSF were (0.75 +/- 0.62)%, (1.32 +/- 0.87)%, (0.15 +/- 0.02)%, and (0.29 +/- 0.23)%, respectively. Compared with control, no significant differences were seen between each group ($P > 0.05$). CONCLUSION: Novel chemokine-like factor 1 can increase the proliferation of CFU-GM, which indicated that CKLF1 could increase the proliferation of human bone marrow hematopoietic progenitor cells and colony formation.

Keirstead, H. S., G. Nistor, et al. (2005). "Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury." J Neurosci **25**(19): 4694-705.

Demyelination contributes to loss of function after spinal cord injury, and thus a potential therapeutic strategy involves replacing myelin-forming cells. Here, we show that transplantation of human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells (OPCs) into adult rat spinal cord injuries enhances remyelination and promotes improvement of motor function. OPCs were injected 7 d or 10 months after injury. In both cases, transplanted cells survived, redistributed over short

distances, and differentiated into oligodendrocytes. Animals that received OPCs 7 d after injury exhibited enhanced remyelination and substantially improved locomotor ability. In contrast, when OPCs were transplanted 10 months after injury, there was no enhanced remyelination or locomotor recovery. These studies document the feasibility of predifferentiating hESCs into functional OPCs and demonstrate their therapeutic potential at early time points after spinal cord injury.

Keller, J. R., S. H. Bartelmez, et al. (1994). "Distinct and overlapping direct effects of macrophage inflammatory protein-1 alpha and transforming growth factor beta on hematopoietic progenitor/stem cell growth." Blood **84**(7): 2175-81.

Both transforming growth factor beta (TGF beta) and macrophage inflammatory protein 1 alpha (MIP-1 alpha) have been shown to be multifunctional regulators of hematopoiesis that can either inhibit or enhance the growth of hematopoietic progenitor cells (HPC). We report here the spectrum of activities of these two cytokines on different hematopoietic progenitor and stem cell populations, and whether these effects are direct or indirect. MIP-1 alpha enhances interleukin-3 (IL-3)/and granulocyte-macrophage colony-stimulating factor (GM-CSF)/induced colony formation of normal bone marrow progenitor cells (BMC) and lineage-negative (Lin-) progenitors, but has no effect on G-CSF or CSF-1/induced colony formation. Similarly, TGF beta enhances GM-CSF/induced colony formation of normal BMC and Lin- progenitors. In contrast, TGF beta inhibits IL-3/ and CSF-1/induced colony formation of Lin- progenitors. The effects of MIP-1 alpha and TGF beta on the growth of Lin- progenitors were direct and correlate with colony formation in soft agar. Separation of the Lin- cells into Thy-1 and Thy-1lo subsets showed that the growth of Thy-1lo Lin- cells is directly inhibited by MIP-1 alpha and TGF beta regardless of the cytokine used to stimulate growth (IL-3), GM-CSF, or CSF-1). In contrast, two other stem cell populations (0% to 15% Hoechst 33342/Rhodamine 123 [Ho/Rh123] and Lin-Sca-1+ cells) were markedly inhibited by TGF beta and unaffected by MIP-1 alpha. Furthermore, MIP-1 alpha has no effect on high proliferative potential colony-forming cells 1 or 2 (HPP-CFC/1 or /2) colony formation in vitro, whereas TGF beta inhibits both HPP-CFC/1 and HPP-CFC/2. Thus, MIP-1 alpha and TGF beta are direct bidirectional regulators of HPC growth, whose effects are dependent on other growth factors present as well as the maturational state of the HPC assayed. The spectrum of their inhibitory and enhancing activities shows overlapping yet distinct effects.

Keller, J. R., M. Ortiz, et al. (1995). "Steel factor (c-kit ligand) promotes the survival of hematopoietic stem/progenitor cells in the absence of cell division." Blood **86**(5): 1757-64.

It is known that the majority of primitive hematopoietic progenitors are in a noncycling quiescent state. In addition, normal hematopoietic progenitors and progenitor cell lines show an absolute dependence on growth factors for their survival in vitro, yet the effect of growth factors on progenitor cell survival has not been separated from effects on both proliferation and differentiation. Using an in vitro assay system, we examined whether growth factors could promote the survival of stem cells in culture in the absence of cell division. These studies show that steel factor (SLF) and, to a lesser extent, interleukin-3 (IL-3) directly promoted the survival of elutriated bone marrow progenitor cells (countercurrent centrifugal elutriation [CCE]-27) that are enriched for primitive hematopoietic progenitors that respond to the combination of SLF plus IL-3. Furthermore, SLF promoted the survival of short-term reconstituting cells (STRC), and long-term reconstituting cells (LTRC) with trilineage reconstitution potential in vivo. In comparison, granulocyte colony-stimulating factor (G-CSF), IL-6, leukemia inhibitory factor, IL-11, IL-1, granulocyte macrophage CSF (GM-CSF), and macrophage CSF (M-CSF) had no effect on the survival of these cells. In the presence of mitotic inhibitors (nocodazole or aphidicolin), SLF promoted the survival of CCE-27 progenitor cells that respond to the combination of SLF plus IL-3 in vitro and STRCs and LTRCs that are detected in vivo. Taken together, these data show that SLF can directly promote the survival of hematopoietic progenitor cells in the absence of cell division.

Kessinger, A. and J. G. Sharp (2003). "The whys and hows of hematopoietic progenitor and stem cell mobilization." Bone Marrow Transplant **31**(5): 319-29.

Intentional mobilization of hematopoietic/stem cells into the circulation has improved the efficiency of their collection. Transplantation of mobilized blood stem cells to patients with marrow aplasia results in a faster pace of hematopoietic recovery than transplantation of marrow-derived stem cells. Autologous and allogeneic hematopoietic stem cell transplantation are increasingly performed with blood-derived cells. Donors of both autologous and allogeneic blood stem cells do not always respond well to therapies designed to produce mobilization. Autologous donors may respond poorly as a result of myelotoxic damage inflicted by prior antitumor therapy, but this

explanation is not valid for allogeneic donors. The mechanism(s) involved in the process of mobilization are incompletely understood. Until these mechanisms are elucidated, methods to improve mobilization vigor on a rational basis will not be obvious. In the meanwhile, clinical observations may provide some hints regarding the whys and hows of mobilization and permit incremental improvements in this process.

Kikushige, Y., G. Yoshimoto, et al. (2008). "Human Flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival." J Immunol **180**(11): 7358-67.

FLT3/FLK2, a member of the receptor tyrosine kinase family, plays a critical role in maintenance of hematopoietic homeostasis, and the constitutively active form of the FLT3 mutation is one of the most common genetic abnormalities in acute myelogenous leukemia. In murine hematopoiesis, Flt3 is not expressed in self-renewing hematopoietic stem cells, but its expression is restricted to the multipotent and the lymphoid progenitor stages at which cells are incapable of self-renewal. We extensively analyzed the expression of Flt3 in human (h) hematopoiesis. Strikingly, in both the bone marrow and the cord blood, the human hematopoietic stem cell population capable of long-term reconstitution in xenogeneic hosts uniformly expressed Flt3. Furthermore, human Flt3 is expressed not only in early lymphoid progenitors, but also in progenitors continuously along the granulocyte/macrophage pathway, including the common myeloid progenitor and the granulocyte/macrophage progenitor. We further found that human Flt3 signaling prevents stem and progenitors from spontaneous apoptotic cell death at least through up-regulating Mcl-1, an indispensable survival factor for hematopoiesis. Thus, the distribution of Flt3 expression is considerably different in human and mouse hematopoiesis, and human FLT3 signaling might play an important role in cell survival, especially at stem and progenitor cells that are critical cellular targets for acute myelogenous leukemia transformation.

Kim, A. C. and G. D. Hammer (2007). "Adrenocortical cells with stem/progenitor cell properties: recent advances." Mol Cell Endocrinol **265-266**: 10-6.

The existence and location of undifferentiated cells with the capability of maintaining the homeostasis of the adrenal cortex have long been sought. These cells are thought to remain mostly quiescent with a potential to commit to self-renewal processes or terminal differentiation to homeostatically repopulate the organ. In addition, in response to physiologic stress, the undifferentiated cells undergo rapid proliferation to accommodate organismic need. Sufficient adrenocortical proliferative capacity lasting the lifespan of the host has been demonstrated through cell transplantation and enucleation experiments. Labeling experiments with tritium, BrdU, or trypan blue, as well as transgenic assays support the clonogenic identity and location of these undefined cells within the gland periphery. We define undifferentiated adrenocortical cells as cells devoid of steroidogenic gene expression, and differentiated cells as cells with steroidogenic capacity. In this review, we discuss historic developmental studies together with recent molecular examinations that aim to characterize such populations of cells.

Kim, H. J., J. F. Tisdale, et al. (2000). "Many multipotential gene-marked progenitor or stem cell clones contribute to hematopoiesis in nonhuman primates." Blood **96**(1): 1-8.

Retroviral insertion site analysis was used to track the contribution of retrovirally transduced primitive progenitors to hematopoiesis after autologous transplantation in the rhesus macaque model. CD34-enriched mobilized peripheral blood cells were transduced with retroviral marking vectors containing the neo gene and were reinfused after total body irradiation. High-level gene transfer efficiency allowed insertion site analysis of individual myeloid and erythroid colony-forming units (CFU) and of highly purified B- and T-lymphoid populations in 2 animals. At multiple time points up to 1 year after transplantation, retroviral insertion sites were identified by performing inverse polymerase chain reaction and sequencing vector-containing CFU or more than 99% pure T- and B-cell populations. Forty-eight unique insertion sequences were detected in the first animal and also in the second animal, and multiple clones contributed to hematopoiesis at 2 or more time points. Multipotential clones contributing to myeloid and lymphoid lineages were identified. These results support the concept that hematopoiesis in large animals is polyclonal and that individual multipotential stem or progenitor cells can contribute to hematopoiesis for prolonged periods. Gene transfer to long-lived, multipotent clones is shown and is encouraging for human gene therapy applications.

Kim, M. K., S. Kim, et al. (2007). "A randomized comparison of peripheral blood hematopoietic progenitor cell level of 5/mm3 versus 50/mm3 as a surrogate marker to initiate efficient autologous blood stem cell collection." J Clin Apher **22**(5): 277-82.

We previously showed that at least 5/mm(3) hematopoietic progenitor cells (HPCs) could be used as a marker for initiating autologous blood stem cell

collection (ABSCC). However, the timing of efficient ABSCC following mobilization is still to be determined. We conducted a prospective, randomized comparison of 5/mm(3) versus 50/mm(3) peripheral blood (PB) HPCs as a surrogate marker to initiate efficient ABSCC. Forty-five consecutive patients, 26 with multiple myeloma (MM) and 19 with non-Hodgkin's lymphoma (NHL), were enrolled between October 2004 and October 2006. Chemotherapy was cyclophosphamide 4 g/m(2) for MM and ESHAP (etoposide, methylprednisolone, high-dose cytarabine, and cisplatin), with or without Rituximab, for NHL. Circulating HPCs were monitored daily with the Sysmex SE9000 automated hematology analyzer, and harvested CD34+ cells were counted by flow cytometry. ABSCC was initiated when HPC levels reached at least 5/mm(3) (HPC5 group) or 50/mm(3) (HPC50 group). The median number of harvested CD34+ cells was 15.0 x 10(6)/kg and 21.0 x 10(6)/kg in the HPC5 and HPC50 groups, respectively (P = 0.23). Optimal collection (>5 x 10(6) CD34+ cells/kg) in a single session (day 1) was attained in 15 HPC5 patients (63%) and in 14 HPC50 patients (67%), and targeted collection of 5 x 10(6) CD34+ cells/kg was achieved in 100 and 95% of HPC5 and HPC50 patients, respectively (P = 0.47), with a median number of 1 apheresis in both groups (P = 0.58). There were no between group differences in optimal collection rate on day 1, median number of aphereses to achieve optimal collection, and overall optimal collection rate. HPC > or = 5/mm(3) and > or =50/mm(3) are both reliable indices for the timing of ABSCC.

Kim, T. S., S. Misumi, et al. (2008). "Increase in dopaminergic neurons from mouse embryonic stem cell-derived neural progenitor/stem cells is mediated by hypoxia inducible factor-1alpha." J Neurosci Res **86**(11): 2353-62.

A reliable method to induce neural progenitor/stem cells (NPCs) into dopaminergic (DAergic) neurons has not yet been established. As well, the mechanism involved remains to be elucidated. To induce DAergic differentiation from NPCs, a cytokine mixture (C-Mix) of interleukin (IL)-1beta, IL-11, leukemia-inhibitory factor (LIF), and glial-derived neurotrophic factor or low oxygen (3.5% O(2): L-Oxy) was used to treat embryonic stem (ES) cell-derived NPCs. Treatment with C-Mix increased the number of tyrosine hydroxylase (TH)-positive cells compared with controls (2.20-fold of control). The C-Mix effect was induced by mainly LIF or IL-1beta treatment. Although L-Oxy caused an increase in TH-positive cells (1.34-fold), the combination of L-Oxy with C-Mix did not show an additive effect. Increases in DA in the medium were shown in the presence of C-Mix, LIF, and L-Oxy by high-performance liquid chromatography. Gene expression patterns of neural markers [tryptophan hydroxylase (TPH), GAD67, GluT1, beta-tubulin III, glial fibrillary acidc protein, and TH] were different in C-Mix and L-Oxy treatments. Because increases in hypoxia-inducible factor (HIF)-1alpha protein were found in both treatments, we investigated the effect of HIF-1alpha on differentiation of NPCs to DAergic neurons. Inhibition of HIF-1alpha by the application of antisense oligodeoxynucleotides (ODNs) to NPCs caused a decrease in TH-positive cells induced by LIF treatment. Gene expressions of TH, GAD67, and GluT1 were decreased, and those of TPH, beta-tubulin III, and S-100beta were increased by treatment with just ODNs, indicating the importance of the endogenous effect of HIF-1alpha on neuronal differentiation. These data suggest that enhanced differentiation into DAergic neurons from ES cell-derived NPCs was induced by C-Mix or L-Oxy mediated by HIF-1alpha.

King, A. G., D. Horowitz, et al. (2001). "Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta." Blood **97**(6): 1534-42.

SB-251353 is an N-terminal truncated form of the human CXC chemokine GRObeta. Recombinant SB-251353 was profiled in murine and rhesus monkey peripheral blood stem cell mobilization and transplantation models. SB-251353 rapidly and transiently mobilized hematopoietic stem cells and neutrophils into the peripheral blood after a single subcutaneous injection. Transplantation of equivalent numbers of hematopoietic stem cells mobilized by SB-251353 into lethally irradiated mice resulted in faster neutrophil and platelet recovery than stem cells mobilized by granulocyte colony-stimulating factor (G-CSF). A single injection of SB-251353 in combination with 4 days of G-CSF administration resulted in augmented stem and progenitor cell mobilization 5-fold greater than G-CSF alone. Augmented stem cell mobilization could also be demonstrated in mice when a single injection of SB-251353 was administered with only one-day treatment with G-CSF. In addition, SB-251353, when used as a single agent or in combination with G-CSF, mobilized long-term repopulating stem cells capable of hematopoietic reconstitution of lethally irradiated mice. In rhesus monkeys, a single injection of SB-251353 induced rapid increases in peripheral blood hematopoietic progenitor cells at a 50-fold lower dose than in mice, which indicates a shift in potency. These

studies provide evidence that the use of SB-251353 alone or in combination with G-CSF mobilizes hematopoietic stem cells with long-term repopulating ability. In addition, this treatment may (1) reduce the number of apheresis sessions and/or amount of G-CSF required to collect adequate numbers of hematopoietic stem cells for successful peripheral blood cell transplantation and (2) improve hematopoietic recovery after transplantation.

Kirouac, D. C., G. J. Madlambayan, et al. (2009). "Cell-cell interaction networks regulate blood stem and progenitor cell fate." Mol Syst Biol **5**: 293.

Communication networks between cells and tissues are necessary for homeostasis in multicellular organisms. Intercellular (between cell) communication networks are particularly relevant in stem cell biology, as stem cell fate decisions (self-renewal, proliferation, lineage specification) are tightly regulated based on physiological demand. We have developed a novel mathematical model of blood stem cell development incorporating cell-level kinetic parameters as functions of secreted molecule-mediated intercellular networks. By relation to quantitative cellular assays, our model is capable of predictively simulating many disparate features of both normal and malignant hematopoiesis, relating internal parameters and microenvironmental variables to measurable cell fate outcomes. Through integrated in silico and experimental analyses, we show that blood stem and progenitor cell fate is regulated by cell-cell feedback, and can be controlled non-cell autonomously by dynamically perturbing intercellular signalling. We extend this concept by demonstrating that variability in the secretion rates of the intercellular regulators is sufficient to explain heterogeneity in culture outputs, and that loss of responsiveness to cell-cell feedback signalling is both necessary and sufficient to induce leukemic transformation in silico.

Kirshenbaum, A. S., C. Akin, et al. (2005). "Thrombopoietin alone or in the presence of stem cell factor supports the growth of KIT(CD117)low/ MPL(CD110)+ human mast cells from hematopoietic progenitor cells." Exp Hematol **33**(4): 413-21.

OBJECTIVES: Thrombopoietin (TPO) is known to promote platelet number, have growth-promoting potential for human megakaryocytes (HuMKs), and increase erythrocyte, monocyte, mast cell, and granulocyte numbers in the presence of additional growth factors. We explored the ability of TPO alone or in the presence of stem cell factor (SCF) to support human mast cells (HuMCs). METHODS: CD34+ pluripotent and CD34+/CD117+/CD13+ HuMC progenitor cells were cultured in rhTPO and examined for HuMCs. Similarly, we added rhTPO to CD34(+) cells cultured in stem cell factor (SCF), which promotes HuMC development. RESULTS: When CD34+ cells were cultured in 10 ng/mL rhTPO and 10 ng/mL rhSCF, TPO enhanced HuMC numbers compared to rhSCF alone. Higher concentrations of rhTPO (50 ng/mL) in the presence of 100 ng/mL rhSCF inhibited the rhSCF-dependent subpopulation of CD117high HuMCs, while promoting CD117low HuMCs. Human CD34+/CD117+/CD13+ cells cultured in rhTPO alone for 1 to 2 weeks differentiated into CD41+/CD110+ HuMKs (85-90%) and FcepsilonRI+/CD117low/CD13+ HuMCs (5-10%). RhTPO-induced HuMCs expressed the TPO (CD110) receptor, tryptase, and chymase and survived when recultured in rhSCF. CONCLUSION: The effect of TPO on HuMCs in the presence of rhSCF varies, depending on the relative concentration of each growth factor, while TPO alone or in combination with rhSCF supports a unique population of CD117low/CD110+ HuMCs.

Kirshenbaum, A. S., J. P. Goff, et al. (1992). "Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells." J Immunol **148**(3): 772-7.

Hemopoietic stem cell factor (SCF), which is the ligand for the proto-oncogene c-kit receptor (allelic with W locus) and the product of Sl locus of the mouse, has recently been cloned. The human homologue has also been cloned, and recombinant protein (human rSCF) expressed and purified to homogeneity. To determine the effect of human rSCF in the presence or absence of human rIL-3 on human bone marrow-derived mast cells and basophils, human CD34+ pluripotent progenitor cells, highly enriched (greater than 99%) from bone marrow mononuclear cells, were cultured over agarose surfaces (interphase cultures) in the presence of human rIL-3, human rIL-3 and increasing concentrations of human rSCF, or human rSCF alone. Over 3 to 4 wk, human rSCF acted synergistically with human rIL-3 at all concentrations, producing a three- to fivefold increase in total, mast cell, and basophil numbers over human rIL-3 alone when used at 100 ng/ml. The percentage of cell types in the human rIL-3 and human rIL-3 plus human rSCF cultures, however, remained the same, with basophils constituting 18 to 35% of the final cultured cells, and mast cells 3% or less of the final cell number. In the presence of human rSCF alone, the combined total percentage of mast cells and basophils was 0 to 1.0%, the majority of cells being macrophages. Mast cells cultured in human rIL-3 plus human rSCF, but not human rIL-3 alone, were berberine sulfate positive, suggesting the presence of heparin proteoglycans within granules. Electron microscopic examination of

cultures supplemented with human rIL-3 and rSCF, but not human rIL-3 alone, revealed that after 3 wk in culture, mast cell granules contained tryptase and exhibited scroll, reticular, and homogeneous patterns as seen previously in CD34+/3T3 fibroblast cocultures. Thus, CD34+ cells cultured in the presence of both human rIL-3 and rSCF give rise to cultures containing increased numbers of basophils and mast cells, with the mast cells by ultrastructural studies showing evidence of maturation although the percentages of basophils and mast cells arising in these cultures remained unchanged.

Kleinman, M. E., M. R. Greives, et al. (2007). "Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma." Arterioscler Thromb Vasc Biol **27**(12): 2664-70.

OBJECTIVE: The mechanism of neovascularization during the proliferative phase of infantile hemangioma is poorly understood. It is known that circulating bone marrow-derived endothelial progenitor cells (EPCs) form new blood vessels in ischemic tissues using mediators regulated by the transcription factor, HIF-1alpha. Mobilization of EPCs is enhanced by VEGF-A, matrix metalloproteinase (MMP)-9, and estrogen, whereas homing is secondary to localized expression of stromal cell-derived factor-1alpha (SDF-1alpha). We examined whether these mediators of EPC trafficking are upregulated during the proliferation of infantile hemangioma. METHODS AND RESULTS: Surgical specimens and blood samples were obtained from children with proliferating hemangioma and age-matched controls (n=10, each group). VEGF-A and MMP-9 levels were measured in blood, and tissue sections were analyzed for SDF-1alpha, MMP-9, VEGF-A, and HIF-1alpha. The role of estrogen as a modulator of hemangioma endothelial cell growth was also investigated. We found that all these mediators of EPC trafficking are elevated in blood and specimens from children with proliferating infantile hemangioma. In vitro, the combination of hypoxia and estrogen demonstrated a synergistic effect on hemangioma endothelial cell proliferation. CONCLUSIONS: These findings demonstrate that proliferating hemangiomas express known mediators of vasculogenesis and suggest that this process may play a role in the initiation or progression of this disease.

Klimchenko, O., M. Mori, et al. (2009). "A common bipotent progenitor generates the erythroid and megakaryocyte lineages in embryonic stem cell-derived primitive hematopoiesis." Blood **114**(8): 1506-17.

The megakaryocytic (MK) and erythroid lineages are tightly associated during differentiation and are generated from a bipotent megakaryocyte-erythroid progenitor (MEP). In the mouse, a primitive MEP has been demonstrated in the yolk sac. In human, it is not known whether the primitive MK and erythroid lineages are generated from a common progenitor or independently. Using hematopoietic differentiation of human embryonic stem cells on the OP9 cell line, we identified a primitive MEP in a subset of cells coexpressing glycophorin A (GPA) and CD41 from day 9 to day 12 of coculturing. This MEP differentiates into primitive erythroid (GPA(+)CD41(-)) and MK (GPA(-)CD41(+)) lineages. In contrast to erythropoietin (EPO)-dependent definitive hematopoiesis, KIT was not detected during erythroid differentiation. A molecular signature for the commitment and differentiation toward both the erythroid and MK lineages was detected by assessing expression of transcription factors, thrombopoietin receptor (MPL) and erythropoietin receptor (EPOR). We showed an inverse correlation between FLI1 and both KLF1 and EPOR during primitive erythroid and MK differentiation, similar to definitive hematopoiesis. This novel MEP differentiation system may allow an in-depth exploration of the molecular bases of erythroid and MK commitment and differentiation.

Knudsen, L. M., T. Rasmussen, et al. (1999). "Reduced bone marrow stem cell pool and progenitor mobilisation in multiple myeloma after melphalan treatment." Med Oncol **16**(4): 245-54.

The content of stem cells was analysed in bone marrow samples from 75 multiple myeloma patients. In unstimulated bone marrow the percentage of CD34+ cells was significantly reduced in 11 patients previously treated with melphalan-prednisolone (MP)(median= 0.15%) compared to median 0.87% in 31 untreated patients (P=0.0001). The bone marrow cellularity in the two groups did not differ. There was no correlation between the number of courses or total dose of melphalan and content of CD34+ cells in the bone marrow. The clonogenicity as well as the ability to expand the marrow stem cell pool during growth factor treatment were also reduced in MP treated patients compared to untreated patients. Analysis of different subsets of CD34+ cells revealed no influence on the pre B cell compartment in the bone marrow by MP treatment, but the committed stem cells (CD34+CD38+) were reduced more than the uncommitted stem cells (CD34+CD38-) in the MP treated group compared to the untreated patients. Mobilisation to and harvest of total number of CD34+ cells from peripheral blood was also reduced in the MP treated group. There was, however, no difference

in the distribution between CD34+CD38+ and CD34+CD38- populations in the leukapheresis products in the untreated and the melphalan-treated group, suggesting selective mobilisation of CD34+CD38+ cells and/or differentiation of CD34+CD38-cells during growth factor stimulation. We conclude that melphalan decreased the number of stem cells in the bone marrow, the ability to expand the stem cell pool and mobilise stem cells to the pheripheral blood.

Koch, K. S., K. H. Son, et al. (2006). "Immune-privileged embryonic Swiss mouse STO and STO cell-derived progenitor cells: major histocompatibility complex and cell differentiation antigen expression patterns resemble those of human embryonic stem cell lines." Immunology **119**(1): 98-115.

Embryonic mouse STO (S, SIM; T, 6-thioguanine resistant; O, ouabain resistant) and 3(8)21-enhanced green fluorescent protein (EGFP) cell lines exhibit long-term survival and hepatic progenitor cell behaviour after xenogeneic engraftment in non-immunosuppressed inbred rats, and were previously designated major histocompatibility complex (MHC) class I- and class II-negative lines. To determine the molecular basis for undetectable MHC determinants, the expression and haplotype of H-2K, H-2D, H-2L and I-A proteins were reassessed by reverse transcriptase-polymerase chain reaction (RT-PCR), cDNA sequencing, RNA hybridization, immunoblotting, quantitative RT-PCR (QPCR), immunocytochemistry and flow cytometry. To detect cell differentiation (CD) surface antigens characteristic of stem cells, apoptotic regulation or adaptive immunity that might facilitate progenitor cell status or immune privilege, flow cytometry was also used to screen untreated and cytokine [interferon (IFN)-gamma]-treated cultures. Despite prior PCR genotyping analyses suggestive of H-2q haplotypes in STO, 3(8)21-EGFP and parental 3(8)21 cells, all three lines expressed H-2K cDNA sequences identical to those of d-haplotype BALB/c mice, as well as constitutive and cytokine-inducible H-2K(d) determinants. In contrast, apart from H-2L(d[LOW]) display in 3(8)21 cells, H-2Dd, H-2Ld and I-Ad determinants were undetectable. All three lines expressed constitutive and cytokine-inducible CD34; however, except for inducible CD117([LOW]) expression in 3(8)21 cells, no expression of CD45, CD117, CD62L, CD80, CD86, CD90.1 or CD95L/CD178 was observed. Constitutive and cytokine-inducible CD95([LOW]) expression was detected in STO and 3(8)21 cells, but not in 3(8)21-EGFP cells. MHC (class I(+[LOW])/class II-) and CD (CD34+/CD80-/CD86-/CD95L-) expression patterns in STO and STO cell-derived progenitor cells resemble patterns reported for human embryonic stem cell lines. Whether these patterns reflect associations with mechanisms that are regulatory of immune privilege or functional tissue-specific plasticity is unknown.

Komitova, M., B. Mattsson, et al. (2005). "Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats." Stroke **36**(6): 1278-82.

BACKGROUND AND PURPOSE: The subventricular zone in the adult brain is identified as an endogenous resource of neuronal precursors that can be recruited to adjacent lesioned areas. The hypothesis was tested that postischemic environmental enrichment might enhance subventricular zone cell genesis. METHODS: A cortical infarct was induced in adult spontaneously hypertensive rats by ligating the middle cerebral artery distal to the striatal branches, after which animals were housed in either standard or enriched environment and allowed to survive for 5 weeks. The thymidine analogue bromodeoxyuridine was administered during the first postischemic week. The generation of neural stem/progenitor cells and neuronal precursors in the subventricular zone were studied with cell specific markers such as Ki67 and phosphorylated histone H3 (cell proliferation), Sox-2 (neural stem/progenitor cells), bromodeoxyuridine (slowly cycling, nonmigratory putative neural stem cells), and doublecortin (newborn immature neurons). RESULTS: Proliferating cells in the subventricular zone were identified as chiefly neural progenitors but also putative neural stem cells and neuronal precursors. Five weeks after stroke, proliferation in the subventricular zone was lower in stroke-lesioned rats housed in standard environment compared with nonlesioned rats. Postischemic environmental enrichment normalized cell proliferation levels, increased the numbers of putative neural stem cells as assessed with bromodeoxyuridine, and increased doublecortin-positive neuroblasts, which extended in migratory chains toward the infarct. CONCLUSIONS: Enriched environment increased the neural stem/progenitor cell pool and neurogenesis in the adult subventricular zone 5 weeks after a cortical stroke. This might be of potential importance for tissue regeneration.

Korbling, M., P. Anderlini, et al. (1996). "Delayed effects of rhG-CSF mobilization treatment and apheresis on circulating CD34+ and CD34+ Thy-1dim CD38- progenitor cells, and lymphoid subsets in normal stem cell donors for allogeneic transplantation." Bone Marrow Transplant **18**(6): 1073-9.

Allogeneic transplantation of peripheral blood progenitor cells (PBPC) is emerging as a new stem cell transplant modality. Rather than undergoing general anesthesia for bone marrow harvest, normal blood stem cell donors are subjected to rhG-CSF mobilization treatment followed by single or multiple apheresis. Whereas the effects of cytokine treatment and apheresis on stem cell peripheralization and collection have been described, little is known about delayed effects of rhG-CSF treatment and apheresis on a normal hematopoietic system, and there are no long-term data that address safety issues. Ten normal, patient-related donors underwent a 3 or 4 day rhG-CSF (filgrastim) treatment (12 micrograms/kg/day) followed by single or tandem apheresis. We monitored peripheral blood (PB) cellularity including CD34+ and lymphoid subsets at baseline, during cytokine treatment, prior to apheresis, and at days 2, 4, 7, 30 and 100 post-apheresis. The PB progenitor cell concentration peak prior to apheresis was followed by a nadir by day 7 and normalized by day 30, with the exception of the most primitive CD34+ Thy-1dim CD38- progenitor subset that reached a nadir by day 30. Lymphoid subsets such as CD3, 4, 8, suppressor cells (CD3+ 4- 8- TCR+ alpha beta), and B cells (CD19+) showed a similar pattern with a nadir concentration by day 7, followed, except for B cells, by a rebound by day 30 and subnormal counts at day 100. The PB concentrations of hemoglobin and platelets dropped mainly due to the apheresis procedure itself, and normalized by day 30. With cytokine treatment, the PB alkaline phosphatase and lactate dehydrogenase concentrations increased 2.2- and 2.8-fold, respectively, over baseline, and returned to normal range by day 30. Based on the preliminary nature of this study, the clinical relevance of these findings is still unclear.

Korbling, M., Y. O. Huh, et al. (1995). "Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease." Blood **86**(7): 2842-8.

Apheresis-derived hematopoietic progenitor cells have recently been used for allogeneic transplantation. Forty-one normal donors were studied to assess the effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) (12 micrograms/kg/d) on the peripheralization of hematopoietic progenitor cells and lymphoid subsets. The white blood cell, polymorphonuclear cell (PMNC), and lymphocyte concentrations at the peak of rhG-CSF effect in the donor's peripheral blood (PB) exceeded baseline by 6.4-, 8.0-, and 2.2-fold, respectively. Corresponding concentrations of PB CD34+ cells and primitive subsets such as CD34+ Thy-1dim, and CD34+ Thy-1dim CD38- cells increased by 16.3-fold, 24.2-fold, and 23.2-fold, respectively in eight normal donors. The percentage of CD34+ Thy-1dim and CD34+ Thy-1dim CD38- cells among CD34+ cells increased as well, suggesting an additional peripheralization effect of rhG-CSF on primitive CD34+ subsets. The preapheresis PB CD34+ and CD34+ Thy-1dim cell concentrations were predictive of their corresponding apheresis yield per liter of donor blood processed PB lymphoid subsets were not significantly affected by rhG-CSF treatment. The mean apheresis-derived yield of CD34+, CD34+ Thy-1dim, and CD34+ Thy-1dim CD38- cells per kilogram of recipient body weight and per liter of donor blood processed was 48.9 x 10(4) (n = 41), 27.2 x 10(4) (n = 10), and 1.9 x 10(4) (n = 10), respectively. As compared with 43 single bone marrow (BM) harvest, the CD34+ cell yield of peripheral blood progenitor cell allografts of 41 normal donors exceeded that of BM allografts by 3.7-fold and that of lymphoid subsets by 16.1-fold (CD3+), 13.3-fold (CD4+), 27.4-fold (CD8+), 11.0-fold (CD19+), and 19.4-fold (CD56+CD3-). All PBPC allografts were cryopreserved before transplantation. The mean recovery of CD34+ cells after freezing, thawing, and washing out dimethylsulfoxide was 86.6% (n = 31) and the recovery of lymphoid subsets was 115.5% (CD3+), 121.4% (CD4+), 105.6% (CD8+), 118.1% (CD19+), and 102.4% (CD56+CD3-). All donors were related to patients: 39 sibling-to-sibling, 1 parent-to-child, and 1 child-to-parent transplant. Thirty-eight transplants were HLA fully identical, two transplants differed in one and two antigens. Engraftment occurred in 38 recipients; two patients died too early to be evaluated, and one patient did not engraft. The lowest CD34+ cell dose transplanted and resulting in complete and sustained engraftment was 2.5 x 10(6)/kg of recipient body weight. There was no significant correlation between the total number of CD34+ cells transfused and the time to reach PMNC >0.5 x 10(9)/L or platelets > 50 x 10(9)/L posttransplant, nor was there a correlation found between the total number of CD3+, CD4+, and CD8+ cells transfused and the development of acute graft-versus-host disease (GVHD). The actuarial probability of developing acute GVHD in 38 evaluable patients was 48%. In 13 patients followed longer than 100 days posttransplant, the actuarial probability of developing chronic GVHD was 66% (median follow-up, 264 days).

Krivtsov, A. V., D. Twomey, et al. (2006). "Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9." Nature **442**(7104): 818-22.

Leukaemias and other cancers possess a rare population of cells capable of the limitless self-renewal necessary for cancer initiation and maintenance. Eradication of these cancer stem cells is probably a critical part of any successful anti-cancer therapy, and may explain why conventional cancer therapies are often effective in reducing tumour burden, but are only rarely curative. Given that both normal and cancer stem cells are capable of self-renewal, the extent to which cancer stem cells resemble normal tissue stem cells is a critical issue if targeted therapies are to be developed. However, it remains unclear whether cancer stem cells must be phenotypically similar to normal tissue stem cells or whether they can retain the identity of committed progenitors. Here we show that leukaemia stem cells (LSC) can maintain the global identity of the progenitor from which they arose while activating a limited stem-cell- or self-renewal-associated programme. We isolated LSC from leukaemias initiated in committed granulocyte macrophage progenitors through introduction of the MLL-AF9 fusion protein encoded by the t(9;11)(p22;q23). The LSC were capable of transferring leukaemia to secondary recipient mice when only four cells were transferred, and possessed an immunophenotype and global gene expression profile very similar to that of normal granulocyte macrophage progenitors. However, a subset of genes highly expressed in normal haematopoietic stem cells was re-activated in LSC. LSC can thus be generated from committed progenitors without widespread reprogramming of gene expression, and a leukaemia self-renewal-associated signature is activated in the process. Our findings define progression from normal progenitor to cancer stem cell, and suggest that targeting a self-renewal programme expressed in an abnormal context may be possible.

Kroger, N., W. Zeller, et al. (1999). "Stem cell mobilization with G-CSF alone in breast cancer patients: higher progenitor cell yield by delivering divided doses (2 x 5 microg/kg) compared to a single dose (1 x 10 microg/kg)." Bone Marrow Transplant **23**(2): 125-9.

We investigated the schedule dependency of G-CSF (10 microg/kg) alone in mobilizing peripheral blood progenitor cells (PBPC) in breast cancer patients. After a median of three cycles (range, 2-6) of anthracycline-based chemotherapy, 49 patients with breast cancer (stage II/III, > or = 10+ Ln n = 36; locally advanced/inflammatory n = 8, stage IV (NED) n = 5) underwent PBPC collection after steady-state mobilization either with 1 x 10 microg/kg (n = 27) or with 2 x 5 microg/kg (n = 22) G-CSF daily for 4 consecutive days until completion of apheresis. Apheresis was started on day 5. Priming with 2 x 5 microg/kg resulted in a higher median number of CD34+ cells (5.8 vs 1.9 x 10(6)/kg, P = 0.003), MNC (6.6 vs 2.6 x 10(8)/kg, P < 0.001) and CFU-GM (6.5 vs 1.3 x 10(4)/kg, P = 0.001) in the first apheresis than with 1 x 10 microg/kg. Also the overall number of collected BFU-E was higher in the 2 x 5 microg group (9.2 vs 3.1 x 10(4)/kg; P = 0.01). After high-dose chemotherapy with cyclophosphamide/thiotepa/mitoxantrone (n = 46) hematopoietic engraftment with leukocyte count > 1.0/nl was reached in both groups after a median of 10 days (range, 8-15) and with platelets count > 50/nl after 12 (range, 9-40) and 13 days (range, 12-41), respectively. A threshold of > 2.5 x 10(6)/kg reinfused CD34+ cells ensured rapid platelet engraftment (12 vs 17 days; P = 0.12). Therefore, the target of collecting > 2.5 x 10(6) CD34+ cells was achieved in 21/27 (80%) patients of the 1 x 10 microg group and in 21/22 (95%) patients of the 2 x 5 microg/kg group with a median of two aphereses (range, 1-4). None in the 10 microg/kg group, but 6/22 (28%) patients in the 2 x 5 microg/kg group required only one apheresis procedure, resulting in fewer apheresis procedures in the 2 x 5 microg/kg group (mean, 1.8 vs 2.3, P = 0.01). These results demonstrate that priming with 10 microg/kg G-CSF alone is well tolerated and effective in mobilizing sufficient numbers of CD34+ cells in breast cancer patients and provide prompt engraftment after CTM high-dose chemotherapy. G-CSF given 5 microg/kg twice daily (2 x 5 microg) leads to a higher harvest of CD34+ cells and required fewer apheresis procedures than when given 10 microg/kg once daily (1 x 10 microg).

Kruse, C., J. Kajahn, et al. (2006). "Adult pancreatic stem/progenitor cells spontaneously differentiate in vitro into multiple cell lineages and form teratoma-like structures." Ann Anat **188**(6): 503-17.

Cells isolated from pancreas have a remarkable potential for self-renewal and multilineage differentiation. We here present a comprehensive characterisation of stem/progenitor cells derived from exocrine parts of the adult rat pancreas. Using purified cells from either single colonies or even single-cell clones, we specifically demonstrate: (i) the cells contain the typical stem/progenitor cell markers alkaline phophatase, SSEA-1, Oct-4, CD9, Nestin, Pax6, CD44, a-Fetoprotein and Brachyury, demonstrated by immunocytochemistry and RT-PCR; (ii) the cells have the potential to differentiate into lineages of all three germ layers in vitro; (iii) a clonal analysis revealed that even cell lines derived from a single cell have stem/progenitor cell properties such as self-renewal and spontaneous differentiation into various cell lineages; (iv) the cells have the propensity

to form three-dimensional, teratoma-like structures in vitro, which contain cells of different lineages; and (v) external stimuli can activate the generation of certain cell types. For instance, cells treated with retinoic acid show an increased expression of alpha-smooth muscle actin. These results suggest that exocrine glands, such as pancreas may be a potential source of adult stem/progenitor cells, suitable for cell therapy of degenerative diseases.

Kubo, F. and S. Nakagawa (2009). "Hairy1 acts as a node downstream of Wnt signaling to maintain retinal stem cell-like progenitor cells in the chick ciliary marginal zone." Development **136**(11): 1823-33.

In the vertebrate retina, stem cell-like progenitor cells are maintained in a distinct region called the ciliary marginal zone (CMZ). Canonical Wnt signaling regulates the maintenance of the progenitor cells in the CMZ. However, its downstream molecular mechanisms have remained largely unclear. Here, we show that chick Hairy1, an established Notch signaling effector, mediates the Wnt-dependent maintenance of CMZ progenitor cells in chicken. Interestingly, unlike other developmental contexts in which Hes gene expression is regulated by Notch signaling, Hairy1 expression in the CMZ is regulated by Wnt signaling. Hairy1 is necessary and sufficient for the expression of a set of molecular markers characteristic of the CMZ, and Wnt2b fails to induce CMZ markers when Hairy1 activity is inhibited. Furthermore, microarray analysis identifies multiple Wnt-responsive transcription factors that activate Hairy1 expression. We thus propose that Hairy1 functions as a node downstream of Wnt signaling to maintain progenitor cells in the chick CMZ.

Kulbatski, I., A. J. Mothe, et al. (2005). "Endogenous and exogenous CNS derived stem/progenitor cell approaches for neurotrauma." Curr Drug Targets **6**(1): 111-26.

Neural stem/progenitor cells capable of generating new neurons and glia, reside in specific areas of the adult mammalian central nervous system (CNS), including the ependymal region of the spinal cord and the subventricular zone (SVZ), hippocampus, and dentate gyrus of the brain. Much is known about the neurogenic regions in the CNS, and their response to various stimuli including injury, neurotrophins (NFs), morphogens, and environmental factors like learning, stress, and aging. This work has shaped our current views about the CNS's potential to recover lost tissue and function post-traumatically and the therapies to support the intrinsic regenerative capacity of the brain or spinal cord. Recently, intensive research has explored the potential of harvesting, culturing, and transplanting neural stem/progenitors as a therapeutic intervention for spinal cord injury (SCI) and traumatic brain injury (TBI). Another strategy has focused on maximizing the potential of this endogenous population of cells by stimulating their recruitment, proliferation, migration, and differentiation in vivo following traumatic lesions to the CNS. The promise of such experimental treatments has prompted tissue and biomaterial engineers to implant synthetic three-dimensional biodegradable scaffolds seeded with neural stem/progenitors into CNS lesions. Although there is no definitive answer about the ideal cell type for transplantation, strong evidence supports the use of region specific neural stem/progenitors. The technical and logistic considerations for transplanting neural stem/progenitors are extensive and crucial to optimizing and maintaining cell survival both before and after transplantation, as well as for tracking the fate of transplanted cells. These issues have been systematically addressed in many animal models, that has improved our understanding and approach to clinical therapeutic paradigms.

Kumagai, G., Y. Okada, et al. (2009). "Roles of ES cell-derived gliogenic neural stem/progenitor cells in functional recovery after spinal cord injury." PLoS One **4**(11): e7706.

Transplantation of neural stem/progenitor cells (NS/PCs) following the sub-acute phase of spinal cord injury (SCI) has been shown to promote functional recovery in rodent models. However, the types of cells most effective for treating SCI have not been clarified. Taking advantage of our recently established neurosphere-based culture system of ES cell-derived NS/PCs, in which primary neurospheres (PNS) and passaged secondary neurospheres (SNS) exhibit neurogenic and gliogenic potentials, respectively, here we examined the distinct effects of transplanting neurogenic and gliogenic NS/PCs on the functional recovery of a mouse model of SCI. ES cell-derived PNS and SNS transplanted 9 days after contusive injury at the Th10 level exhibited neurogenic and gliogenic differentiation tendencies, respectively, similar to those seen in vitro. Interestingly, transplantation of the gliogenic SNS, but not the neurogenic PNS, promoted axonal growth, remyelination, and angiogenesis, and resulted in significant locomotor functional recovery after SCI. These findings suggest that gliogenic NS/PCs are effective for promoting the recovery from SCI, and provide essential insight into the mechanisms through which cellular transplantation leads to functional improvement after SCI.

Kurata, H., G. C. Mancini, et al. (1998). "Stem cell factor induces proliferation and differentiation of fetal

progenitor cells in the mouse." Br J Haematol **101**(4): 676-87.

We have investigated the kinetics of the amplification of the progenitor cell compartments (CFC) in haemopoietic organs during murine ontogenesis and compared the growth requirements of fetal and adult CFC. Two haemopoietic phases were recognized in the fetal liver (FL): an exponential growth phase, from 11.5 to 15.5 d post conception (p.c.), during which the mean number of nucleated cells and of CFC in the FL increased from 4.9 x 10(5) to 7.0 x 10(7) and from 4.5 x 10(3) to 2.7 x 10(5), respectively, and a recessive phase after 15.5 d p.c., during which the CFC number in the FL gradually decreased, although some CFC were still detectable in the liver after birth. In serum-deprived cultures, FL and adult marrow (AM) CFC had similar responses to GM-CSF, and did not respond to G-CSF or IL-3. In contrast, FL, but not AM, erythroid colonies grew Epo-independently whereas SCF alone induced formation of maximal numbers of erythroid bursts from FL, but not from AM cells. The proliferative and differentiative effect of SCF alone on fetal cells was confirmed in serum-deprived cultures of purified early progenitor cells isolated by cell sorting on the basis of multiple parameters from FL and AM light-density cells. In culture of purified FL cells, SCF alone induced a similar amplification of total cells (maximal amplification at day 12: 800-300-fold) and total CFC (11-38-fold of maximal amplification at day 6) to the combination of SCF plus IL-3 (1300-800-fold amplification of total cells and 31-88-fold amplification of CFC). In contrast, SCF alone allowed only survival of purified AM early progenitor cells. Therefore FL early progenitor cells have an intrinsic higher potential than their adult counterpart to respond to SCF, confirming the potent role of this growth factor in the development of the murine haemopoietic system.

Lardon, F., H. W. Snoeck, et al. (1994). "Transforming growth factor-beta regulates the cell cycle status of interleukin-3 (IL-3) plus IL-1, stem cell factor, or IL-6 stimulated CD34+ human hematopoietic progenitor cells through different cell kinetic mechanisms depending on the applied stimulus." Exp Hematol **22**(9): 903-9.

The immediate cell kinetic response of highly purified human bone marrow progenitor cells (CD34+ sorted fraction) to the inhibitory effects of transforming growth factor-beta (TGF-beta) was studied using the BrdU-Hoechst flow-cytometric technique. The progenitor cells were stimulated with either interleukin-3 (IL-3) alone or with IL-3 in combination with IL-1, stem cell factor (SCF), or IL-6, and the inhibitory action of TGF-beta was evaluated in each phase of the first three consecutive cell cycles. Semisolid methylcellulose cultures were also performed to compare these initial events to the effects observed after 7, 14, and 21 days of incubation. Within the CD34+ compartment, the progenitor cells can be discriminated on a functional basis, i.e., in terms of TGF-beta sensitivity. Very primitive progenitors, recruited out of the G0 phase by IL-3 plus an early-acting factor (IL-1, SCF) are, upon addition of TGF-beta, arrested specifically in the G1 phase of the second cell cycle. In the clonogenic assays, the increased colony formation due to IL-1 or SCF was completely abolished by the counteracting effect of TGF-beta that diminished colony output back to the level of TGF-beta-plus-IL-3 supplemented colony growth. Addition of TGF-beta to CD34+ progenitors responding to IL-3 alone resulted in an overall retardation, but without an apparent specific accumulation of cells in any of the cell cycles. Finally, within the CD34+ compartment, there exists a subset of IL-3-responsive, but TGF-beta-insensitive, progenitor cells that were, upon addition of TGF-beta, not arrested at all. In conclusion, our results demonstrate that TGF-beta exerts different cell kinetic effects on CD34+ progenitor cell growth depending on the applied stimulus.

Lassmann, H. (2005). "Stem cell and progenitor cell transplantation in multiple sclerosis: the discrepancy between neurobiological attraction and clinical feasibility." J Neurol Sci **233**(1-2): 83-6.

Recent developments in our understanding of stem- and progenitor cell differentiation raises hopes that brain damage in chronic neurological diseases may become repaired by systemic or focal transplantation of such cells. In this review the potential of such an approach is discussed, but it is also highlighted that many aspects regarding its feasibility or safety are currently unresolved. Furthermore, recent findings on the pathogenesis of multiple sclerosis lesions indicate that major problems in this disease rather are related to axonal pathology and neurodegeneration rather than to the absence of oligodendrocyte progenitor cells within the lesions. In light of this complex situation, it is concluded that clinical trials of stem- or progenitor cell transplantation in multiple sclerosis are currently premature.

Laterveer, L., J. M. Zijlmans, et al. (1996). "Improved survival of lethally irradiated recipient mice transplanted with circulating progenitor cells mobilized by IL-8 after pretreatment with stem cell factor." Exp Hematol **24**(12): 1387-93.

We have demonstrated previously that a single bolus-injection of interleukin (IL)-8 induces

instant mobilization of hematopoietic progenitor cells (HPC) in mice and primates. To further improve the mobilization of HPC, we treated mice with hematopoietic growth factors (HGF) before IL-8-administration. The mobilized HPC were transplanted into lethally irradiated recipient mice to study the effects on survival. Male donor mice (age 8-12 weeks, weight 20-25 grams) were pretreated intraperitoneally (ip) with a fixed dose of 2.5 micrograms of either granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, stem cell factor (SCF), or saline administered twice daily for 2 to 4 days. Then a fixed dose of 30 micrograms of IL-8 was administered ip at various time intervals before harvesting blood, bone marrow, and spleen. Cell counts and numbers of colony-forming units granulocyte/macrophage (CFU-GM) of these organs were assessed. Donor mice pretreated with HGF for 2 days and subsequently injected with IL-8 showed an increase in the numbers of circulating CFU-GM per mL blood from 168 +/- 98 to 402 +/- 201 (mean +/- SD, CFU-GM/mL blood) when GM-CSF was used, 314 +/- 133 to 2502 +/- 513 with G-CSF, and 27 +/- 15 to 524 +/- 339 with SCF compared with saline-pretreated controls (28 +/- 17 to 462 +/- 335 CFU-GM/mL blood, mean +/- SD; n = 42 and 40 per interval). Donor-mice pretreated for 4 days with IL-3 or GM-CSF showed an increase in the numbers of circulating HPC from 62 +/- 52 to 368 +/- 118 and 859 +/- 387 to 1034 +/- 421, respectively (CFU-GM/mL, mean +/- SD, n = 4 per group). Lethally irradiated (8.5 Gy) female Balb/c mice were then injected with decreasing numbers of peripheral blood mononuclear cells (PBMNC). Transplantation of 1.5 x 10(5) MNC obtained from donors pretreated with SCF for 2 days prior to IL-8 mobilization resulted in a significantly enhanced survival of 100% of the recipients, whereas recipients of PBM-NCs derived from donors treated with SCF only or IL-8 as a single injection had a survival rate at day 60 of only 50% and 60% respectively. When equal numbers of IL-8 mobilized MNCs from G-CSF, GM-CSF, or IL-3 pretreated donors were transplanted into lethally irradiated recipients, no such survival-advantage was observed. We conclude that pretreatment with SCF for 2 days improves the mobilizing effect induced by IL-8 and that transplantation of these cells enhances survival of lethally irradiated recipients.

Lee, C., C. A. Evans, et al. (2003). "Generation of a conditionally immortalized myeloid progenitor cell line requiring the presence of both interleukin-3 and stem cell factor to survive and proliferate." Br J Haematol **122**(6): 985-95.

The H-2Kappab temperature-sensitive (ts) A58 transgenic (Immorto) mouse has been used previously to generate conditionally immortalized cells from a number of tissues. The present study aimed to investigate characteristics of primitive myeloid precursor cells derived from H-2Kappab-tsA58 bone marrow. Cell populations were enriched for granulocyte/macrophage progenitors by centrifugal elutriation, and were cultured in the presence and absence of cytokines at the permissive and restrictive temperatures for the A58 oncogene. Cells derived from H-2Kappab-tsA58 mice required both A58 activation and the growth factors, stem cell factor (SCF) and interleukin-3 (IL-3), for long-term cell survival and growth; cells were maintained for > 300 d in culture under these conditions. IL-3- and SCF-dependent clonal cell lines were derived with a phenotype (lin-, Sca-1+, CD34+, ER-MP 58+, ER-MP 12+, ER-MP 20-) characteristic of primitive myeloid progenitors. These cells differentiated on addition of granulocyte/macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor (M-CSF) and acquired mature cell morphology with some upregulation of differentiation markers. In conclusion, the A58 oncogene can immortalize haemopoietic progenitor cells. These cells require two cytokines for growth, IL-3 and SCF; as such, they constitute a useful resource for the study of synergistic interactions between growth factors. The ability to develop monocytic cell characteristics also permits the investigation of cytokine-mediated early haemopoietic progenitor cell development.

Lee, J. L., S. B. Kim, et al. (2003). "Clinical usefulness of the hematopoietic progenitor cell counts in predicting the optimal timing of peripheral blood stem cell harvest." J Korean Med Sci **18**(1): 27-35.

Although enumeration of CD34+ cells in the peripheral blood (PB) on the day of apheresis predicts the quantity of those cells collected, the flow cytometric techniques used are complex and expensive, and several hours are required to obtain the result in the clinical practice setting. The Sysmex SE-9000 automated haematology analyzer provides an estimate of immature cells, called hematopoietic progenitor cells (HPC). The aim of this study was to evaluate the clinical usefulness of HPC in predicting the optimal timing of peripheral blood progenitor cells (PBPC) harvest. Studies were performed on 628 aphereses from 160 patients with hematologic or solid malignancies. Spearman's rank statistics was used to assess correlation between HPC, WBC, mononuclear cells (MNC), and CD34+ cells. A receiver operating characteristic (ROC) curve was drawn for cutoff value of HPC, and predictive values of the chosen cutoff value of HPC for different target CD34+ cell collections were calculated. The PB HPC had a stronger correlation (rho=0.592, $p<0.001$) with

collected CD34+ cells than did PB WBC and PB MNC. The ROC curve showed that the best cutoff value of HPC was 50 x 10(6)/L for the target CD34+ cells > or =1 x 10(6)/kg with sensitivity of 75%. Positive and negative predictive values of HPC > or =50 x 10(6)/L for CD34+ cells > or =1 x 10(6)/kg were 59.7% and 81.1%, respectively. In the clinical practice setting, applying variable cutoff values of HPC would be a useful tool to predict the optimal timing of PBPC collection.

Lefrere, F., F. Audat, et al. (1999). "The timing of granulocyte-colony-stimulating factor administration after chemotherapy does not affect stem and progenitor cell apheresis yield: a retrospective study of 65 cases." Transfusion **39**(6): 561-4.

BACKGROUND: The optimal time for postchemotherapy granulocyte-colony stimulating factor (G-CSF) administration before peripheral blood stem and progenitor cell (PBPC) collection is not well defined. The impact of G-CSF scheduling on the number of CD34+ cells collected by leukapheresis from 65 patients with malignant disease was studied retrospectively. STUDY DESIGN AND METHODS: Chemotherapy was performed on Days 1 and 2 and was followed by G-CSF to mobilize PBPCs. In Group 1, 30 patients received the first dose of G-CSF immediately after the end of chemotherapy, as commonly recommended. In Group 2, 35 patients received the first G-CSF dose after the end of chemotherapy (Days 7 or 8). RESULTS: No difference was observed between the two groups in white cell recovery and the median number of CD34+ cells harvested. The number of leukapheresis procedures necessary to obtain the minimal number of 3 x 10(6) CD34+ cells per kg was the same. The proportion of patients with a failure of PBPC collection was similar, and G-CSF consumption was reduced in Group 2 without increasing infectious risks. CONCLUSION: Early administration of G-CSF after chemotherapy appears not to be a prerequisite for satisfactory PBPC collection. This approach could allow significant savings in terms of medical cost. A randomized and prospective study would be necessary, however, to assess the validity of these conclusions.

Li, J., X. Meng, et al. (2008). "Prolongation of cardiac allograft survival by syngeneic hematopoietic stem/progenitor cell transplantation in mice." Adv Ther **25**(9): 935-42.

INTRODUCTION: Organ transplantation is a rapidly developing field, being the only effective treatment for end-stage organ disease. However, the associated immunosuppressant therapy has numerous direct and indirect adverse effects. Hematopoietic stem cell transplantation (HSCT), via immune reconstitution, offers an alternative method of treatment. In this study, we determined the cardiac allograft survival in mice treated with syngeneic HSCT or hematopoietic progenitor cell transplantation (HPCT). METHODS: BALB/c and C57BL/6 mice were randomly divided into three groups. Mice in Group A (n=7) were untreated while those in Group B (n=8) and C (n=7) were treated with HPCT and HSCT, respectively. Cervical heterotopic cardiac transplantation models were established in all groups and cardiac grafts were observed throughout. Regulatory T (Treg) cell expression in peripheral blood was analyzed by flow cytometry. We recorded the cardiac allograft survival time and constructed Kaplan-Meier survival curves. RESULTS: The number of Treg cells in Group B was significantly higher than that in Group C ($P<0.05$). The survival time of mice from each group differed significantly according to Kaplan-Meier/log-rank analysis ($P<0.01$). A total of 62.5% of the grafts in Group B showed long-term survival (>100 days); all the mice in Group A died within 9 days, compared with 59 days in Group C. CONCLUSION: We conclude that syngeneic HSCT and HPCT can prolong cardiac allograft survival in mice. These two methods could be promising ways to induce immune tolerance in future organ transplantation.

Li, J., D. P. Sejas, et al. (2006). "Nucleophosmin regulates cell cycle progression and stress response in hematopoietic stem/progenitor cells." J Biol Chem **281**(24): 16536-45.

Nucleophosmin (NPM) is a multifunctional protein frequently overexpressed in actively proliferating cells. Strong evidence indicates that NPM is required for embryonic development and genomic stability. Here we report that NPM enhances the proliferative potential of hematopoietic stem cells (HSCs) and increases their survival upon stress challenge. Both short term liquid culture and clonogenic progenitor cell assays show a selective expansion of NPM-overexpressing HSCs. Interestingly, HSCs infected with NPM retrovirus show significantly reduced commitment to myeloid differentiation compared with vector-transduced cells, and majority of the NPM-overexpressing cells remains primitive during a 5-day culture. Bone marrow transplantation experiments demonstrate that NPM promotes the self-renewal of long term repopulating HSCs while attenuated their commitment to myeloid differentiation. NPM overexpression induces rapid entry of HSCs into the cell cycle and suppresses the expression of several negative cell cycle regulators that are associated with G(1)-to-S transition. NPM knockdown elevates expression of these negative

regulators and exacerbates stress-induced cell cycle arrest. Finally, overexpression of NPM promotes the survival and recovery of HSCs and progenitors after exposure to DNA damage, oxidative stress, and hematopoietic injury both in vivo and in vitro. DNA repair kinetics study suggests that NPM has a role in reducing the susceptibility of chromosomal DNA to damage rather than promoting DNA damage repair. Together, these results indicate that NPM plays an important role in hematopoiesis via mechanisms involving modulation of HSC/progenitor cell cycle progression and stress response.

Li, L., Y. H. El-Hayek, et al. (2008). "Direct-current electrical field guides neuronal stem/progenitor cell migration." Stem Cells **26**(8): 2193-200.

Direct-current electrical fields (EFs) promote nerve growth and axon regeneration. We report here that at physiological strengths, EFs guide the migration of neuronal stem/progenitor cells (NSPCs) toward the cathode. EF-directed NSPC migration requires activation of N-methyl-d-aspartate receptors (NMDARs), which leads to an increased physical association of Rho GTPase Rac1-associated signals to the membrane NMDARs and the intracellular actin cytoskeleton. Thus, this study identifies the EF as a directional guidance cue in controlling NSPC migration and reveals a role of the NMDAR/Rac1/actin signal transduction pathway in mediating EF-induced NSPC migration. These results suggest that as a safe physical approach in clinical application, EFs may be developed as a practical therapeutic strategy for brain repair by directing NSPC migration to the injured brain regions to replace cell loss. Disclosure of potential conflicts of interest is found at the end of this article.

Li, L., O. Piloto, et al. (2007). "FLT3/ITD expression increases expansion, survival and entry into cell cycle of human haematopoietic stem/progenitor cells." Br J Haematol **137**(1): 64-75.

Activating mutation of FLT3 by internal tandem duplications (ITDs) in the juxtamembrane region is the most common molecular aberration found in acute myeloid leukaemia (AML). In this study, a lentiviral vector containing two promoters achieved consistent and efficient co-expression of FLT3/ITD and GFP in transduced human CD34(+) haematopoietic stem/progenitor cells (HSPCs). When cultured in medium containing stem cell factor, thrombopoietin and FLT3 ligand (FL), FLT3/ITD-transduced cells demonstrated enhanced self-renewal and survival potential, unaffected by the withdrawal of FL. These cells retained a CD34(+)CD38(-/dim) immunophenotype, typical of HSPCs. Compared to cells transduced with a vector expressing GFP alone, FLT3/ITD-transduced HSPCs had a higher fraction of cells in active cell cycle. FLT3/ITD-transduced HSPCs were more sensitive to the induction of cytotoxicity by CEP-701, a selective FLT3 inhibitor, indicating a rapid 'addiction' to signalling through this oncogenic pathway. The FLT3/ITD-transduced HSPCs showed increased expression of Pim-1, c-Myc and Cyclin D3 (CCND3), each of which may contribute to the altered genetic programme instituted by FLT3/ITD signalling. Taken together, these results indicate that FLT3/ITD mutations may contribute to leukaemic transformation of normal HSPCs by prolonging survival, promoting proliferation and partially blocking differentiation. CEP-701 may act as a potent therapeutic agent for AML stem cells harbouring FLT3/ITD mutations.

Li, X., B. Z. Barkho, et al. (2008). "Epigenetic regulation of the stem cell mitogen Fgf-2 by Mbd1 in adult neural stem/progenitor cells." J Biol Chem **283**(41): 27644-52.

Whether and how mechanisms intrinsic to stem cells modulate their proliferation and differentiation are two central questions in stem cell biology. Although exogenous basic fibroblast growth factor 2 (FGF-2/Fgf-2) is commonly used to expand adult neural stem/progenitor cells (NSPCs) in vitro, we do not yet understand the functional significance or the molecular regulation of Fgf-2 expressed endogenously by adult NSPCs. We previously demonstrated that methylated CpG binding protein 1 (MBD1/Mbd1) is a transcriptional repressor of Fgf-2 and is enriched in adult brains. Mbd1 deficiency in mice selectively affected adult neurogenesis and the differentiation of NSPCs. Here we show that an Mbd1 and DNA methylation-mediated epigenetic mechanism regulated the expression of stem cell mitogen Fgf-2 in adult NSPCs. Mbd1 bound to the Fgf-2 promoter and regulates its expression in adult NSPCs. In the absence of functional Mbd1, the Fgf-2 promoter was hypomethylated, and treatment with a DNA methylation inhibitor resulted in increased Fgf-2 expression in adult NSPCs. We further demonstrated that both acute knockdown of Mbd1 or overexpression of Fgf-2 in adult NSPCs inhibited their neuronal differentiation, which could be responsible for the neurogenic deficits observed in Mbd1-deficient mice. These data indicate that intrinsic epigenetic mechanisms play critical roles in the regulation of adult NSPC functions.

Li, X., P. A. Plett, et al. (2003). "Fanconi anemia type C-deficient hematopoietic stem/progenitor cells exhibit aberrant cell cycle control." Blood **102**(6): 2081-4.

The pathogenesis of bone marrow failure in Fanconi anemia is poorly understood. Suggested mechanisms include enhanced apoptosis secondary to DNA damage and altered inhibitory cytokine signaling. Recent data determined that disrupted cell cycle control of hematopoietic stem and/or progenitor cells disrupts normal hematopoiesis with increased hematopoietic stem cell cycling resulting in diminished function and increased sensitivity to cell cycle-specific apoptotic stimuli. Here, we used Fanconi anemia complementation type C-deficient (Fancc-/-) mice to demonstrate that Fancc-/- phenotypically defined cell populations enriched for hematopoietic stem and progenitor cells exhibit increased cycling. In addition, we established that the defect in cell cycle regulation is not a compensatory mechanism from enhanced apoptosis occurring in vivo. Collectively, these data provide a previously unrecognized phenotype in Fancc-/- hematopoietic stem/progenitor cells, which may contribute to the progressive bone marrow failure in Fanconi anemia.

Liao, H., W. Huang, et al. (2008). "Cross-talk between the epidermal growth factor-like repeats/fibronectin 6-8 repeats domains of Tenascin-R and microglia modulates neural stem/progenitor cell proliferation and differentiation." J Neurosci Res **86**(1): 27-34.

Mounting evidence has demonstrated that the microenvironment of stem/progenitor cells plays an important role in their proliferation and commitment to their fate. However, it remains unclear how all elements, such as astrocytes, microglia, extracellular matrix molecules, soluble factors, and their cross-talk interactions in the microenvironments, affect neural stem/progenitor cell fate. This work explored the influences of cross-talk between Tenascin-R (TN-R) and microglia on neural stem/progenitor cell proliferation and differentiation. Our results show that microglia triggered by TN-R distinct domains EGF-like repeats (EGFL) and fibronectin 6-8 repeats (FN6-8) significantly enhanced the proliferation of neural stem/progenitor cells and also obviously induced the differentiation into neurons but not oligodendrocytes. Neurite processes of neurons generated from neural progenitor cells were promoted by both EGFL and FN6-8 domains-activated microglia. Microglia triggered by EGFL and FN6-8 secreted brain-derived neurotrophic factor (BDNF) and transforming growth factor-beta (TGF-beta); interestingly, FN6-8 could activate microglia to secrete nerve growth factor in addition to BDNF and TGF-beta, but EGFL domain could not. All these data implied that the cross-talk between TN-R distinct domains EGFL/FN6-8 and microglia promoted neural stem/progenitor cell proliferation and induced their differentiation into neurons.

Liao, H., W. Huang, et al. (2008). "Beta 1 integrin-mediated effects of tenascin-R domains EGFL and FN6-8 on neural stem/progenitor cell proliferation and differentiation in vitro." J Biol Chem **283**(41): 27927-36.

Neural stem/progenitor cells (NSCs) have the capacity for self-renewal and differentiation into major classes of central nervous system cell types, such as neurons, astrocytes, and oligodendrocytes. The determination of fate of NSCs appears to be regulated by both intrinsic and extrinsic factors. Mounting evidence has shown that extracellular matrix molecules contribute to NSC proliferation and differentiation as extrinsic factors. Here we explore the effects of the epidermal growth factor-like (EGFL) and fibronectin type III homologous domains 6-8 (FN6-8) of the extracellular matrix molecule tenascin-R on NSC proliferation and differentiation. Our results show that domain FN6-8 inhibited NSC proliferation and promoted NSCs differentiation into astrocytes and less into oligodendrocytes or neurons. The EGFL domain did not affect NSC proliferation, but promoted NSC differentiation into neurons and reduced NSC differentiation into astrocytes and oligodendrocytes. Treatment of NSCs with beta 1 integrin function-blocking antibody resulted in attenuation of inhibition of the effect of FN6-8 on NSC proliferation. The influence of EGFL or FN6-8 on NSCs differentiation was inhibited by beta 1 integrin antibody application, implicating beta 1 integrin in proliferation and differentiation induced by EGFL and FN6-8 mediated triggering of NSCs.

Lie, A. K., T. P. Rawling, et al. (1996). "Progenitor cell yield in sequential blood stem cell mobilization in the same patients: insights into chemotherapy dose escalation and combination of haemopoietic growth factor and chemotherapy." Br J Haematol **95**(1): 39-44.

Between April 1988 and March 1994 a total of 23 patients with haematological or non-haematological malignancies received serial peripheral blood stem cell (PBSC) mobilization to attain sufficient harvest for PBSC transplant at our institution. There was no improvement in yield with the second mobilization for group A patients (n = 12) who had the same dose of cyclophosphamide twice as mobilizing agent. For group B patients (n = 6). who had a higher dose of cyclophosphamide with the second mobilization, there was significant increase in CFU-GM yield. CD34+ cell yield was not measured. For group C patients, who received interleukin-3 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) with the first mobilization and chemotherapy plus GM-CSF with the second, there

was significant increase in CFU-GM as well as CD34+ cell yield. Our results demonstrate that, at the doses studied, chemotherapy dose escalation and combining haemopoietic growth factor with chemotherapy improve progenitor cell yield in PBSC mobilization.

Link, H. and L. Arseniev (1997). "CD34 positive blood cells for allogeneic progenitor and stem cell transplantation." Leuk Lymphoma **26**(5-6): 451-65.

The transplantation of allogeneic peripheral blood progenitor cells (PBPC) provides complete and sustained hematopoietic and lymphopoietic engraftment. In healthy donors, large amounts of PBPC can be mobilized with hematopoietic growth factors. However, the high content of immunocompetent T-cells in apheresis products may expose recipients of allogeneic PBPC to an elevated risk of acute and chronic graft-versus-host disease. Thus, the use of appropriate T-cell reduction, but not depletion might reduce this risk. The hazards of graft rejection and a higher relapse rate can be avoided by maintaining a portion of the T-cells in the graft. The positive selection of CD34+ cells from peripheral blood preparations simultaneously provides an approximately 1000-fold reduction of T-cells. These purified CD34+ cells containing committed and pluripotent stem cells are suitable for allogeneic transplantation and can be used in the following instances: 1. As hematopoietic stem and progenitor cell transplantation instead of bone marrow cells, from HLA-identical family donors; 2. for increasing the stem cell numbers from HLA-mismatched or three HLA-loci different family donors in order to reduce the incidence of rejection but without increasing the T-cell number; 3. boosting of poor marrow graft function with stem cells from the same family donors; 4. transplantation from volunteer matched unrelated donors; 5. split transplantation of CD34+ and T-cells; 6. addition of ex vivo expanded CD34+ cells to blood cell or bone marrow transplantation; 7. generation of antigen specific immune effector cells and antigen presenting cells for cell therapy.

Linnekin, D., C. S. DeBerry, et al. (1997). "Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells." J Biol Chem **272**(43): 27450-5.

Stem cell factor (SCF) is a cytokine critical for normal hematopoiesis. The receptor for SCF is c-Kit, a receptor tyrosine kinase. Our laboratory is interested in delineating critical components of the SCF signal transduction pathway in hematopoietic tissue. The present study examines activation of Src family members in response to SCF. Stimulation of cell lines as well as normal progenitor cells with SCF rapidly increased tyrosine phosphorylation of the Src family member Lyn. Peak responses were noted 10-20 min after SCF treatment, and phosphorylation of Lyn returned to basal levels 60-90 min after stimulation. SCF also induced increases in Lyn kinase activity in vitro. Lyn coimmunoprecipitated with c-Kit, and studies with GST fusion proteins demonstrated that Lyn readily associated with the juxtamembrane region of c-Kit. Treatment of cells with either Lyn antisense oligonucleotides or PP1, a Src family inhibitor, resulted in dramatic inhibition of SCF-induced proliferation. These data demonstrate that SCF rapidly activates Lyn and suggest that Lyn is critical in SCF-induced proliferation in hematopoietic cells.

Liu, S., C. Ginestier, et al. (2008). "BRCA1 regulates human mammary stem/progenitor cell fate." Proc Natl Acad Sci U S A **105**(5): 1680-5.

Although it is well established that women with germ-line mutations in the BRCA1 gene have a greatly increased lifetime incidence of breast and ovarian cancer, the molecular mechanisms responsible for this tissue-specific carcinogenesis remain undefined. The majority of these breast cancers are of the basal-like phenotype characterized by lack of expression of ER, PR, and ERBB2. Because this phenotype has been proposed to resemble that of normal breast stem cells, we examined the role of BRCA1 in human mammary stem cell fate. Using both in vitro systems and a humanized NOD/SCID mouse model, we demonstrate that BRCA1 expression is required for the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells. Knockdown of BRCA1 in primary breast epithelial cells leads to an increase in cells displaying the stem/progenitor cell marker ALDH1 and a decrease in cells expressing luminal epithelial markers and estrogen receptor. In breast tissues from women with germ-line BRCA1 mutations, but not normal controls, we detect entire lobules that, although histologically normal, are positive for ALDH1 expression but are negative for the expression of ER. Loss of heterozygosity for BRCA1 was documented in these ALDH1-positive lobules but not in adjacent ALDH1-negative lobules. Taken together, these studies demonstrate that BRCA1 plays a critical role in the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells. Because BRCA1 also plays a role in DNA repair, our work suggests that loss of BRCA1 may result in the accumulation of genetically unstable breast stem cells, providing prime targets for further carcinogenic events.

Liu, X. and J. F. Engelhardt (2008). "The glandular stem/progenitor cell niche in airway development and repair." Proc Am Thorac Soc **5**(6): 682-8.

Airway submucosal glands (SMGs) are major secretory structures that lie beneath the epithelium of the cartilaginous airway. These glands are believed to play important roles in normal lung function and airway innate immunity by secreting antibacterial factors, mucus, and fluid into the airway lumen. Recent studies have suggested that SMGs may additionally serve as a protective niche for adult epithelial stem/progenitor cells of the proximal airways. As in the case of other adult stem cell niches, SMGs are believed to provide the localized environmental signals required to both maintain and mobilize stem/progenitor cells, in the setting of normal cellular turnover or injury. Aberrant proliferation and differentiation of glandular stem/progenitor cells may be associated with several hypersecretory lung diseases, including chronic bronchitis, asthma, and cystic fibrosis. To better understand the molecular mechanisms that regulate the specification and proliferation of glandular stem/progenitor cells in lung diseases associated with SMG hypertrophy and hyperplasia, researchers have begun to search for the molecular signals and cell types responsible for establishing the glandular stem/progenitor cell niche, and to dissect how these determinants of the niche change in the setting of proximal airway injury and repair. Such studies have revealed certain similarities between stem/progenitor cell niches of the distal conducting airways and the SMGs of the proximal airways.

Liu, Z. and L. J. Martin (2006). "The adult neural stem and progenitor cell niche is altered in amyotrophic lateral sclerosis mouse brain." J Comp Neurol **497**(3): 468-88.

Amyotrophic lateral sclerosis (ALS) is a fatal adult human disease caused by motor neuron degeneration. Stem cell therapy might be a treatment for ALS. The adult mammalian forebrain has neural stem cells (NSCs) and neural progenitor cells (NPCs) in the anterior subventricular zone (SVZa), rostral migratory stream (RMS), olfactory bulb (OB) core, and dentate gyrus (DG). These cells could be used to rescue or replace degenerating upper and lower motor neurons through endogenous recruitment or autologous/allogenic transplantation. We evaluated the competency of forebrain NSCs and NPCs in transgenic (tg) mice harboring human mutant superoxide dismutase-1 (mSOD1), a model of ALS. Tg human wild-type SOD1 (wtSOD1) mice and non-tg mice were controls. Bromodeoxyuridine (BrdU) labeling of cells, a marker for cell proliferation and other events, was reduced in a niche-specific pattern in presymptomatic and symptomatic mice, with the SVZa having greater reductions than the RMS, OB, and DG. Different NSC and NPC complements were evaluated by localizing nestin, neural cell adhesion molecule, distalless-2 transcription factor, vimentin, and glial fibrillary acidic protein. In symptomatic mice, NSC markers were reduced, whereas NPC markers were unchanged or elevated. Neurogenesis was preserved in symptomatic mSOD1 mice. NSC/NPC competence assessment in vitro revealed that mSOD1 SVZa cells had the ability to proliferate and form neurospheres but had an impaired response to mitogen stimulation. We conclude that adult mSOD1 ALS mice have abnormalities in forebrain NSCs, but the essential features of NSC/NPCs remained in presymptomatic and symptomatic mice.

Lock, L. T. and E. S. Tzanakakis (2007). "Stem/Progenitor cell sources of insulin-producing cells for the treatment of diabetes." Tissue Eng **13**(7): 1399-412.

Patients with diabetes experience decreased insulin secretion that is linked to a significant reduction in the number of islet cells. Reversal of diabetes can be achieved through islet transplantation, but the scarcity of donor islets severely hinders wide application of this therapeutic modality. Toward that end, embryonic stem cells, adult tissue-residing progenitor cells, and regenerating native beta-cells may serve as sources of islet cell surrogates. Insulin-producing cells generated from stem or progenitor cells display subsets of native beta-cell attributes, indicating the need for further development of methods for differentiation to completely functional beta-cells. Pharmacological approaches aiming at stimulating the in vivo/ex vivo regeneration of beta-cells have also been proposed as a way of augmenting islet cell mass. We review the current state of the generation of insulin-producing cells from different sources with emphasis on embryonic stem cells and adult progenitor cells. Challenges for the clinical use of these sources are also discussed.

Loizou, J. I., G. Oser, et al. (2009). "Histone acetyltransferase cofactor Trrap is essential for maintaining the hematopoietic stem/progenitor cell pool." J Immunol **183**(10): 6422-31.

The pool of hematopoietic stem/progenitor cells, which provide life-long reconstitution of all hematopoietic lineages, is tightly controlled and regulated by self-renewal and apoptosis. Histone modifiers and chromatin states are believed to govern establishment, maintenance, and propagation of distinct patterns of gene expression in stem cells, however the underlying mechanism remains poorly understood. In this study, we identified a role for the

histone acetytransferase cofactor Trrap in the maintenance of hematopietic stem/progenitor cells. Conditional deletion of the Trrap gene in mice resulted in ablation of bone marrow and increased lethality. This was due to the depletion of early hematopoietic progenitors, including hematopoietic stem cells, via a cell-autonomous mechanism. Analysis of purified bone marrow progenitors revealed that these defects are associated with induction of p53-independent apoptosis and deregulation of Myc transcription factors. Together, this study has identified a critical role for Trrap in the mechanism that maintains hematopoietic stem cells and hematopoietic system, and underscores the importance of Trrap and histone modifications in tissue homeostasis.

Lord-Grignon, J., M. Abdouh, et al. (2006). "Identification of genes expressed in retinal progenitor/stem cell colonies isolated from the ocular ciliary body of adult mice." Gene Expr Patterns **6**(8): 992-9.

Rare pigmented cells showing retinal stem cell characteristics have been identified in the ocular ciliary body (CB) of adult mammals. In vitro, these cells were reported to clonally proliferate and generate pigmented sphere colonies (PSC) containing multipotent retinal progenitor-like cells. Because these cells may have important clinical applications and because their embryonic origin is unclear, we have analyzed their local environment and gene expression profile. We found that transcription factors Pax6, Six3, and Rx, all involved in early eye morphogenesis, were expressed in the CB of adult mice. By sequencing a PSC cDNA library, we found that PSC expressed at high levels transcripts involved in the control of redox metabolism and cellular proliferation. PSC also expressed the retinal transcription factor Six6, which expression was not detected in the CB epithelium. By in situ hybridization screen, we found that Palmdelphin (Palm), Hmga2, and a novel transcript were expressed in the central nervous system of early embryos. Palm expression delineated the pigmented epithelium of the future CB and the developing myotome. Hmga2 was expressed in the ventricular zone of the telencephalon, the developing retinal ciliary margin and lens. Several genes expressed in PSC were also expressed in the nasal anlagen. Taken together, our study reveals that PSC isolated from the ocular CB express genes involved in the control of embryonic development, retinal identity, redox metabolism, and cellular proliferation.

Luis, T. C., F. Weerkamp, et al. (2009). "Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation." Blood **113**(3): 546-54.

Canonical Wnt signaling has been implicated in various aspects of hematopoiesis. Its role is controversial due to different outcomes between various inducible Wnt-signaling loss-of-function models and also compared with gain-of-function systems. We therefore studied a mouse deficient for a Wnt gene that seemed to play a nonredundant role in hematopoiesis. Mice lacking Wnt3a die prenatally around embryonic day (E) 12.5, allowing fetal hematopoiesis to be studied using in vitro assays and transplantation into irradiated recipient mice. Here we show that Wnt3a deficiency leads to a reduction in the numbers of hematopoietic stem cells (HSCs) and progenitor cells in the fetal liver (FL) and to severely reduced reconstitution capacity as measured in secondary transplantation assays. This deficiency is irreversible and cannot be restored by transplantation into Wnt3a competent mice. The impaired long-term repopulation capacity of Wnt3a(-/-) HSCs could not be explained by altered cell cycle or survival of primitive progenitors. Moreover, Wnt3a deficiency affected myeloid but not B-lymphoid development at the progenitor level, and affected immature thymocyte differentiation. Our results show that Wnt3a signaling not only provides proliferative stimuli, such as for immature thymocytes, but also regulates cell fate decisions of HSC during hematopoiesis.

Luo, Y., J. Cai, et al. (2003). "Designing, testing, and validating a focused stem cell microarray for characterization of neural stem cells and progenitor cells." Stem Cells **21**(5): 575-87.

Fetal neural stem cells (NSCs) have received great attention not only for their roles in normal development but also for their potential use in the treatment of neurodegenerative disorders. To develop a robust method of assessing the state of stem cells, we have designed, tested, and validated a rodent NSC array. This array consists of 260 genes that include cell type-specific markers for embryonic stem (ES) cells and neural progenitor cells as well as growth factors, cell cycle-related genes, and extracellular matrix molecules known to regulate NSC biology. The 500-bp polymerase chain reaction products amplified and validated by using gene-specific primers were arrayed along with positive controls. Blanks were included for quality control, and some genes were arrayed in duplicate. No cross-hybridization was detected. The quality of the arrays and their sensitivity were also examined by using probes prepared by conventional reverse transcriptase or by using amplified probes prepared by linear polymerase replication (LPR). Both methods showed good reproducibility, and probes prepared by LPR labeling

appeared to detect expression of a larger proportion of expressed genes. Expression detected by either method could be verified by RT-PCR with high reproducibility. Using these stem cell chips, we have profiled liver, ES, and neural cells. The cell types could be readily distinguished from each other. Nine markers specific to mouse ES cells and 17 markers found in neural cells were verified as robust markers of the stem cell state. Thus, this focused neural stem array provides a convenient and useful tool for detection and assessment of NSCs and progenitor cells and can reliably distinguish them from other cell populations.

Ma, W., W. Fitzgerald, et al. (2004). "CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels." Exp Neurol **190**(2): 276-88.

The mammalian central nervous system (CNS) has little capacity for self-repair after injury, and neurons are not capable of proliferating. Therefore, neural tissue engineering that combines neural stem and progenitor cells and biologically derived polymer scaffolds may revolutionize the medical approach to the treatment of damaged CNS tissues. Neural stem and progenitor cells isolated from embryonic rat cortical or subcortical neuroepithelium were dispersed within type I collagen, and the cell-collagen constructs were cultured in serum-free medium containing basic fibroblast growth factor. The collagen-entrapped stem and progenitors actively expanded and efficiently generated neurons, which developed neuronal polarity, neurotransmitters, ion channels/receptors, and excitability. Ca2+ imaging showed that differentiation from BrdU+/TuJ1- to BrdU-/TuJ1+ cells was accompanied by a shift in expression of functional receptors for neurotransmitters from cholinergic and purinergic to predominantly GABAergic and glutamatergic. Spontaneous postsynaptic currents were recorded by patch-clamping from precursor cell-derived neurons and these currents were partially blocked by 10-microM bicuculline, and completely blocked by additional 10 microM of the kainate receptor antagonist CNQX, indicating an appearance of both GABAergic and glutamatergic synaptic activities. Staining with endocytotic marker FM1-43 demonstrated active synaptic vesicle recycling occurring among collagen-entrapped neurons. These results show that neural stem and progenitor cells cultured in 3D collagen gels recapitulate CNS stem cell development; this is the first demonstration of CNS stem and progenitor cell-derived functional synapse and neuronal network formation in a 3D matrix. The proliferative capacity and neuronal differentiating potential of neural progenitors in 3D collagen gels suggest their potential use in attempts to promote neuronal regeneration in vivo.

Machold, R., S. Hayashi, et al. (2003). "Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches." Neuron **39**(6): 937-50.

To directly test the requirement for hedgehog signaling in the telencephalon from early neurogenesis, we examined conditional null alleles of both the Sonic hedgehog and Smoothened genes. While the removal of Shh signaling in these animals resulted in only minor patterning abnormalities, the number of neural progenitors in both the postnatal subventricular zone and hippocampus was dramatically reduced. In the subventricular zone, this was partially attributable to a marked increase in programmed cell death. Consistent with Hedgehog signaling being required for the maintenance of stem cell niches in the adult brain, progenitors from the subventricular zone of floxed Smo animals formed significantly fewer neurospheres. The loss of hedgehog signaling also resulted in abnormalities in the dentate gyrus and olfactory bulb. Furthermore, stimulation of the hedgehog pathway in the mature brain resulted in elevated proliferation in telencephalic progenitors. These results suggest that hedgehog signaling is required to maintain progenitor cells in the postnatal telencephalon.

Maciejewski, J. P., C. O'Keefe, et al. (2007). "Immune-mediated bone marrow failure syndromes of progenitor and stem cells: molecular analysis of cytotoxic T cell clones." Folia Histochem Cytobiol **45**(1): 5-14.

The unique structure of the T cell receptor (TCR) enables molecular identification of individual T cell clones and provides an unique opportunity for the design of molecular diagnostic tests based on the structure of the rearranged TCR chain e.g., using the TCR CDR3 region. Initially, clonal T cell malignancies, including T cell large granular lymphocyte leukemia (T-LGL), mucosis fungoides and peripheral T cell lymphoma were targets for the TCR-based analytic assays such as detection of clonality by T-gamma rearrangement using y-chain-specific PCR or Southern Blotting. Study of these disorders facilitated further analytic concepts and application of rational methods of TCR analysis to investigations of polyclonal T cell-mediated diseases. In hematology, such conditions include graft versus host disease (GvHD) and immune-mediated bone marrow failure syndromes. In aplastic anemia (AA), myelodysplastic syndrome (MDS) or paroxysmal nocturnal hemoglobinuria (PNH), cytotoxic T cell responses may be directed against certain antigens

located on stem or more lineage-restricted progenitor cells in single lineage cytopenias. The nature of the antigenic targets driving polyclonal CTL responses remains unclear. Novel methods of TCR repertoire analysis, include VB flow cytometry, peptide-specific tetramer staining, in vitro stimulation assays and TCR CDR3-specific PCR. Such PCR assay can be either VB family-specific or multiplexed for all VB families. Amplified products can be characterized and quantitated to facilitate detection of the most immunodominant clonotypes. Such clonotypes may serve as markers for the global polyclonal T cell response. Identification of these clonotypes can be performed in blood and tissue biopsy material by various methods. Once immunodominant clonotypes corresponding to pathogenic CTL clones are identified they can serve as surrogate markers for the activity of the pathophysiologic process or even indicate the presence of specific antigens. The relevance of the individual clonotypes can be ascertained from clinical correlations with the activity of the disease. Quantitative clonotypic assays such as sequencing of multiple CDR3 clones or clonotypic Taqman PCR can be applied for the monitoring of the immunosuppressive therapy and prediction of relapse. Future technologies may allow for the design of clonotypic microarrays or other more clinically applicable methods of clonotypic diagnostics. Similarly, identification of immunodominant clonotypes may facilitate targeting of autoimmune or malignant clones with vaccination and induction of anti-idiotypic responses.

Madlambayan, G. J., J. M. Butler, et al. (2009). "Bone marrow stem and progenitor cell contribution to neovasculogenesis is dependent on model system with SDF-1 as a permissive trigger." Blood **114**(19): 4310-9.

Adult bone marrow (BM) contributes to neovascularization in some but not all settings, and reasons for these discordant results have remained unexplored. We conducted novel comparative studies in which multiple neovascularization models were established in single mice to reduce variations in experimental methodology. In different combinations, BM contribution was detected in ischemic retinas and, to a lesser extent, Lewis lung carcinoma cells, whereas B16 melanomas showed little to no BM contribution. Using this spectrum of BM contribution, we demonstrate the necessity for site-specific expression of stromal-derived factor-1alpha (SDF-1alpha) and its mobilizing effects on BM. Blocking SDF-1alpha activity with neutralizing antibodies abrogated BM-derived neovascularization in lung cancer and retinopathy. Furthermore, secondary transplantation of single hematopoietic stem cells (HSCs) showed that HSCs are a long-term source of neovasculogenesis and that CD133(+)CXCR4(+) myeloid progenitor cells directly participate in new blood vessel formation in response to SDF-1alpha. The varied BM contribution seen in different model systems is suggestive of redundant mechanisms governing postnatal neovasculogenesis and provides an explanation for contradictory results observed in the field.

Madras, N., A. L. Gibbs, et al. (2002). "Modeling stem cell development by retrospective analysis of gene expression profiles in single progenitor-derived colonies." Stem Cells **20**(3): 230-40.

The process of development of various cell types is often based on a linear or deterministic paradigm. This is true, for example, for osteoblast development, a process that occurs through the differentiation of a subset of primitive fibroblast progenitors called colony-forming unit-osteoblasts (CFU-Os). CFU-O differentiation has been subdivided into three stages: proliferation, extracellular matrix development and maturation, and mineralization, with characteristic changes in gene expression at each stage. Few analyses have asked whether CFU-O differentiation, or indeed stem cell differentiation in general, may follow more complex and nondeterministic paths, a possibility that may underlie the substantial number of discrepancies in published reports of progenitor cell developmental sequences. We analyzed 99 single colonies of osteoblast stem/primitive progenitor cells cultured under identical conditions. The colonies were analyzed by global amplification poly(A) polymerase chain reaction to determine which of nine genes had been expressed. We used the expression profiles to develop a statistically rigorous map of the cell fate decisions that occur during osteoprogenitor differentiation and show that different developmental routes can be taken to achieve the same end point phenotype. These routes appear to involve both developmental "dead ends" (leading to the expression of genes not correlated with osteoblast-associated genes or the mature osteoblast phenotype) and developmental flexibility (the existence of multiple gene expression routes to the same developmental end point). Our results provide new insight into the biology of primitive progenitor cell differentiation and introduce a powerful new quantitative method for stem cell lineage analysis that should be applicable to a wide variety of stem cell systems.

Maeda, K., M. Takemura, et al. (2008). "E-cadherin prolongs the moment for interaction between intestinal stem cell and its progenitor cell to ensure Notch

signaling in adult Drosophila midgut." Genes Cells **13**(12): 1219-27.

Intestinal stem cells (ISCs) are required for maintenance of the proper cell composition in the adult intestine. To ensure permanent recruitment of newly differentiated cells, the ISC undergoes asymmetric cell division that generates an ISC itself and a progenitor cell. In the Drosophila midgut, cell fate for the absorptive cell is determined by Notch (N) signal in the progenitor cells that receive a ligand Delta (Dl) produced by the ISCs. Although most of the ISCs and progenitor cells are distantly located, they should retain their attachment when N is activated because the Dl-N interaction requires cell adhesion. Furthermore, N cannot be activated before completion of cell division. Thus, the moment after cell division and before cell separation should be prolonged for certain N activation, although the mechanism for this remains unclear. Here, we demonstrate that E-cadherin (E-cad) is required for stable attachment between the two cells. When E-cad does not function, N is not activated and cell differentiation is attenuated. We also show that the ISC tumor by N inactivation is assisted by a defect in E-cad down-regulation. These findings reveal one of the normal N functions used to inhibit tumorigenesis through lowering of E-cad for proper midgut cell turnover.

Mahmud, D. L., G. A. M, et al. (2002). "Phosphorylation of forkhead transcription factors by erythropoietin and stem cell factor prevents acetylation and their interaction with coactivator p300 in erythroid progenitor cells." Oncogene **21**(10): 1556-62.

The mammalian forkhead transcription factors, FOXO3a (FKHRL1), FOXO1a (FKHR) and FOXO4 (AFX) are negatively regulated by PKB/Akt kinase. In the present study we examined the engagement of forkhead family of transcription factors in erythropoietin (Epo)- and stem cell factor (SCF)-mediated signal transduction. Our data show that all three forkhead family members, FOXO3a, FOXO1a and FOXO4 are phosphorylated in human primary erythroid progenitors. Experiments performed to determine various upstream signaling pathways contributing to phosphorylation of forkhead family members show that only PI-3-kinase pathway is required for inactivation of FOXO3a. Our data also demonstrate that during Epo deprivation FOXO3a interacts with the transcriptional coactivator p300 and such interaction is disrupted by stimulation of cells with Epo. To determine the domains in FOXO3a, mediating its interaction with p300, we performed GST pull-down assays and found that the N-terminus region containing the first 52 amino acids was sufficient for binding p300. Finally, our data demonstrate that FOXO3a and FOXO1a are acetylated during growth factor deprivation and such acetylation is reversed by stimulation with Epo. Thus mammalian forkhead transcription factors are involved in Epo and SCF signaling in primary erythroid progenitors and may play a role in the induction of apoptotic and mitogenic signals.

Mahmud, N., N. Katayama, et al. (1996). "A possible change in doubling time of haemopoietic progenitor cells with stem cell development." Br J Haematol **94**(2): 242-9.

We separated haemopoietic progenitors derived from marrow cells of 5-fluorouracil (5-FU)-treated mice into three groups, based on the stages of stem cell development and studied doubling time, using a serum-free clonal culture system. Stage I progenitors were those present in primary marrow cells from 5-FU-treated mice. Stages II and III progenitors were early and late progenies in culture of stage I progenitors respectively. The morphological analysis of colonies derived from stage I, II and III progenitors demonstrated an association of progression of stages with loss of multipotentiality. The doubling time of haemopoietic progenitors was estimated by sequential analysis of colony formation and studies of growth fraction. The time required for haemopoietic progenitors to double shortened as their stage of development progressed. Alteration in one doubling time of haemopoietic progenitors at progressive stages of stem cell development was seen in cultures supported by various combinations of growth factors, including interleukin-3 (IL-3), IL-11. and steel factor (SF), Cell-cycle analysis suggested that reduction of the doubling time of haemopoietic progenitors is probably due to a decrease in the time spent in the G1 phase of the cell cycle. Our results suggest that in early haemopoiesis the doubling time of haemopoietic progenitors may change with stem cell development.

Mahmut, N., Y. Katayama, et al. (1999). "Analysis of circulating hematopoietic progenitor cells after peripheral blood stem cell transplantation." Int J Hematol **69**(1): 36-42.

We investigated the kinetics of posttransplant circulating progenitor cells (PTCPC) in the early phase after autologous (auto-) and allogeneic (allo-) peripheral blood stem cell transplantation (PBSCT). We analyzed the number of myeloid progenitor cells (CFU-GM) per 10 ml of peripheral blood (PB) on days 0 (just prior to transplantation), 1 (12-15 hours after completion of first transplantation), 2, 3, 5, 7, 10, 14, 17, 21 and 28 (after auto-PBSCT), and also additionally on day 35 after allo-PBSCT. A standard

methylcellulose colony assay was used for analysing the number of CFU-GGM and BFU-E on all of the days. In addition, high proliferative potential-colony forming cells (HPP-CFC) of the harvested PBSC from donors and day 1 PB from recipients were assayed in 5 allo-PBSCT patients. Furthermore, a proportion of CD38- cells among CD34+ cells in the harvested PBSC and day 1 PB was evaluated by two-color flow cytometric analysis in 5 allo-PBSCT patients. The number of CFU-GM on day 1 ranged from 7 to 119 per 10 ml PB after auto-PBSCT, and from 15 to 61 per 10 ml PB after allo-PBSCT. After these transient increases, PTCPC diminished rapidly. Then, PTCPC emerged again on day 7 after auto-PBSCT and on day 10 or 14 after allo-PBSCT along with neutrophil recovery. A proportion of HPP-CFC among myeloid colonies from day 1 PB of recipients was significantly higher than that from the harvested PBSC from donors (65.6 +/- 12.7% vs. 17.4 +/- 13.0%, respectively, n = 5, P = 0.0013). In addition, two-color flow cytometric analysis revealed that the proportion of CD34+CD38- cells was significantly higher in day 1 PB of recipients than in the harvested PBSC from donors (57.5 +/- 17.6% vs. 11.7 +/- 4.9%, n = 5, P = 0.005). These observations suggest that both primitive and committed transplanted myeloid progenitor cells may circulate in the very early period following PBSCT.

Malaterre, J., T. Mantamadiotis, et al. (2008). "c-Myb is required for neural progenitor cell proliferation and maintenance of the neural stem cell niche in adult brain." Stem Cells **26**(1): 173-81.

Ongoing production of neurons in adult brain is restricted to specialized neurogenic niches. Deregulated expression of genes controlling homeostasis of neural progenitor cell division and/or their microenvironment underpins a spectrum of brain pathologies. Using conditional gene deletion, we show that the proto-oncogene c-myb regulates neural progenitor cell proliferation and maintains ependymal cell integrity in mice. These two cellular compartments constitute the neurogenic niche in the adult brain. Brains devoid of c-Myb showed enlarged ventricular spaces, ependymal cell abnormalities, and reduced neurogenesis. Neural progenitor cells lacking c-Myb showed a reduced intrinsic proliferative capacity and reduction of Sox-2 and Pax-6 expression. These data point to an important role for c-Myb in the neurogenic niche of the adult brain.

Marley, S. B. and M. Y. Gordon (2005). "Chronic myeloid leukaemia: stem cell derived but progenitor cell driven." Clin Sci (Lond) **109**(1): 13-25.

The biology of CML (chronic myeloid leukaemia) has been extensively investigated as the disease is a paradigm of neoplasms induced when a translocation results in expression of a novel fusion protein, in this instance p210(BCR-ABL). Although CML manifests itself principally as unregulated expansion of the myeloid lineage, the lesion is present in the stem cell population and it has long been assumed that disregulated stem cell kinetics must underlie the basic pathology of the disease. In this review, we present evidence that, in normal haemopoiesis, less primitive precursor cells retain considerable flexibility in their capacity to undergo self-renewal, allowing them to maintain lineage-specific homoeostasis without inflicting proliferative stress upon the stem cell population. This mechanism is dysregulated in CML and we have developed a self-renewal assay for CFU-GM (colony-forming unit-granulocyte/macrophage) which demonstrates that, in CML, the PI (proliferative index) of the myeloid progenitor cell population is increased. The ability to measure the PI as an endpoint of p210(BCR-ABL) expression gives considerable versatility to the in vitro investigation of putative therapeutic regimes in CML.

Marquez-Curtis, L., A. Jalili, et al. (2008). "Carboxypeptidase M expressed by human bone marrow cells cleaves the C-terminal lysine of stromal cell-derived factor-1alpha: another player in hematopoietic stem/progenitor cell mobilization?" Stem Cells **26**(5): 1211-20.

Carboxypeptidase M (CPM) is a membrane-bound zinc-dependent protease that cleaves C-terminal basic residues, such as arginine or lysine, from peptides/proteins. We examined whether CPM is expressed by hematopoietic and stromal cells and could degrade stromal cell-derived factor (SDF)-1alpha, a potent chemoattractant for hematopoietic stem/progenitor cells (HSPC). We found that (a) CPM transcript is expressed by bone marrow (BM) and mobilized peripheral blood CD34(+) cells, myeloid, erythroid, and megakaryocytic cell progenitors, mononuclear cells (MNC), polymorphonuclear cells (PMN), and stromal cells, including mesenchymal stem cells; and that (b) granulocyte-colony-stimulating factor (G-CSF) significantly increases its expression at the gene and protein levels in MNC and PMN. Moreover, we found that recombinant CPM cleaves full-length SDF-1alpha (1-68) rapidly, removing the C-terminal lysine and yielding des-lys SDF-1alpha (1-67). We demonstrated that such CPM treatment of SDF-1alpha reduced the in vitro chemotaxis of HSPC, which, however, was preserved when the CPM was exposed to the carboxypeptidase inhibitor dl-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid. Thus, we present evidence that CPM is expressed by cells occurring in the BM microenvironment and that the mobilizing agent G-CSF strongly upregulates it in MNC and PMN. We

suggest that cleavage of the C-terminal lysine residue of SDF-1alpha by CPM leads to attenuated chemotactic responses and could facilitate G-CSF-induced mobilization of HSPC from BM to peripheral blood.

Martin, C. H., P. S. Woll, et al. (2008). "Differences in lymphocyte developmental potential between human embryonic stem cell and umbilical cord blood-derived hematopoietic progenitor cells." Blood **112**(7): 2730-7.

Hematopoietic progenitor cells derived from human embryonic stem cells (hESCs) develop into diverse mature hematopoietic lineages, including lymphocytes. Whereas functional natural killer (NK) cells can be efficiently generated in vitro from hESC-derived CD34(+) cells, studies of T- and B-cell development from hESCs have been much more limited. Here, we demonstrate that despite expressing functional Notch-1, CD34(+) cells from hESCs did not derive T cells when cocultured with OP9 cells expressing Delta-like 1, or in fetal thymus organ culture. hESC-derived CD34(+) cells also did not produce B cells in vitro. In contrast, CD34(+) cells isolated from UCB routinely generated T and B cells when cultured in the same conditions. Notably, both undifferentiated hESCs, and sorted hESC-derived populations with hematopoietic developmental potential exhibited constitutive expression of ID family genes and of transcriptional targets of stem cell factor-induced signaling. These pathways both inhibit T-cell development and promote NK-cell development. Together, these results demonstrate fundamental differences between hESC-derived hematopoietic progenitors and analogous primary human cells. Therefore, hESCs can be more readily supported to differentiate into certain cell types than others, findings that have important implications for derivation of defined lineage-committed populations from hESCs.

Martinez-Serrano, A., F. J. Rubio, et al. (2001). "Human neural stem and progenitor cells: in vitro and in vivo properties, and potential for gene therapy and cell replacement in the CNS." Curr Gene Ther **1**(3): 279-99.

The generation of unlimited quantities of neural stem and/or progenitor cells derived from the human brain holds great interest for basic and applied neuroscience. In this article we critically review the origins and recent developments of procedures developed for the expansion, perpetuation, identification, and isolation of human neural precursors, as well as their attributes. Factors influencing their in vitro properties, both under division and after differentiation conditions, are evaluated, with the aim of identifying properties common to the different culture systems reported. This analysis suggests that different culture procedures result in cells with different properties, or even in different cells being isolated. With respect to in vivo performance, present evidence obtained in rodents indicate that cultured human neural precursors, in general, are endowed with excellent integrative properties. Differentiation of the implanted cells, in particular in the case of adult recipients, seems not to be complete, and functionality still needs to be demonstrated. In relation to gene transfer and therapy, aspects currently underexplored, initial data support the view that human neural stem and progenitor cells may serve a role as a platform cell for the delivery of bioactive substances to the diseased CNS. Although a large deal of basic research remains to be done, available data illustrate the enormous potential that human neural precursors isolated, expanded, and characterized in vitro hold for therapeutic applications. In spite of this potential, maintaining a critical view on many unresolved questions will surely help to drive this research field to a good end, that is, the development of real therapies for diseases of the human nervous system.

Masaka, T., M. Miyazaki, et al. (2008). "Derivation of hepato-pancreatic intermediate progenitor cells from a clonal mesenchymal stem cell line of rat bone marrow origin." Int J Mol Med **22**(4): 447-52.

We have recently established a clonal mesenchymal stem cell line (rBM25/S3) from adult rat bone marrow. The cells have practically unlimited proliferation capacity (over 300 PDL), maintaining multipotency for differentiation. In the present study, we examined the potential for rBM25/S3 cells to differentiate into insulin-secreting cells. When cultured in the presence of HGF and FGF-4 on Matrigel, rBM25/S3 cells expressed genes specific to pancreatic beta-cells as well as those specific to hepatocytes. They still maintained proliferation capacity with a doubling time of approximately 30 h. These hepato-pancreatic intermediate progenitor cells, but not the original undifferentiated rBM25/S3 cells, were induced by the overexpression of PDX-1 to produce significant amounts of insulin in a manner responding to glucose concentration in medium. The present culture system indicates a direction for further studies aimed at the realization of cell transplantation therapy for type I diabetes mellitus.

Maslov, A. Y., K. J. Bailey, et al. (2007). "Stem/progenitor cell-specific enhanced green fluorescent protein expression driven by the endogenous Mcm2 promoter." Stem Cells **25**(1): 132-8.

Previous studies have demonstrated expression of the minichromosome maintenance protein Mcm2 in cells that remain competent to divide, including stem/progenitor cells of the subventricular zone (SVZ) within the brain. Here, a transgenic mouse line in which the Mcm2 gene drives expression of enhanced green fluorescent protein (EGFP) was constructed by insertion of an internal ribosomal entry site (IRES)-EGFP cassette into the last exon of the gene, 3' to the stop codon. In these mice, expression of EGFP is observed in the SVZ and several other tissues with high proliferative activity, including the spleen, intestine, hair follicles, and bone marrow. These observations suggest that EGFP fluorescence in this mouse line provides an index of the proliferative capacity of different tissues. Immunohistological analysis demonstrates a direct concordance between expression of EGFP and Mcm2, consistent with a transcriptional level downregulation of Mcm2 expression in postmitotic cells. To test the utility of EGFP expression for recovery of live cells retaining the capacity to divide, EGFP-expressing and -nonexpressing cells from bone marrow and brain were isolated from an adult Mcm2(IRES-EGFP) mouse by fluorescence-activated cell sorting and assayed for clonal growth. The EGFP-positive fraction contained the entire clonogenic population of the bone marrow and greater than 90% of neurosphere-forming cells from the brain. Brain-derived clonogenic cells were shown to remain competent to differentiate towards all three neural lineages. These studies demonstrate that the Mcm2(IRES-EGFP) transgenic line constructed here can be used for recovery of proliferation competent cells from different tissue types.

Mathieu, C., K. Sii-Felice, et al. (2008). "Endothelial cell-derived bone morphogenetic proteins control proliferation of neural stem/progenitor cells." Mol Cell Neurosci **38**(4): 569-77.

Neurogenesis persists in the adult brain subventricular zone where neural stem/progenitor cells (NSPCs) lie close to brain endothelial cells (BECs). We show in mouse that BECs produce bone morphogenetic proteins (BMPs). Coculture of embryonic and adult NSPCs with BECs activated the canonical BMP/Smad pathway and reduced their proliferation. We demonstrate that coculture with BECs in the presence of EGF and FGF2 induced a reversible cell cycle exit of NSPCs (LeX+) and an increase in the amount of GFAP/LeX-expressing progenitors thought to be stem cells. Levels of the phosphatidylinositol phosphatase PTEN were upregulated in NSPCs after coculture with BECs, or treatment with recombinant BMP4, with a concomitant reduction in Akt phosphorylation. Silencing Smad5 with siRNA or treatment with Noggin, a BMP antagonist, demonstrated that upregulation of PTEN in NSPCs required BMP/Smad signaling and that this pathway regulated cell cycle exit of NSPCs. Therefore, BECs may provide a feedback mechanism to control the proliferation of NSPCs.

McNiece, I. K., R. A. Briddell, et al. (1994). "The role of stem cell factor in mobilization of peripheral blood progenitor cells." Leuk Lymphoma **15**(5-6): 405-9.

Stem cell factor (SCF) is a hematopoietic growth factor which acts on both primitive and mature progenitors cells. In animals, high doses of SCF alone stimulate increases in cells of multiple lineages and mobilize peripheral blood progenitor cells (PBPC). Phase I studies of rhSCF have demonstrated dose related side effects which are consistent with mast cell activation. Based upon in vitro synergy between SCF and G-CSF we have demonstrated the potential of low doses of SCF to synergize with G-CSF to give enhanced mobilization of PBPC. These PBPC have increased potential for both short and long term engraftment in lethally irradiated mice and lead to more rapid recovery of platelets. On going Phase I/II studies with rhSCF plus rhG-CSF for mobilization of PBPC, demonstrated similar increases in PBPC compared to rhG-CSF alone. These data suggest a clinical role of rhSCF in combination with rhG-CSF for optimal mobilization of PBPC.

McNiece, I. K., K. E. Langley, et al. (1991). "Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and epo to stimulate human progenitor cells of the myeloid and erythroid lineages." Exp Hematol **19**(3): 226-31.

The cDNA for human stem cell factor (hSCF) has been cloned and expressed in mammalian and bacterial hosts and recombinant protein purified. We have examined the stimulatory effect of recombinant human SCF (rhSCF) on human bone marrow cells alone and in combination with recombinant human colony stimulating factors (CSFs) and erythropoietin (rhEpo). RhSCF alone resulted in no significant colony formation, however, in the presence of rhGM-CSF, rhG-CSF or rhIL-3, rhSCF stimulated a synergistic increase in colony numbers. In addition, increased colony size was stimulated by all combinations. The morphology of cells in the colonies obtained with the CSFs plus rhSCF was identical to the morphology obtained with rhGM-CSF, rhG-CSF or rhIL-3 alone. RhEpo also synergised with rhSCF to stimulate the formation of large compact hemoglobinized colonies which stained positive for spectrin and transferrin receptor and had a morphological appearance consistent with

normoblasts. RhSCF stimulation of low density non-adherent, antibody depleted, CD34+ cells suggests that rhSCF directly stimulates progenitor cells capable of myeloid and erythroid differentiation.

McQuaker, I. G., A. P. Haynes, et al. (1997). "Stem cell mobilization in resistant or relapsed lymphoma: superior yield of progenitor cells following a salvage regimen comprising ifosphamide, etoposide and epirubicin compared to intermediate-dose cyclophosphamide." Br J Haematol **98**(1): 228-33.

We analysed the factors influencing the efficacy of peripheral blood stem cell (PBSC) collection in patients with lymphoma. Sixty-six patients underwent initial PBSC collection following mobilization with chemotherapy plus recombinant granulocyte colony-stimulating factor (300 microg/d). Patients were mobilized with one of two chemotherapy regimens, either cyclophophamide (3 g/m2 or 4 g/m2) (n = 50) or ifosphamide, etoposide and epirubicin (IVE; n = 16). The target of collecting > 2.0 x 10(6) CD34+ cells/kg was achieved in 43/66 (65%) patients with a median of two apheresis procedures. The IVE plus G-CSF mobilization regimen gave a significantly higher median yield of CD34+ cells (8.62 x 10(6)/kg) compared with cyclophosphamide plus G-CSF (3.59 x 10(6)/kg) (P = 0.045). The median yield of CD34+ cells per leukapheresis was almost twice as high in patients receiving IVE (1.94 x 10(6)/kg) compared to cyclophosphamide (1.03 x 10(6)/kg) (P = 0.035). In a univariate analysis of the factors affecting mobilization, the subtype of lymphoma (high-grade NHL) and the mobilization regimen were the only factors associated with high CD34+ cell yield. However, in a multivariate analysis of factors affecting mobilization including age, lymphoma subtype, previous chemotherapy and radiotherapy, only the use of the IVE protocol was predictive of a high yield of CD34+ cells. In 13 patients undergoing a second mobilization procedure the use of IVE was associated with a significantly higher yield of CD34+ cells compared to cyclophosphamide; three patients who failed cyclophosphamide plus G-CSF mobilization were able to proceed to transplantation following successful mobilization with IVE + G-CSF. These results demonstrate that IVE is a highly effective mobilization regimen which is superior to cyclophophamide and has the benefit of being effective salvage therapy for lymphoma patients.

Mebius, R. E., S. van Tuijl, et al. (1998). "Transfer of primitive stem/progenitor bone marrow cells from LT alpha-/- donors to wild-type hosts: implications for the generation of architectural events in lymphoid B cell domains." J Immunol **161**(8): 3836-43.

To analyze whether the phenotypic abnormalities observed in lymphotoxin-alpha(-/-) (LT alpha-/-) mice are intrinsic to the hemolymphoid system itself or dependent on stromal elements, wild-type (WT) mice were reconstituted with bone marrow (BM) cells enriched for hemopoietic stem cells from LT alpha-/- animals. WT mice reconstituted with LT alpha-/- c-kit+ Lin- Sca-1+ BM cells do not maintain follicular dendritic cell (FDC) networks and do not form primary follicles, while clear segregation of B and T cells could be observed. Furthermore, IgM+ IgD- B cells, MOMA-1 (anti-metallophilic macrophages), ERTR-9 (anti-marginal zone macrophages), and MECA-367 (anti-MAdCAM-1) were all absent from the splenic marginal zone. Surprisingly, however, the expression of MOMA-1, ERTR-9, and MAdCAM-1 was normal in the lymph nodes of mice reconstituted with LT alpha-/- cells. In addition, peanut agglutinin-positive germinal centers were observed in both the spleen and mesenteric lymph nodes, although in the absence of detectable FDC. Furthermore, in animals reconstituted with a mixture of LT alpha-/- and WT c-kit+ Lin- Sca-1+, GC contained either predominantly LT alpha-/- B cells or WT B cells. These results suggest that although the formation of primary follicles, FDC networks, and the splenic marginal zone are all dependent on hemopoietically derived LT alpha, germinal center formation and the expression of MAdCAM-1, MOMA-1, and ERTR-9 in lymph nodes are not. Our results also suggest that the disturbed B-T cell separation in LT alpha-/- mice is unrelated to defects in the marginal zone.

Meehan, K. R., U. N. Verma, et al. (1997). "Suppression of progenitor cell growth by vancomycin following autologous stem cell transplantation." Bone Marrow Transplant **19**(10): 1029-32.

The occurrence of hematologic side-effects resulting from the use of vancomycin is rare. Prior to this report, vancomycin-induced neutropenia was believed to be due to a hypersensitivity reaction since antibodies directed against circulating neutrophils have been discovered in the serum of some patients. We demonstrate suppression of hematopoietic bone marrow progenitor cells in a patient experiencing vancomycin-induced neutropenia after an autologous hematopoietic stem cell transplantation for multiple myeloma. A bone marrow (BM) specimen obtained at the time of neutropenia demonstrated direct suppression of progenitor cell growth in vitro when vancomycin was added at increasing concentrations (1, 10 and 50 microg/ml). No such trend was noted in a BM sample from the same patient obtained 11 months prior to transplantation and a normal control BM. The decrease in the total number of colony-

forming units (CFU) was statistically significant at all the dose levels of vancomycin when compared to the number of CFU in the baseline BM sample ($P < 0.05$). The myeloid maturation arrest observed in the bone marrow sample obtained during the period of neutropenia and the dose dependent growth inhibition by vancomycin observed in vitro suggest a novel nonimmune mechanism of hematologic effects due to suppression of bone marrow progenitor cell growth.

Meisenberg, B. R., M. Callaghan, et al. (1997). "Complications associated with central venous catheters used for the collection of peripheral blood progenitor cells to support high-dose chemotherapy and autologous stem cell rescue." Support Care Cancer **5**(3): 223-7.

The purpose of this study was to review the incidence and type of complications associated with the insertion and use of central venous catheters for leukapheresis and high-dose chemotherapy with stem cell rescue. One hundred sixty-seven central venous catheters placed either at the transplant center or by various community surgeons were studied for insertion complications, inability to perform leukapheresis and incidence of infection. The overall incidence of hemo- or pneumothorax was 3.6%. Inability to pherese occurred in 13% of catheters placed by outside surgeons and 6.5% of catheters inserted at the transplant institution. Most often, these were due to malposition of the catheter too high in the superior vena cava or in other veins. Deep venous thrombosis was often related to this malposition and occurred in 4.8% of all patients. Pulmonary embolism was not seen in these patients despite the fact the catheters were often left in place during the thrombotic episode. Early or late-onset infections occurred in 6.5% of patients and were most often exit site infections. The incidence of complications of pheresis catheters is high but might be reduced by more attention to proper placement of the catheter closer to the right atrial/superior vena cava junction, and limiting insertion to a cadre of surgeons familiar with leukapheresis requirements.

Metcalf, D., S. Mifsud, et al. (2002). "Stem cell factor can stimulate the formation of eosinophils by two types of murine eosinophil progenitor cells." Stem Cells **20**(5): 460-9.

There are only three known stimuli for eosinophil formation-GM-CSF, interleukin-5 (IL-5), and IL-3. Because mice with inactivation of the gene encoding the common beta receptor chain for GM-CSF, IL-5, and IL-3 (betac(-/-) mice) cannot respond to GM-CSF or IL-5 and do not produce IL-3, they should lack eosinophils. However, they produce reduced numbers of eosinophils, indicating the existence of at least one additional stimulatory factor. Use of betac(-/-) mouse marrow cells failed to detect a novel eosinophil-stimulating factor in cell line- or organ-conditioned media, but stem cell factor (SCF) was found to have a previously unrecognized ability to stimulate the formation of eosinophil colonies or mixed neutrophil-eosinophil colonies. This action of SCF was also observable with marrow cells from other mouse strains and was enhanced by the addition of G-CSF but no other factor tested. Recloning of SCF-stimulated blast colonies showed that progenitors forming pure eosinophil or mixed neutrophil-eosinophil colonies can have a common ancestor but many appear to arise independently from different preprogenitor cells. The observed activity of SCF in marrow cultures was relatively weak, but its action may be stronger in vivo, and SCF needs to be added to GM-CSF, IL-5, and IL-3 in the list of cytokines able to stimulate eosinophil formation.

Mielcarek, M., E. Bryant, et al. (1999). "Haemopoietic reconstitution by donor-derived myelodysplastic progenitor cells after haemopoietic stem cell transplantation." Br J Haematol **105**(2): 361-5.

A 50-year-old woman who was retrospectively diagnosed with an early asymptomatic myelodysplastic syndrome (MDS) served as a haemopoietic stem cell donor for her HLA-identical sister who had chemotherapy-refractory non-Hodgkin's lymphoma. The MDS of the donor was classified as refractory anaemia (RA) and cytogenetically characterized by deletion of the long arm of chromosome 20 [del(20q)]. Donor cell engraftment in marrow and peripheral blood was analysed over a period of 5 months after transplant using conventional cytogenetics, fluorescence in situ hybridization, and variable number of tandem repeats. Neutrophil counts >0.5 x 109/l and platelet counts >20 x 109/l were reached promptly on days 12 and 24, respectively. Throughout the period of observation the percentage of cells with the del(20q) abnormality in the recipient's marrow and peripheral blood was comparable to the proportion of these cells in the donor. These data indicate that the abnormal clone was capable of homing to the marrow, proliferating, differentiating, and therefore contributing to haemopoiesis in a relatively efficient manner. This implies that MDS progenitor cells may not have homing and growth deficiencies, a finding that has particular relevance for autologous transplantation in MDS patients where tumour cells potentially contaminate the graft.

Migliaccio, A. R., M. Baiocchi, et al. (1994). "Stem cell factor and the amplification of progenitor cells

from CD34+ cord blood cells." Blood Cells **20**(1): 129-38; discussion 138-9.

We have studied the frequency of colony-forming cells (CFC) in fetal and neonatal blood in comparison with adult blood and marrow. Fetal or neonatal blood contains at least as many CFC as adult marrow and higher numbers of the more primitive CFC--those CFC (mixed-cell CFC) giving rise to colonies composed of erythroid and myeloid cells. CD34+ cord blood cells (selected by one of several means) proliferate in culture over time and generate more CFC (from pre-CFC) and differentiated cells in response to stem cell factor (SCF) plus different hematopoietic growth factors. For its effect, SCF requires the synergistic action of erythropoietin (Epo), granulocyte colony-stimulating factor (G-CSF), or interleukin-3 (IL-3). In the presence of Epo or G-CSF, CFC and differentiated cells are generated for 15 days and are mainly erythroid or granulocytic, respectively. In contrast, SCF plus IL-3 generate multilineage CFC and differentiated cells for more than 1 month. When the conditions for these long-term suspension cultures were optimized, CFC and differentiated cells were generated for more than 2 months. At this time, CFC were no longer detectable, but cells continued to be generated, and the cells had a mast cell phenotype. These cells have been maintained and propagated for more than 8 months in the presence of IL-3 and SCF and may represent a useful tool to study human mast cell differentiation.

Migliaccio, G., A. R. Migliaccio, et al. (1991). "Effects of recombinant human stem cell factor (SCF) on the growth of human progenitor cells in vitro." J Cell Physiol **148**(3): 503-9.

We have studied the effect of recombinant human Stem Cell Factor (SCF) on the growth of human peripheral blood, bone marrow, and cord blood progenitor cells in semisolid medium. While SCF alone had little colony-stimulating activity under fetal bovine serum (FBS)-deprived culture conditions, SCF synergized with erythropoietin (Epo), granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin 3 (IL-3) to stimulate colony growth. Colony morphology was determined by the late-acting growth factor added along with SCF. Of all the combinations of growth factors, SCF plus IL-3 and Epo resulted in the largest number of mixed-cell colonies--a larger number than observed with IL-3 and Epo alone even in FBS-supplemented cultures. These results suggest that SCF is a growth factor that more specifically targets early progenitor cells (mixed-cell colony-forming cells) and has the capacity to synergize with a wide variety of other hematopoietic growth factors to cause the proliferation and differentiation of committed progenitor cells. Our studies indicate that SCF may be the earliest acting growth factor described to date.

Mimeault, M. and S. K. Batra (2008). "Recent progress on normal and malignant pancreatic stem/progenitor cell research: therapeutic implications for the treatment of type 1 or 2 diabetes mellitus and aggressive pancreatic cancer." Gut **57**(10): 1456-68.

Recent progress on pancreatic stem/progenitor cell research has revealed that the putative multipotent pancreatic stem/progenitor cells and/or more committed beta cell precursors may persist in the pancreatic gland in adult life. The presence of immature pancreatic cells with stem cell-like properties offers the possibility of stimulating their in vivo expansion and differentiation or to use their ex vivo expanded progenies for beta cell replacement-based therapies for type 1 or 2 diabetes mellitus in humans. In addition, the transplantation of either insulin-producing beta cells derived from embryonic, fetal and other tissue-resident adult stem/progenitor cells or genetically modified adult stem/progenitor cells may also constitute alternative promising therapies for treating diabetic patients. The genetic and/or epigenetic alterations in putative pancreatic adult stem/progenitor cells and/or their early progenies may, however, contribute to their acquisition of a dysfunctional behaviour as well as their malignant transformation into pancreatic cancer stem/progenitor cells. More particularly, the activation of distinct tumorigenic signalling cascades, including the hedgehog, epidermal growth factor-epidermal growth factor receptor (EGF-EGFR) system, wingless ligand (Wnt)/beta-catenin and/or stromal cell-derived factor-1 (SDF-1)-CXC chemokine receptor 4 (CXCR4) pathways may play a major role in the sustained growth, survival, metastasis and/or drug resistance of pancreatic cancer stem/progenitor cells and their further differentiated progenies. The combination of drugs that target the oncogenic elements in pancreatic cancer stem/progenitor cells and their microenvironment, with the conventional chemotherapeutic regimens, could represent promising therapeutic strategies. These novel targeted therapies should lead to the development of more effective treatments of locally advanced and metastatic pancreatic cancers, which remain incurable with current therapies.

Mimeault, M. and S. K. Batra (2008). "Targeting of cancer stem/progenitor cells plus stem cell-based therapies: the ultimate hope for treating and curing aggressive and recurrent cancers." Panminerva Med **50**(1): 3-18.

The rapid progression from aggressive primary cancers into locally advanced and invasive

and/or metastatic diseases remains a big obstacle for an early diagnosis and curative therapeutic intervention for cancer patients. The late-stage leukemias and disseminated and metastatic sarcomas, melanomas, brain tumors and epithelial cancers are the devastating diseases associated with a high rate of recurrence after treatment with the conventional clinical therapies including surgery, ionizing radiation, hormonal therapy and systemic chemotherapy, which generally lead to the death of patients. Therefore, the establishment of the molecular events underlying cancer initiation and progression into locally invasive and metastatic diseases is of major interest in basic cancer research as well as for the development of new effective clinical therapeutic options against the recurrent and lethal cancers. Recent advances have led to the identification of specific oncogenic products that are implicated in the malignant transformation of adult stem/progenitor cells into leukemic or tumorigenic and migrating cancer stem/progenitor cells during cancer progression. Of therapeutic interest, the molecular targeting of deregulated signaling elements in cancer stem/progenitor cells and their local microenvironment represents a new potential strategy for the development of more effective clinical treatments against aggressive cancers. Particularly, the combined use of chemotherapeutic drugs to eradicate cancer-initiating cells with hematopoietic stem cell or genetically-modified stem cell transplant is emerging as potential cancer treatments that hold great promise in the area of clinical cancer research. These targeting and stem cell-based therapies may offer the ultimate hope for treating and even curing the patients diagnosed with locally advanced cancers at high risk of recurrence, metastatic and/or relapsed cancers in the clinics.

Mimeault, M., R. Hauke, et al. (2007). "Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers." J Cell Mol Med **11**(5): 981-1011.

Overcoming intrinsic and acquired resistance of cancer stem/progenitor cells to current clinical treatments represents a major challenge in treating and curing the most aggressive and metastatic cancers. This review summarizes recent advances in our understanding of the cellular origin and molecular mechanisms at the basis of cancer initiation and progression as well as the heterogeneity of cancers arising from the malignant transformation of adult stem/progenitor cells. We describe the critical functions provided by several growth factor cascades, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), stem cell factor (SCF) receptor (KIT), hedgehog and Wnt/beta-catenin signalling pathways that are frequently activated in cancer progenitor cells and are involved in their sustained growth, survival, invasion and drug resistance. Of therapeutic interest, we also discuss recent progress in the development of new drug combinations to treat the highly aggressive and metastatic cancers including refractory/relapsed leukaemias, melanoma and head and neck, brain, lung, breast, ovary, prostate, pancreas and gastrointestinal cancers which remain incurable in the clinics. The emphasis is on new therapeutic strategies consisting of molecular targeting of distinct oncogenic signalling elements activated in the cancer progenitor cells and their local microenvironment during cancer progression. These new targeted therapies should improve the efficacy of current therapeutic treatments against aggressive cancers, and thereby preventing disease relapse and enhancing patient survival.

Miura, Y., Z. Gao, et al. (2006). "Mesenchymal stem cell-organized bone marrow elements: an alternative hematopoietic progenitor resource." Stem Cells **24**(11): 2428-36.

Bone marrow-derived mesenchymal stem cells (BMMSCs) are multipotent postnatal stem cells that have been used for the treatment of bone defects and graft-versus-host diseases in clinics. In this study, we found that subcutaneously transplanted human BMMSCs are capable of organizing hematopoietic progenitors of recipient origin. These hematopoietic cells expressed multiple lineages of hematopoietic cell associated markers and were able to rescue lethally irradiated mice, with successful engraftment in the recipient, suggesting a potential bone marrow (BM) resource for stem cell therapies. Furthermore, we found that platelet-derived growth factor (PDGF) promotes the formation of BMMSC-generated BM niches through upregulation of beta-catenin, implying that the PDGF pathway contributes to the formation of ectopic BM. These results indicate that the BMMSC-organized BM niche system represents a unique hematopoietic progenitor resource possessing potential clinical value.

Miyazaki, T., I. Matsuda, et al. (2001). "Flow cytometric analysis of hemetopoietic progenitor cells in peripheral blood stem cell harvest from patients with CD34 positive acute leukemia." J Immunol Methods **247**(1-2): 9-15.

We analyzed CD34 positive cells in peripheral blood stem cell harvest (PBSCH) using flow cytometry. PBSCH from CD34 positive acute myelogeous leukemia (AML-M2) patient contained 1.87% CD34 positive cells, of which 1.21% was represented by MRD.PBSCH from CD34 positive acute lymphoblast leukemia (ALL) patient contained

3.14% CD34 positive cells, of which 0.11% was accounted for by minimal residual disease (MRD). If PBSCH from CD34 positive acute leukemia patient is analyzed for CD34 monoclonal antibody alone, the presence of CD34 positive MRD may escape attention so that CD34 positive hematopoietic progenitor cells may be overestimated. To avoid this risk, it is necessary to analyze PBSCH using both CD34 monoclonal antibody and characteristic markers of leukemia cells that were found pre-treatment.

Molofsky, A. V., R. Pardal, et al. (2003). "Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation." Nature **425**(6961): 962-7.

Stem cells persist throughout life by self-renewing in numerous tissues including the central and peripheral nervous systems. This raises the issue of whether there is a conserved mechanism to effect self-renewing divisions. Deficiency in the polycomb family transcriptional repressor Bmi-1 leads to progressive postnatal growth retardation and neurological defects. Here we show that Bmi-1 is required for the self-renewal of stem cells in the peripheral and central nervous systems but not for their survival or differentiation. The reduced self-renewal of Bmi-1-deficient neural stem cells leads to their postnatal depletion. In the absence of Bmi-1, the cyclin-dependent kinase inhibitor gene p16Ink4a is upregulated in neural stem cells, reducing the rate of proliferation. p16Ink4a deficiency partially reverses the self-renewal defect in Bmi-1-/- neural stem cells. This conserved requirement for Bmi-1 to promote self-renewal and to repress p16Ink4a expression suggests that a common mechanism regulates the self-renewal and postnatal persistence of diverse types of stem cell. Restricted neural progenitors from the gut and forebrain proliferate normally in the absence of Bmi-1. Thus, Bmi-1 dependence distinguishes stem cell self-renewal from restricted progenitor proliferation in these tissues.

Moskowitz, C. H., P. Stiff, et al. (1997). "Recombinant methionyl human stem cell factor and filgrastim for peripheral blood progenitor cell mobilization and transplantation in non-Hodgkin's lymphoma patients--results of a phase I/II trial." Blood **89**(9): 3136-47.

To examine the safety and efficacy of recombinant-methionyl human stem cell factor (r-metHuSCF), 38 patients with intermediate-grade or immunoblastic high-grade non-Hodgkin's lymphoma who were eligible for autologous transplantation were randomized to receive r-metHuSCF (5, 10, 15, or 20 microg/kg/d) plus Filgrastim (10 microg/kg/d) or Filgrastim (10 microg/kg/d) alone to mobilize peripheral blood progenitor cells. Subcutaneous administration of r-metHuSCF was well tolerated in conjunction with a multi-agent pre-medication regimen; local injection site reactions were the most commonly seen adverse event. The total mononuclear cell count, CD34+ cell content, granulocyte-macrophage colony-forming cells (GM-CFC), and burst-forming units-erythroid (BFU-E) per kilogram in the apheresis product was similar when all patients were analyzed by treatment cohort and mobilization regimen (Filgrastim or r-metHuSCF in combination with Filgrastim); however, when prior chemotherapy was taken into account in a supplementary analysis, clinically important differences were observed. Extensive prior therapy was defined as the amount of exposure to specific stem cell toxic chemotherapeutic agents that patients received. These agents include procarbazine, nitrogen mustard, melphalan, nitrosoureas (> or = 2 cycles of any of these drugs) or greater than 7.5 g of cytosine arabinoside. In these patients, there was an increased number of CD34+ cells (1.76 v 0.28 x 10(6)/kg), GM-CFC (20.5 v 5.0 x 10(4)/kg), and BFU-E (36.9 v 8.9 x 10(4)/kg) in patients receiving r-metHuSCF and Filgrastim (N = 18) compared with Filgrastim alone (N = 5). These patients also had a decreased time to an untransfused platelet count of 20 x 10(9)/L that was 10.5 days shorter in the patients who received r-metHuSCF and Filgrastim (12.5 v 23 days). These differences were not found to be statistically significant, possibly because of small size, but are clinically important.

Muschler, G. F., R. J. Midura, et al. (2003). "Practical Modeling Concepts for Connective Tissue Stem Cell and Progenitor Compartment Kinetics." J Biomed Biotechnol **2003**(3): 170-193.

Stem cell activation and development is central to skeletal development, maintenance, and repair, as it is for all tissues. However, an integrated model of stem cell proliferation, differentiation, and transit between functional compartments has yet to evolve. In this paper, the authors review current concepts in stem cell biology and progenitor cell growth and differentiation kinetics in the context of bone formation. A cell-based modeling strategy is developed and offered as a tool for conceptual and quantitative exploration of the key kinetic variables and possible organizational hierarchies in bone tissue development and remodeling, as well as in tissue engineering strategies for bone repair.

Musso, M., F. Porretto, et al. (1999). "Successful treatment of resistant thrombotic thrombocytopenic purpura/hemolytic uremic syndrome with autologous peripheral blood stem and progenitor (CD34+) cell

transplantation." Bone Marrow Transplant **24**(2): 207-9.

The first-line treatment of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS syndrome) induces a response and survival rate of approximately 85%, even if a considerable number of patients relapse; nevertheless, a number of these patients are resistant to conventional management. Immunoablation followed by stem cell transplantation has been shown to be capable of inducing remissions in a large spectrum of experimental autoimmune disorders. We report here the case of a 20-year-old male patient with the TTP-HUS syndrome who was resistant to conventional treatment and was transplanted with autologous immunoselected CD34+ PBPC after conditioning with cyclosphosphamide, anti-T lymphocyte globulin and prednisone. Seven months after transplant the patient is alive and well, without any further treatment being given.

Musso, M., F. Porretto, et al. (1998). "Autologous peripheral blood stem and progenitor (CD34+) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome." Lupus **7**(7): 492-4.

Immunoablation followed by allogeneic stem cell (SC) transplantation has been shown to be capable of curing a large spectrum of experimental autoimmune disorders, hereditary and/or induced. Superimposable results, albeit with some exceptions, have been obtained in human patients affected by coincidental autoimmune and blood diseases. However, both because of encouragine experimental results and of the procedure's greater safety, autologous SC are being increasingly utilized worldwide. Case reports are being collected in the registry of the European Group for Blood and Marrow Transplantation (EBMT)/European League against Rheumatism (EULAR) Autoimmune Disease Stem Cell Project. Among the severe autoimmune diseases (SADs), which are the target of autologous transplantation, severe refractory systemic lupus erythematosus (SLE) is a condition which may benefit from this procedure. We report here the case of a 19 year old female patient with a six year history of SLE with secondary antiphospholipid syndrome (APS), who later developed refractory Evans syndrome. She was transplanted with autologous mobilized CD34+ SC and progenitor cells after conditioning with cyclosphosphamide, anti-T lymphocyte globulin and prednisone. Eight months after transplant, the patient is alive and well, with normal blood counts and persistent low-titre direct antiglobulin (DAT, Coombs) and anti-nuclear antibody (ANA) tests. Anti-double stranded DNA antibody (Anti-dsDNA), lupus anticoagulant tests and anti-cardiolipin antibody (ACA) test are negative.

Muta, K., S. B. Krantz, et al. (1995). "Stem cell factor retards differentiation of normal human erythroid progenitor cells while stimulating proliferation." Blood **86**(2): 572-80.

Stem cell factor (SCF), the ligand for the c-kit tyrosine kinase receptor, markedly stimulates the accumulation of erythroid progenitor cells in vitro. We now report that SCF delays erythroid differentiation among the progeny of individual erythroid progenitors while greatly increasing the proliferation of these progeny. These effects appear to be independent of an effect on maintenance of cell viability. Highly purified day-6 erythroid colony-forming cells (ECFC), consisting mainly of colony-forming units-erythroid (CFU-E), were generated from human peripheral blood burst-forming units-erythroid (BFU-E). Addition of SCF to the ECFC in serum-free liquid culture, together with erythropoietin (EP) and insulin-like growth factor 1 (IGF-1), resulted in a marked increase in DNA synthesis, associated with a delayed peak in cellular benzidine positivity and a delayed incorporation of 59Fe into hemoglobin compared with cultures without SCF. In the presence of SCF, the number of ECFC was greatly expanded during this culture period, and total production of benzidine-positive cells plus hemoglobin synthesis were ultimately increased. To determine the effect of SCF on individual ECFC, single-cell cultures were performed in both semisolid and liquid media. These cultures demonstrated that SCF, in the presence of EP and IGF-1, acted on single cells and their descendants to delay erythroid differentiation while substantially stimulating cellular proliferation, without an enhancement of viability of the initial cells. This was also evident when the effect of SCF was determined using clones of ECFC derived from single BFU-E. Our experiments demonstrate that SCF acts on individual day-6 ECFC to retard erythroid differentiation while simultaneously providing enhanced proliferation by a process apparently independent of an effect on cell viability or programmed cell death.

Muta, K., S. B. Krantz, et al. (1994). "Distinct roles of erythropoietin, insulin-like growth factor I, and stem cell factor in the development of erythroid progenitor cells." J Clin Invest **94**(1): 34-43.

Erythropoietin (EP), insulin-like growth factor I (IGF-I) and stem cell factor (SCF) each reduce apoptosis of human erythroid progenitor cells. To determine if these growth factors have additional roles in stimulating erythropoiesis, the proliferation, maturation, and survival of highly purified human

erythroid colony-forming cells (ECFCs) were studied during the application of different combinations of these growth factors in a serum-free liquid culture. EP maintained cell viability and supported heme synthesis during erythroid maturation, with little increase in viable cell number or stimulation of DNA synthesis. The addition of SCF with EP resulted in a substantial increase in DNA synthesis, which was greater than that seen with the addition of EP and was associated with a large expansion in the number of ECFCs. Thus EP, by itself, produces little increase in cell proliferation, and expansion of the number of erythroid cells depends upon the presence of SCF with EP. The addition of IGF-I with EP led to enhanced heme synthesis and moderate cellular proliferation, but also greatly enhanced nuclear condensation and enucleation in the late erythroblasts. Thus EP, by itself, is not sufficient for complete end-terminal nuclear condensation/enucleation and the presence of IGF-I is necessary for this complete process. While EP greatly reduced apoptosis during 16 h of incubation at 37 degrees C, the addition of SCF and IGF-I with EP had little additional effect, but these additions enhanced DNA synthesis > 3.4-fold. Thus SCF may have an additional role in directly stimulating proliferation through a process that is distinct from apoptosis. Our observations indicate that EP prevents apoptosis and maintains erythroid cell viability and development. IGF-I enhances erythroid maturation and proliferation, but the proliferation of erythroid progenitors is mainly controlled by the addition of SCF with EP, independent of an effect on apoptosis.

Myers, S. E., R. Mick, et al. (1994). "High-dose chemotherapy with autologous stem cell rescue in women with metastatic breast cancer with involved bone marrow: a role for peripheral blood progenitor transplant." Bone Marrow Transplant **13**(4): 449-54.

This retrospective analysis was done to determine the response rate and survival of women with metastatic breast cancer with bone marrow involvement treated with high-dose cyclophosphamide and thiotepa and peripheral progenitor cell rescue. Eligibility criteria included histologically-documented metastatic breast cancer and either stable disease, a partial response or a complete response to conventional dose chemotherapy. Due to bone marrow involvement, all patients received peripheral progenitor cell reinfusion. Purging of the stem cell product was not performed. Cyclophosphamide (CY) 7.5 gm/m2 total dose and thiotepa 675 mg/m2 total dose was used as the intensification regimen. Of 27 treated patients, 4 (14%) died of treatment-related toxicity. Three patients were in complete remission after induction chemotherapy and remained so after high-dose chemotherapy. Three patients converted from a partial response after induction to a complete response after transplant. This yielded a complete remission rate of 21%. Five of these six patients continue in CR at 5, 6, 11, 14, and 26 months post-transplant. Eight patients (29%) are alive with stable disease post transplant. Ten patients developed disease progression. Six patients died shortly after disease progression; however, four patients are alive with disease at 18, 26, 33, and 53 months post-transplant. The median time to treatment failure is 12 months. In women with metastatic breast cancer with bone marrow involvement, durable responses after high-dose chemotherapy are possible utilizing peripheral blood progenitor support rather than marrow purging.

Nagamatsu, G., M. Ohmura, et al. (2006). "A CTX family cell adhesion molecule, JAM4, is expressed in stem cell and progenitor cell populations of both male germ cell and hematopoietic cell lineages." Mol Cell Biol **26**(22): 8498-506.

Stem cells are maintained in an undifferentiated state by interacting with a microenvironment known as the "niche," which is comprised of various secreted and membrane proteins. Our goal was to identify niche molecules participating in stem cell-stem cell and/or stem cell-supporting cell interactions. Here, we isolated genes encoding secreted and membrane proteins from purified male germ stem cells using a signal sequence trap approach. Among the genes identified, we focused on the junctional adhesion molecule 4 (JAM4), an immunoglobulin type cell adhesion molecule. JAM4 protein was actually localized to the plasma membrane in male germ cells. JAM4 expression was downregulated as cells differentiated in both germ cell and hematopoietic cell lineages. To analyze function in vivo, we generated JAM4-deficient mice. Histological analysis of testes from homozygous nulls did not show obvious abnormalities, nor did liver and kidney tissues, both of which strongly express JAM4. The numbers of hematopoietic stem cells in bone marrow were indistinguishable between wild-type and mutant mice, as was male germ cell development. These results suggest that JAM4 is expressed in stem cells and progenitor cells but that other cell adhesion molecules may substitute for JAM4 function in JAM4-deficient mice both in male germ cell and hematopoietic lineages.

Naito, H., H. Kidoya, et al. (2009). "Induction and expression of anti-angiogenic vasohibins in the hematopoietic stem/progenitor cell population." J Biochem **145**(5): 653-9.

Haematopoiesis and blood vessel formation are closely associated, with several molecules

employed by both systems. Recently, vasohibin-1 (VASH1), an endothelium-derived negative feedback regulator of angiogenesis, has been isolated and characterized. VASH1 is induced by VEGF or bFGF in endothelial cells (ECs) and inhibits their proliferation and migration. However, there are no data on the induction and expression of VASH1 in haematopoietic cells (HCs). Here, we show that the haematopoietic stem cell (HSC) population, but not haematopoietic progenitors (HPs) or mature HCs from adult bone marrow (BM) constitutively express VASH1. However, HPs, but not HSCs, can be induced to express VASH1 after BM suppression by 5-FU. Knock-down of the VASH1 gene in VASH1(+) leukaemia cells induced cell proliferation. These results suggest a role for VASH1 in negative feedback regulation of HP proliferation during recovery following BM ablation.

Nakano, I., J. D. Dougherty, et al. (2007). "Phosphoserine phosphatase is expressed in the neural stem cell niche and regulates neural stem and progenitor cell proliferation." Stem Cells **25**(8): 1975-84.

Phosphoserine phosphatase (PSP) metabolizes the conversion of l-phosphoserine to l-serine, classically known as an amino acid necessary for protein and nucleotide synthesis and more recently suggested to be involved in cell-to-cell signaling. Previously, we identified PSP as being enriched in proliferating neural progenitors and highly expressed by embryonic and hematopoietic stem cells, suggesting a general role in stem cells. Here we demonstrate that PSP is highly expressed in periventricular neural progenitors in the embryonic brain. In the adult brain, PSP expression was observed in slowly dividing or quiescent glial fibrillary acidic protein (GFAP)-positive cells and CD24-positive ependymal cells in the forebrain germinal zone adjacent to the lateral ventricle and within GFAP-positive cells of the hippocampal subgranular zone, consistent with expression in adult neural stem cells. In vitro, PSP overexpression promoted proliferation, whereas small interfering RNA-induced knockdown inhibited proliferation of neural stem cells derived from embryonic cortex and adult striatal subventricular zone. The effects of PSP knockdown were partially rescued by exogenous l-serine. These data support a role for PSP in neural stem cell proliferation and suggest that in the adult periventricular germinal zones, PSP may regulate signaling between neural stem cells and other cells within the stem cell niche. Disclosure of potential conflicts of interest is found at the end of this article.

Nakayama, N., D. Duryea, et al. (2003). "Macroscopic cartilage formation with embryonic stem-cell-derived mesodermal progenitor cells." J Cell Sci **116**(Pt 10): 2015-28.

The totipotent embryonic stem cell generates various mesodermal cells when stimulated with BMP4. Among the resulting cells, those expressing flk-1 and/or PDGFRalpha displayed chondrogenic activity in the presence of TGFbeta3 and expressed cartilage-specific genes in 7 to 16 day pellet cultures. Depositions of cartilage matrix and type II collagen were detected by day 14. TGFbeta-stimulated chondrogenesis was synergistically enhanced by PDGF-BB, resulting in a larger cartilage particle filled with a cartilaginous area containing type II collagen, with a surface cell layer expressing type I collagen. In contrast, noggin inhibited both the TGFbeta- and TGFbeta+PDGF-stimulated cartilage formation, suggesting that a BMP-dependent pathway is involved. In fact, replacement of TGFbeta3 with BMP4 on days 10 to 12 markedly elevated the cartilage matrix deposition during the following 7 to 8 days. Moreover, culture with TGFbeta3 and PDGF-BB, followed by the incubation with BMP4 alone, resulted in a cartilage particle lacking type I collagen in the matrix and the surface layer, which suggests hyaline cartilage formation. Furthermore, such hyaline cartilage particles were mineralized. These studies indicate that the PDGFRalpha+ and/or flk-1+ cells derived from embryonic stem cells possess the full developmental potential toward chondrocytes, in common with embryonic mesenchymal cells.

Nalapareddy, K., H. Jiang, et al. (2008). "Determining the influence of telomere dysfunction and DNA damage on stem and progenitor cell aging: what markers can we use?" Exp Gerontol **43**(11): 998-1004.

The decline in organ maintenance and function is one of the major problems limiting quality of life during aging. The accumulation of telomere dysfunction and DNA damage appears to be one of the underlying causes. Uncapping of chromosome ends in response to critical telomere shortening limits the proliferative capacity of human cells by activation of DNA damage checkpoints inducing senescence or apoptosis. Telomere shortening occurs in the vast majority of human tissues during aging and in chronic diseases that increase the rate of cell turnover. There is emerging evidence that telomere shortening can limit the maintenance and function of adult stem cells -- a cell type of utmost importance for organ maintenance and regeneration. In mouse models, telomere dysfunction leads to a depletion of adult stem cell compartments suggesting that stem cells are very sensitive to DNA damage. Both the rarity of stem and progenitor cells in adult organs and their removal in

response to damage make it difficult to assess the impact of telomere dysfunction and DNA damage on stem and progenitor cell aging. Such approaches require the development of sensitive biomarkers recognizing low levels of telomere dysfunction and DNA damage in stem and progenitor cells. Here, we review experimental data on the prevalence of telomere dysfunction and DNA damage during aging and its possible impact on stem and progenitor cell aging.

Neben, S., K. Marcus, et al. (1993). "Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor." Blood **81**(7): 1960-7.

Committed progenitor cells and primitive stem cells mediate early and sustained engraftment, respectively, after lethal irradiation and stem cell transplantation. Peripheral blood stem cells (PBSC) from unstimulated mice are deficient in both cell types. To study techniques to mobilize both progenitor cells and primitive stem cells from the marrow to the blood, we collected peripheral blood from C57BL/6 mice 6 to 7 days after a single dose of cyclophosphamide (CY; 200 mg/kg intraperitoneally), after recombinant human granulocyte colony-stimulating factor (rhG-CSF) (250 micrograms/kg/d twice per day subcutaneously for 4 days), or after CY followed by G-CSF. Significant increases in white blood cell counts (1.6- to 2.7-fold) and circulating day 8 colony-forming unit spleen (CFU-S) (11- to 36-fold) were seen with all three mobilization methods compared with unstimulated control mice. Transplantation of mobilized blood stem cells into lethally irradiated hosts decreased the time to erythroid engraftment. Blood stem cells were analyzed for primitive stem cell content by Rs, an assay for CFU-S self-renewal, and competitive repopulation index (CRI), an assay of long-term repopulating ability. The primitive stem cell content of unstimulated blood was clearly deficient, but was significantly increased following mobilization, approaching normal bone marrow levels. These results were confirmed by an in vitro limiting dilution long-term culture assay that measures the frequency of progenitor cells and primitive stem cells. Mobilization following CY + G-CSF was accompanied by a marked loss of both progenitor cells and primitive stem cells in the marrow. In contrast, following G-CSF alone the progenitor cell and primitive stem cell content of the marrow was unchanged. Stem cell mobilization following CY + G-CSF was not affected by previous exposure of donors to cytosine arabinoside or cyclophosphamide, but was significantly reduced by previous exposure to busulfan. These data show that stem cell content in the blood may reach near-normal marrow levels after mobilization, the mobilization from the marrow to the blood is temporary and reversible, the specific technique used may mobilize different subpopulations of stem cells, and the type of prior chemotherapy may influence the ability to mobilize stem cells into the blood.

Nikolova, T., J. Czyz, et al. (2005). "Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells." Faseb J **19**(12): 1686-8.

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

Nilsson, L., I. Astrand-Grundstrom, et al. (2000). "Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level." Blood **96**(6): 2012-21.

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterized by ineffective hematopoiesis and frequent progression to acute myeloid leukemia. Within MDS, 5q-

syndrome constitutes a distinct clinical entity characterized by an isolated deletion of the long arm of chromosome 5 (5q-), a relatively good prognosis, and infrequent transformation to acute leukemia. The cell of origin in 5q- syndrome as well as in other 5q-deleted MDS patients has not been established, but evidence for involvement of multiple myeloid (but not lymphoid) lineages has suggested that a myeloid-restricted progenitor rather than a pluripotent (lympho-myeloid) stem cell might be the primary target in most patients. Although in 9 patients no evidence of peripheral blood T-cell and only 1 case of B-cell involvement was found, the data herein support that 5q deletions occur in hematopoietic stem cells (HSCs) with a combined lympho-myeloid potential. First, in all investigated patients a minimum of 94% of cells in the minor CD34(+)CD38(-) HSC compartment were 5q deleted as determined by fluorescence in situ hybridization. Second, in 3 of 5 patients 5q aberrations were detected in a large fraction (25% to 90%) of purified CD34(+)CD19(+) pro-B cells. Furthermore, extensive functional characterization with regard to responsiveness to early-acting cytokines, long-term culture-initiating cells, and nonobese diabetic/severe combined immunodeficiency repopulating cells supported that MDS HSCs in 5q-deleted patients are CD34(+)CD38(-), but inefficient at reconstituting hematopoiesis.

Nishihara, M., Y. Wada, et al. (1998). "A combination of stem cell factor and granulocyte colony-stimulating factor enhances the growth of human progenitor B cells supported by murine stromal cell line MS-5." Eur J Immunol **28**(3): 855-64.

We have developed a long-term culture system using the murine bone marrow stromal cells MS-5 to support the growth of progenitor B cells with CD34-, CD10+, CD19+, and cytoplasmic mu chain (C mu)-negative surface phenotype from human CD34+ cells purified from umbilical cord blood (CB). When 10(3) CD34+ cells/well were seeded on MS-5 stromal cells at the beginning of culture in the absence of exogenously added cytokines, progenitor B cells first appeared after 14 days, and the maximal cell production was achieved during the 6th week of culture. Intriguingly, the addition of recombinant human stem cell factor (rhSCF) and granulocyte colony-stimulating factor (rhG-CSF), but not rhIL-7, strikingly enhanced the growth of progenitor B cells from CB CD34+ population cultured on MS-5 stromal cells. The culture of progenitor B cells could be maintained until the 6th week of culture when some cells were revealed to have a C mu phenotype, and a small number of cells had immunoglobulin mu chain on their cell surface in the presence of both rhSCF and rhG-CSF. When CD34+ cells were cultured physically separated from the stromal layer by membrane, supportive effects of MS-5 stromal cells for the growth of progenitor B cells were not observed. These results suggest that the present culture system could generate progenitor B cells to proliferate from CB CD34+ cells, that some of these progenitor B cells could differentiate into immature B cells in conjunction with rhSCF and rhG-CSF, and that a species-cross-reactive membrane-bound factor(s), which stimulates early human B lymphopoiesis, may exist in MS-5 stromal cells. Further studies are required to investigate the mechanism how rhG-CSF acts on progenitor B cells to allow their proliferation and differentiation.

Noble, M., D. Wren, et al. (1992). "The O-2A(adult) progenitor cell: a glial stem cell of the adult central nervous system." Semin Cell Biol **3**(6): 413-22.

Systematic comparison of the properties of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells derived from optic nerves of perinatal and adult rats has revealed that these two populations differ in many fundamental properties. In particular, O-2A(perinatal) progenitor cells are rapidly dividing cells capable of generating large numbers of oligodendrocytes over a relatively short time span. Oligodendrocyte differentiation generally occurs synchronously in all members of a clone, thus leading to elimination of that clone from the pool of dividing cells. However, some O-2A(perinatal) progenitors are also capable of giving rise to O-2A(adult) progenitors. These latter cells express many of the characteristics of stem cells of adult animals, including the capacity to undergo asymmetric division and differentiation. We suggest that precursors which function during early development give rise to terminally differentiated end-stage cells and to a second generation of precursors with properties more appropriate for later developmental stages. It is this second generation of precursors which express the properties of stem cells in adult animals, and we therefore further suggest that our work offers novel insights into the possible developmental origin of stem cells.

Oertel, M., A. Menthena, et al. (2006). "Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells." Gastroenterology **130**(2): 507-20; quiz 590.

BACKGROUND & AIMS: A critical property of stem cells is their ability to repopulate an organ or tissue under nonselective conditions. The aims of this study were to determine whether we could obtain reproducible, high levels of liver repopulation by transplanted fetal liver stem/progenitor cells in

normal adult liver and the mechanism by which liver replacement occurred. METHODS: Wild-type (dipeptidyl peptidase IV [DPPIV(+)]) embryonic day (ED) 14 fetal liver cells underwent transplantation into DPPIV(-) mutant F344 rats to follow the fate and differentiation of transplanted cells. To determine the mechanism for repopulation, proliferation and apoptosis of transplanted and host liver cells were also followed. RESULTS: Transplanted ED 14 fetal liver cells proliferated continuously for 6 months, differentiated into mature hepatocytes, and replaced 23.5% of total liver mass. The progeny of transplanted cells were morphologically and functionally indistinguishable from host hepatocytes and expressed unique liver-specific genes commensurate with their location in the hepatic lobule. Repopulation was based on greater proliferative activity of transplanted cells and reduced apoptosis of their progeny compared with host hepatocytes, coupled with increased apoptosis of host hepatocytes immediately adjacent to transplanted cells. This process, referred to as cell-cell competition, has been described previously in Drosophila during wing development. CONCLUSIONS: We show for the first time that cell-cell competition, a developmental paradigm, can be used to replace functional organ tissue in an adult mammalian species under nonselective conditions and may serve as a strategy for tissue reconstitution in a wide variety of metabolic and other disorders involving the liver, as well as other organs.

Ogawa, D., Y. Okada, et al. (2009). "Evaluation of human fetal neural stem/progenitor cells as a source for cell replacement therapy for neurological disorders: properties and tumorigenicity after long-term in vitro maintenance." J Neurosci Res **87**(2): 307-17.

It is expected that human neural stem/progenitor cells (hNS/PCs) will some day be used in cell replacement therapies. However, their availability is limited because of ethical issues, so they have to be expanded to obtain sufficient amounts for clinical application. Moreover, in-vitro-maintained hNS/PCs may have a potential for tumorigenicity that could be manifested after transplantation in vivo. In the present study, we demonstrate the in vitro and in vivo properties of long-term-expanded hNS/PCs, including a 6-month bioluminescence imaging (BLI) study of their in vivo tumorigenicity. hNS/PCs cultured for approximately 250 days in vitro (hNS/PCs-250) exhibited a higher growth rate and greater neurogenic potential than those cultured for approximately 500 days in vitro (hNS/PCs-500), which showed greater gliogenic potential. In vivo, both hNS/PCs-250 and -500 differentiated into neurons and astrocytes 4 weeks after being transplanted into the striatum of immunodeficient mice, and hNS/PCs-250 exhibited better survival than hNS/PCs-500 at this time point. We also found that the grafted hNS/PCs-250 survived stably and differentiated properly into neurons and astrocytes even 6 months after the surgery. Moreover, during the 6-month observation period by BLI, we did not detect any evidence of rapid tumorigenic growth of the grafted hNS/PCs, and neither PCNA/Ki67-positive proliferating cells nor significant malignant invasive features were detected histologically. These findings support the idea that hNS/PCs may represent a nontumorigenic, safe, and appropriate cell source for regenerative therapies for neurological disorders.

Ohta, K., T. Yamane, et al. (1999). "An effective method for recovering CD34 positive progenitor cells from peripheral blood stem cell apheresis products cryopreserved with simplified method." Osaka City Med J **45**(2): 139-48.

The use of small volume collection chamber (SVCC) during peripheral blood stem cell (PBSC) apheresis, combined with simplified cryopreservation without rate-controlled freezing, have successfully been applied to clinical PBSC transplantation following myeloablative chemotherapies. However, the method to effectively select CD34+ progenitor cells from frozen apheresis products obtained with these simplified methods has not been reported. For this goal, after washing the thawed apheresis products with medium containing Dnase I, two different approaches to purify CD34+ progenitor cells from washed WBCs were compared. In method I, CD34+ cells were purified on the same day using immunomagnetic method. In method II, the cells after wash were cultured for overnight in RPMI-1640/10% FCS containing SCF and IL-3, followed by enrichment of CD34+ cells as in method I on the next day. In both methods, CD34+ cells were recovered with high viability. However, subsequent liquid culture revealed that the cells obtained from method II have clearly higher growth potential compared with the cells from method I. In addition, these CD34+ cells from method II well-tolerated to further refreeze and thaw. Thus, allowing to "rest" overnight after thaw may be critical for processing of the simply cryopreserved apheresis products as in method II.

Oikawa, T., A. Kamiya, et al. (2009). "Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells." Gastroenterology **136**(3): 1000-11.

BACKGROUND & AIMS: Fetal hepatic stem/progenitor cells, called hepatoblasts, differentiate into both hepatocytes and cholangiocytes. The molecular mechanisms regulating this lineage segmentation process remain unknown. Sall4 has been

shown to be among the regulators of organogenesis, embryogenesis, maintenance of pluripotency, and early embryonic cell fate decisions in embryonic stem cells. The expression and functional roles of Sall4 during liver development have not been elucidated. We here provide their first description in hepatoblasts. METHODS: To investigate functions of Sall4 in fetal liver development, Dlk(+)CD45(-)Ter119(-) hepatoblasts derived from embryonic day 14 mouse livers were purified, and in vitro gain and loss of function analyses and in vivo transplantation analyses were performed using retrovirus- or lentivirus-mediated gene transfer. RESULTS: We demonstrated that Sall4 was expressed in fetal hepatoblasts but not adult hepatocytes. The expression level of Sall4 gradually fell during liver development. Overexpression of Sall4 in hepatoblasts significantly inhibited maturation induced by oncostatin M and extracellular matrix in vitro, as evidenced by morphologic changes and suppression of hepatic maturation marker gene expression. When bile duct-like structures were induced by collagen gel-embedded culture, overexpression of Sall4 markedly augmented size and number of cytokeratin19(+)-branching structures. Knockdown of Sall4 inhibited formation of these branching structures. With in vivo transplantation, Sall4 enhanced differentiation of cytokeratin19(+)-bile ducts derived from transplanted hepatoblasts. CONCLUSIONS: These results suggest that Sall4 plays a crucial role in controlling the lineage commitment of hepatoblasts not only inhibiting their differentiation into hepatocytes but also driving their differentiation toward cholangiocytes.

Okada, Y., A. Matsumoto, et al. (2008). "Spatiotemporal recapitulation of central nervous system development by murine embryonic stem cell-derived neural stem/progenitor cells." Stem Cells **26**(12): 3086-98.

Neural stem/progenitor cells (NS/PCs) can generate a wide variety of neural cells. However, their fates are generally restricted, depending on the time and location of NS/PC origin. Here we demonstrate that we can recapitulate the spatiotemporal regulation of central nervous system (CNS) development in vitro by using a neurosphere-based culture system of embryonic stem (ES) cell-derived NS/PCs. This ES cell-derived neurosphere system enables the efficient derivation of highly neurogenic fibroblast growth factor-responsive NS/PCs with early temporal identities and high cell-fate plasticity. Over repeated passages, these NS/PCs exhibit temporal progression, becoming epidermal growth factor-responsive gliogenic NS/PCs with late temporal identities; this change is accompanied by an alteration in the epigenetic status of the glial fibrillary acidic protein promoter, similar to that observed in the developing brain. Moreover, the rostrocaudal and dorsoventral spatial identities of the NS/PCs can be successfully regulated by sequential administration of several morphogens. These NS/PCs can differentiate into early-born projection neurons, including cholinergic, catecholaminergic, serotonergic, and motor neurons, that exhibit action potentials in vitro. Finally, these NS/PCs differentiate into neurons that form synaptic contacts with host neurons after their transplantation into wild-type and disease model animals. Thus, this culture system can be used to obtain specific neurons from ES cells, is a simple and powerful tool for investigating the underlying mechanisms of CNS development, and is applicable to regenerative treatment for neurological disorders.

Okumura, N., K. Tsuji, et al. (1996). "Chemotactic and chemokinetic activities of stem cell factor on murine hematopoietic progenitor cells." Blood **87**(10): 4100-8.

We investigated the effects of stem cell factor (SCF) on the migration of murine bone marrow hematopoietic progenitor cells (HPC) in vitro using a modification of the checkerboard assay. Chemotactic and chemokinetic activities of SCF on HPC were evaluated by the numbers of HPC migrated on positive and negative gradients of SCF, respectively. On both positive and negative gradients of SCF, HPC began to migrate after 4 hours incubation, and their numbers then increased time-dependently. These results indicated that SCF functions as a chemotactic and chemokinetic agent for HPC. Analysis of types of colonies derived from the migrated HPC showed that SCF had chemotactic and chemokinetic effects on all types of HPC. When migrating activities of other cytokines were examined, interleukin (IL)-3 and IL-11 also affected the migration of HPC, but the degrees of each effect were lower than that of SCF. The results of the present study demonstrated that SCF is one of the most potent chemotactic and chemokinetic factors for HPC and suggest that SCF may play an important role in the flow of HPC into bone marrow where stromal cells constitutively produce SCF.

Olweus, J., L. W. Terstappen, et al. (1996). "Expression and function of receptors for stem cell factor and erythropoietin during lineage commitment of human hematopoietic progenitor cells." Blood **88**(5): 1594-607.

The aim of the present study was to determine whether stem cell factor (SCF) and erythropoietin (EPO) act differently on defined subsets of progenitor cells, and if potential differences correlate with the receptor density on each subset. To investigate this possibility directly, we optimized

conditions for the identification and purification of homogeneous progenitor cell subpopulations from human bone marrow. Populations containing 40% and 44% colony forming cells (CFCs) with 99% and 95% purity for the granulomonocytic and erythroid lineage, respectively, were sorted on the basis of differential expression of CD34, CD64, and CD71. In addition, a population containing 67% CFCs, of which 29-43% were CFU-MIX, was sorted from CD34hi CD38loCD50+ cells. Purified progenitor cell subsets were compared directly for responsiveness to SCF and EPO using a short-term proliferation assay. Expression of the receptors for SCF and EPO were then examined on each subset using a flow cytometer modified for high-sensitivity fluorescence measurements. The results show that EPO induces extensive proliferation of erythroid progenitor cells, but has no effect on the proliferation or survival of primitive or granulomonocytic progenitors, even when used in combination with other cytokines. The majority of erythroid progenitor cells furthermore stained positively with anti-EPO receptor (EPO-R) monoclonal antibodies, whereas other progenitor cells were negative. SCF alone induced extensive proliferation of erythroid progenitor cells, and had a stronger synergistic effect on primitive than on granulo-monocytic progenitors. In spite of these differences in SCF activity, there were no significant differences in SCF-R expression between the progenitor subsets. These results suggest that the selective action of EPO on erythropoiesis is determined by lineage-restricted receptor expression, whereas there are additional cell-type specific factors that influence progenitor cell responses to SCF.

Oritani, K., K. Aoyama, et al. (2000). "Stromal cell CD9 and the differentiation of hematopoietic stem/progenitor cells." Leuk Lymphoma **38**(1-2): 147-52.

CD9 belongs to the tetraspan family of proteins that facilitates the regulation of cell proliferation, motility, and adhesion. In mouse hematopoietic organs, CD9 is expressed by myeloid and stromal cells. Although the precise mechanisms are not clear, antibody ligation of CD9 on stromal cells regulates the adhesion between stromal cells and hematopoietic stem cells, the production of myeloid cells in long term bone marrow cultures and the differentiation of hematopoietic stem cells. A 100 kD protein co-precipitated with CD9 is distinct from several previously reported CD9-associated molecules with respect to size and distribution. Identification and analysis of this interesting protein may clarify the molecular mechanisms through which CD9 bearing stromal cells control the differentiation of hematopoietic stem cells and/or allow them to maintain their vital self-renewal capacity.

Paczesny, S., Y. P. Li, et al. (2007). "Efficient generation of CD34+ progenitor-derived dendritic cells from G-CSF-mobilized peripheral mononuclear cells does not require hematopoietic stem cell enrichment." J Leukoc Biol **81**(4): 957-67.

As a result of their potent antigen-presentation function, dendritic cells (DC) are important tools for cell therapy programs. In vitro-generated DC from enriched CD34+ hematopoietic stem cells (HSC; enriched CD34 DC) have already proven their efficiency in Phase I/II clinical trials. Here, we investigated whether enrichment of CD34+ HSC before the onset of culture was absolutely required for their differentiation into DC. With this aim, we developed a new two-step culture method. PBMC harvested from G-CSF-mobilized, healthy patients were expanded for 7 days during the first step, with early acting cytokines, such as stem cell factor, fetal liver tyrosine kinase 3 ligand (Flt-3L), and thrombopoietin. During the second step, expanded cells were then induced to differentiate into mature DC in the presence of GM-CSF, Flt-3L, and TNF-alpha for 8 days, followed by LPS exposure for 2 additional days. Our results showed that the rate of CD34+/CD38+/lineageneg cells increased 19.5+/-10-fold (mean+/-sd) during the first step, and the expression of CD14, CD1a, CD86, CD80, and CD83 molecules was up-regulated markedly following the second step. When compared with DC generated from enriched CD34+ cells, which were expanded for 7 days before differentiation, DC derived from nonenriched peripheral blood stem cells showed a similar phenotye but higher yields of production. Accordingly, the allogeneic stimulatory capacity of the two-step-cultured DC was as at least as efficient as that of enriched CD34 DC. In conclusion, we report herein a new two-step culture method that leads to high yields of mature DC without any need of CD34+ HSC enrichment.

Padmanabhan, A., R. Reich-Slotky, et al. (2009). "Use of the haematopoietic progenitor cell parameter in optimizing timing of peripheral blood stem cell harvest." Vox Sang **97**(2): 153-9.

BACKGROUND AND OBJECTIVES: Timing of peripheral blood stem cell (PBSC) harvest is typically based on quantification of peripheral blood (PB) CD34+ cells. CD34 enumeration is expensive, requires expertise and takes a minimum of 1-2 h to perform. The Sysmex XE2100 is an automated haematology analyser that can rapidly and inexpensively identify haematopoietic progenitor cell (HPC) populations in PB. The aim of this study was to

examine if HPC can be used to optimize timing of PBSC harvest. MATERIALS AND METHODS: White blood cell (WBC), HPC and CD34 counts were determined in a total of 60 mobilized donors. Data were analysed to examine the utility of WBC and HPC counts in predicting preharvest CD34+ counts. RESULTS: In adults presenting for autologous collection, a PB HPC threshold of > 30/microl predicts a preharvest CD34+ count of > 20/microl with sensitivity of 86% and positive predictive value (PPV) of 100%. Among paediatric patients with a diagnosis of neuroblastoma, an HPC threshold of > 16/microl yielded sensitivity and PPV of 100%, while in children with other diagnoses, an HPC cut-off of > 44/microl yielded sensitivity and PPV of 67% and 100%, respectively. Eighty per cent of adequately mobilized allogeneic donors were identified using an HPC threshold > 15/microl, with a PPV of 100%. PB WBC can also aid in predicting CD34 counts in most patient groups, albeit with lower sensitivity than HPC. CONCLUSION: By virtue of being a sensitive and accurate predictor of preharvest CD34+ counts, our data support the use of the HPC parameter in optimizing the timing of PBSC harvest.

Paguirigan, A., D. J. Beebe, et al. (2007). "Simulating mouse mammary gland development: cell ageing and its relation to stem and progenitor activity." Cell Prolif **40**(1): 106-24.

BACKGROUND: Somatic stem and progenitor cell division is likely to be an important determinant of tumor development. Each division is accompanied by a risk of fixing genetic mutations, and/or generating innately immortal cells that escape normal physiological controls. AIM: Using biological information, we aimed to devise a theoretical model for mammary gland development that described the effect of various stem/progenitor cells activities on the demographics of adult mammary epithelial cell populations. RESULTS: We found that mammary ductal trees should develop in juvenile mice despite widely variant levels of activity in the progenitor compartment. Sequestration (inactivation) of progenitor cells dramatically affected the aging-maturation of the population without affecting the total regenerative capacity of the gland. Our results showed that if stem and progenitor cells can be demonstrated in glands regenerated by serial transplantation, they originated in a canonical primary stem cell (providing a functional definition of mammary stem cells). Finally, when the probability of symmetric division of stem cells increased above a threshold, the mammary epithelial population overall was immortal during serial transplantation. CONCLUSIONS: This model provides, (1) a theoretical framework for testing whether the phenotypes of genetically modified mice (many of which are breast cancer models) derive from changes of stem and progenitor activity, and (2) a means to evaluate the resolving power of functional assays of regenerative capacity in mammary epithelial cell populations.

Panzenbock, B., P. Bartunek, et al. (1998). "Growth and differentiation of human stem cell factor/erythropoietin-dependent erythroid progenitor cells in vitro." Blood **92**(10): 3658-68.

Stem cell factor (SCF) and erythropoietin (Epo) effectively support erythroid cell development in vivo and in vitro. We have studied here an SCF/Epo-dependent erythroid progenitor cell from cord blood that can be efficiently amplified in liquid culture to large cell numbers in the presence of SCF, Epo, insulin-like growth factor-1 (IGF-1), dexamethasone, and estrogen. Additionally, by changing the culture conditions and by administration of Epo plus insulin, such progenitor cells effectively undergo terminal differentiation in culture and thereby faithfully recapitulate erythroid cell differentiation in vitro. This SCF/Epo-dependent erythroid progenitor is also present in CD34(+) peripheral blood stem cells and human bone marrow and can be isolated, amplified, and differentiated in vitro under the same conditions. Thus, highly homogenous populations of SCF/Epo-dependent erythroid progenitors can be obtained in large cell numbers that are most suitable for further biochemical and molecular studies. We demonstrate that such cells express the recently identified adapter protein p62(dok) that is involved in signaling downstream of the c-kit/SCF receptor. Additionally, cells express the cyclin-dependent kinase (CDK) inhibitors p21(cip1) and p27(kip1) that are highly induced when cells differentiate. Thus, the in vitro system described allows the study of molecules and signaling pathways involved in proliferation or differentiation of human erythroid cells.

Papayannopoulou, T. (1999). "Hematopoietic stem/progenitor cell mobilization. A continuing quest for etiologic mechanisms." Ann N Y Acad Sci **872**: 187-97; discussion 197-9.

The physiologic egress of mature hemopoietic cells and of hemopoietic stem/progenitor cells from bone marrow to the circulation are poorly understood processes. Likewise, the mechanism of their enforced emigration or mobilization through the use of several agents has not been unraveled. Although mobilization is suspected to be a multi-step process, involving sequential and/or overlapping changes in adhesion and migratory capacity, a model of molecular hierarchy, like the one governing the

extravasation of mature leukocytes to tissues of inflammation, has not been worked out. Understanding the in vivo mechanism of mobilization has been a challenge. Signals emanating from both stromal cells and from hemopoietic cells are likely involved. However, dissecting out their roles, specificity, and interactions has been difficult. Nevertheless insightful information is rapidly emerging, especially with the current availability of many mouse models bearing targeted disruptions of cytoadhesion or signaling molecules.

Papayannopoulou, T. (2000). "Mechanisms of stem-/progenitor-cell mobilization: the anti-VLA-4 paradigm." Semin Hematol **37**(1 Suppl 2): 11-8.

Since the introduction of mobilized peripheral blood stem cells for transplantation purposes, many studies have been performed using mobilized cells with different mobilization schemes, primarily to optimize mobilization protocols. Studies aiming at mechanisms of mobilization have been few, but have provided useful insights. However, conclusions about mobilization mechanisms were largely inferential. We have attempted to analyze the mobilization process Involving the VLA-4/VCAM-1 pathway. Our findings are summarized and an attempt is made to put our experience into a general model of mobilization.

Papayannopoulou, T. (2004). "Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization." Blood **103**(5): 1580-5.

Uncovering the molecular mechanisms governing the exit of stem/progenitor cells from bone marrow to peripheral blood at steady state or after their enforced migration has been an ongoing challenge. Recently, however, several new avenues or paradigms in mobilization have emerged from ever-expanding work in humans subjected to granulocyte colony-stimulating factor (G-CSF) mobilization, as well as from studies in normal and gene-deficient mouse models. Although these developments represent notable advances that met with considerable excitement, they have been quenched by surprising vacillations in subsequent research. This perspective highlights recent developments in mobilization along with their controversies. A full understanding of the directional cues that control the migratory behavior and the fate of stem/progenitor cells once they migrate out of bone marrow will await further experimentation, aiming to bridge our current gaps in knowledge.

Papayannopoulou, T., G. V. Priestley, et al. (2003). "The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization." Blood **101**(12): 4739-47.

The directed migration of mature leukocytes to inflammatory sites and the lymphocyte trafficking in vivo are dependent on G protein-coupled receptors and delivered through pertussis toxin (Ptx)-sensitive Gi-protein signaling. In the present study, we explored the in vivo role of G-protein signaling on the redistribution or mobilization of hematopoietic stem/progenitor cells (HPCs). A single injection of Ptx in mice elicits a long-lasting leukocytosis and a progressive increase in circulating colony-forming unit-culture (CFU-C) and colony-forming unit spleen (CFU-S). We found that the prolonged effect is sustained by a continuous slow release of Ptx bound to red blood cells or other cells and is potentially enhanced by an indirect influence on cell proliferation. Plasma levels of certain cytokines (interleukin 6 [IL-6], granulocyte colony-stimulating factor [G-CSF]) increase days after Ptx treatment, but these are unlikely initiators of mobilization. In addition to normal mice, mice genetically deficient in monocyte chemotactic protein 1 (MCP-1), matrix metalloproteinase 9 (MMP-9), G-CSF receptor, beta2 integrins, or selectins responded to Ptx treatment, suggesting independence of Ptx-response from the expression of these molecules. Combined treatments of Ptx with anti-very late activation antigen (anti-VLA-4), uncovered potentially important insight in the interplay of chemokines/integrins, and the synergy of Ptx with G-CSF appeared to be dependent on MMP-9. As Ptx-mobilized kit+ cells display virtually no response to stromal-derived factor 1 (SDF-1) in vitro, our data suggest that disruption of CXCR4/SDF-1 signaling may be the underlying mechanism of Ptx-induced mobilization and indirectly reinforce the notion that active signaling through this pathway is required for continuous retention of cells within the bone marrow. Collectively, our data unveil a novel example of mobilization through pharmacologic modulation of signaling.

Partlow, K. C., J. Chen, et al. (2007). "19F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon nanobeacons." Faseb J **21**(8): 1647-54.

MRI has been employed to elucidate the migratory behavior of stem/progenitor cells noninvasively in vivo with traditional proton (1H) imaging of iron oxide nanoparticle-labeled cells. Alternatively, we demonstrate that fluorine (19F) MRI of cells labeled with different types of liquid perfluorocarbon (PFC) nanoparticles produces unique and sensitive cell markers distinct from any tissue background signal. To define the utility for cell tracking, mononuclear cells harvested from human

umbilical cord blood were grown under proendothelial conditions and labeled with nanoparticles composed of two distinct PFC cores (perfluorooctylbromide and perfluoro-15-crown-5 ether). The sensitivity for detecting and imaging labeled cells was defined on 11.7T (research) and 1.5T (clinical) scanners. Stem/progenitor cells (CD34+ CD133+ CD31+) readily internalized PFC nanoparticles without aid of adjunctive labeling techniques, and cells remained functional in vivo. PFC-labeled cells exhibited distinct 19F signals and were readily detected after both local and intravenous injection. PFC nanoparticles provide an unequivocal and unique signature for stem/progenitor cells, enable spatial cell localization with 19F MRI, and permit quantification and detection of multiple fluorine signatures via 19F MR spectroscopy. This method should facilitate longitudinal investigation of cellular events in vivo for multiple cell types simultaneously.

Passegue, E., A. J. Wagers, et al. (2005). "Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates." J Exp Med **202**(11): 1599-611.

Knowledge of the molecular networks controlling the proliferation and fate of hematopoietic stem cells (HSC) is essential to understand their function in maintaining blood cell production during normal hematopoiesis and upon clinical transplantation. Using highly purified stem and progenitor cell populations, we define the proliferation index and status of the cell cycle machinery at discrete stages of hematopoietic differentiation and during cytokine-mediated HSC mobilization. We identify distinct sets of cell cycle proteins that specifically associate with differentiation, self-renewal, and maintenance of quiescence in HSC and progenitor cells. Moreover, we describe a striking inequality of function among in vivo cycling and quiescent HSC by demonstrating that their long-term engraftment potential resides predominantly in the G(0) fraction. These data provide a direct link between HSC proliferation and function and identify discrete molecular targets in regulating HSC cell fate decisions that could have implications for both the therapeutic use of HSC and the understanding of leukemic transformation.

Pearson, T., L. D. Shultz, et al. (2008). "A new immunodeficient hyperglycaemic mouse model based on the Ins2Akita mutation for analyses of human islet and beta stem and progenitor cell function." Diabetologia **51**(8): 1449-56.

AIMS/HYPOTHESIS: To develop and validate a new immunodeficient mouse strain that spontaneously develops a non-autoimmune hyperglycaemia to serve as a diabetic host for human islets and human beta stem and progenitor cells without the need for induction of hyperglycaemia by toxic chemicals with their associated side effects. METHODS: We generated and characterised a new strain of immunodeficient spontaneously hyperglycaemic mice, the NOD-Rag1null Prf1null Ins2Akita strain and compared this strain with the NOD-scid Il2rgammanull (also known as Il2rg) immunodeficient strain rendered hyperglycaemic by administration of a single dose of streptozotocin. Hyperglycaemic mice were transplanted with human islets ranging from 1,000 to 4,000 islet equivalents (IEQ) and were monitored for normalisation of blood glucose levels. RESULTS: NOD-Rag1null Prf1null Ins2Akita mice developed spontaneous hyperglycaemia, similar to Ins2Akita-harbouring strains of immunocompetent mice. Histological examination of islets in the host pancreas validated the spontaneous loss of beta cell mass in the absence of mononuclear cell infiltration. Human islets transplanted into spontaneously diabetic NOD-Rag1null Prf1null Ins2Akita and chemically diabetic NOD-scid Il2rgammanull mice resulted in a return to euglycaemia that occurred with transplantation of similar beta cell masses. CONCLUSIONS/INTERPRETATION: The NOD-Rag1null Prf1null Ins2Akita mouse is the first immunodeficient, spontaneously hyperglycaemic mouse strain described that is based on the Ins2Akita mutation. This strain is suitable as hosts for human islet and human beta stem and progenitor cell transplantation in the absence of the need for pharmacological induction of diabetes. This strain of mice also has low levels of innate immunity and can be engrafted with a human immune system for the study of human islet allograft rejection.

Pellegatta, S., P. Tunici, et al. (2006). "The therapeutic potential of neural stem/progenitor cells in murine globoid cell leukodystrophy is conditioned by macrophage/microglia activation." Neurobiol Dis **21**(2): 314-23.

Twitcher (GALC(twi/twi)) is the murine model of globoid cell leukodystrophy (GLD or Krabbe disease), a disease caused by mutations of the lysosomal enzyme galactocerebrosidase (GALC). To verify the therapeutic potential on twitcher of neural stem/progenitor cells (NSPC), we transduced them with a GALC lentiviral vector. Brain injection of NSPC-GALC increased survival of GALC(twi/twi) from 36.1 +/- 4.1 to 52.2 +/- 5.6 days ($P < 0.0001$). Detection of GALC activity and flow cytometry showed that NSPC-GALC and NSPC expressing the green fluorescent protein were attracted to the posterior area of twitcher brain, where demyelination

occurs first. GALC(twi/twi) microglia, also more abundant in posterior regions of the brain, released significant amounts of the cytotoxic cytokine TNF-alpha when matched with NSPC-GALC. Thus, in murine GLD, and possibly in other demyelinating diseases, NSPC are attracted to regions of active demyelination but have limited survival and therapeutic potential if attacked by activated macrophages/microglia.

Perino, M. G., S. Yamanaka, et al. (2008). "Cardiomyogenic stem and progenitor cell plasticity and the dissection of cardiopoiesis." J Mol Cell Cardiol **45**(4): 475-94.

Cell-based therapies hold promise of repairing an injured heart, and the description of stem and progenitor cells with cardiomyogenic potential is critical to its realization. At the vanguard of these efforts are analyses of embryonic stem cells, which clearly have the capacity to generate large numbers of cardiomyocytes in vitro. Through the use of this model system, a number of signaling mechanisms have been worked out that describes at least partially the process of cardiopoiesis. Studies on adult stem and on progenitor cells with cardiomyogenic potential are still in their infancy, and much less is known about the molecular signals that are required to induce the differentiation to cardiomyocytes. It is also unclear whether the pathways are similar or different between embryonic and adult cell-induced cardiomyogenesis, partly because of the continued controversies that surround the stem cell theory of cardiac self-renewal. Irrespective of any perceived or actual limitations, the study of stem and progenitor cells has provided important insights into the process of cardiomyogenesis, and it is likely that future research in this area will turn the promise of repairing an injured heart into a reality.

Pierelli, L., M. Marone, et al. (2002). "Transforming growth factor-beta1 causes transcriptional activation of CD34 and preserves haematopoietic stem/progenitor cell activity." Br J Haematol **118**(2): 627-37.

Stem/progenitor cells endowed with in vitro and in vivo haematopoietic activity express the surface protein CD34. Transforming growth factor beta1 (TGF-beta1) is one of the soluble molecules that regulate cell cycle and differentiation of haematopoietic cells, but has pleiotropic activities depending on the state of responsiveness of the target cells. It has previously been shown that TGF-beta1 maintains human CD34+ haematopoietic progenitors in an undifferentiated state, independently of any cell cycle effect. Here, we have shown that TGF-beta1 upregulates the human CD34, an effect that was evident in primary stem/progenitor cells (CD34+lin-) both at the transcriptional and protein levels, and was not associated with any relevant effect on cell growth. The presence of TGF-beta1 influenced differentiation, maintaining primary CD34+/Lin- in an undifferentiated state. This effect was associated with Smad activation and with a dramatic decrease in p38 phosphorylation. Moreover, blocking p38 phosphorylation by the SB202190 inhibitor increased CD34 RNA levels but did not enhance CD34 protein expression in CD34+/Lin- cells, suggesting that modulation of multiple signalling pathways is necessary to reproduce TGF-beta1 effects. These data establish the role that TGF-beta1 has in the modulation of the CD34 stem/progenitor protein and stem/progenitor functions, providing important clues for understanding haematopoietic development and a potential tool for the modulation of human haematopoiesis.

Pierre-Louis, O., D. Clay, et al. (2009). "Dual SP/ALDH functionalities refine the human hematopoietic Lin-CD34+CD38- stem/progenitor cell compartment." Stem Cells **27**(10): 2552-62.

Identification of prevalent specific markers is crucial to stem/progenitor cell purification. Determinants such as the surface antigens CD34 and CD38 are traditionally used to analyze and purify hematopoietic stem/progenitor cells (HSCs/HPCs). However, the variable expression of these membrane antigens poses some limitations to their use in HSC/HPC purification. Techniques based on drug/stain efflux through the ATP-binding cassette (ABC)G2 pump (side population [SP] phenotype) or on detection of aldehyde dehydrogenase (ALDH) activity have been independently developed and distinguish the SP and ALDH(Bright) (ALDH(Br)) cell subsets for their phenotype and proliferative capability. In this study, we developed a multiparametric flow cytometric method associating both SP and ALDH activities on human lineage negative (Lin(-)) bone marrow cells and sorted different cell fractions according to their SP/ALDH activity level. We find that Lin(-)CD34(+)CD38(Low/-) cells are found throughout the spectrum of ALDH expression and are enriched especially in ALDH(Br) cells when associated with SP functionality (SP/ALDH(Br) fraction). Furthermore, the SP marker identified G(0) cells in all ALDH fractions, allowing us to sort quiescent cells regardless of ALDH activity. Moreover, we show that, within the Lin(-)CD34(+)CD38(-)ALDH(Br) population, the SP marker identifies cells with higher primitive characteristics, in terms of stemness-related gene expression and in vitro and in vivo proliferative potential, than the Lin(-)CD34(+) CD38(-)ALDH(Br)

main population cells. In conclusion, our study shows that the coexpression of SP and ALDH markers refines the Lin(-)CD34(+)CD38(-) hematopoietic compartment and identifies an SP/ALDH(Br) cell subset enriched in quiescent primitive HSCs/HPCs.

Pituch-Noworolska, A., M. Majka, et al. (2003). "Circulating CXCR4-positive stem/progenitor cells compete for SDF-1-positive niches in bone marrow, muscle and neural tissues: an alternative hypothesis to stem cell plasticity." Folia Histochem Cytobiol **41**(1): 13-21.

The trans-differentiation hypothesis of adult tissue-specific stem cells has been recently questioned because of insufficient proof that the so-called plasticity experiments were performed on pure populations of tissue-specific stem cells. It was shown recently, for example, that the formation of haematopoietic colonies by muscle cells depended on the presence of haematopoietic stem/progenitor cells residing within the muscle tissue and hence was not related to the plasticity of the muscle stem cells. The explanation for the presence in, or homing into, muscles of haematopoietic stem cells is, however, not clear. In our study, we hypothesised that muscle tissues secrete stromal-derived factor (SDF)- 1, an alpha-chemokine for haematopoietic stem cells (HSC), which could attract HSC circulating in peripheral blood into muscle tissue. We found, using RT-PCR and immunocytochemistry, that SDF-1 was expressed in human heart and skeletal muscles. Moreover, muscle satellite cells, which are pivotal for regeneration of muscle, highly expressed on their surface CXCR4, a G-protein-coupled receptor that binds SDF-1. To determine whether the CXCR4 receptor is functional on muscle satellite/progenitor cells, we stimulated murine satellite cells (the C2C12 cell line) with SDF-1 and demonstrated the phosphorylation of p42/44 MAPK and AKT serine-threonine kinase in these cells. Moreover, we showed that SDF-1 gradient chemoattracts these cells. We postulate that the CXCR4-positive muscle satellite and CXCR4-positive HSC circulating in the peripheral blood compete for occupancy of SDF-1-positive stem cell niches that are present in bone marrow and muscle tissues. Thus, we suggest that competition for common niches by various circulating CXCR4-positive stem cells and their ability to home to the SDF-1-positive niches in various organs, is a better explanation than stem cell plasticity of why (i) haematopoietic colonies can be cultured from muscles and (ii) early muscle progenitors could be cultured from bone marrow.

Ploemacher, R. E., P. L. van Soest, et al. (1993). "Autocrine transforming growth factor beta 1 blocks colony formation and progenitor cell generation by hemopoietic stem cells stimulated with steel factor." Stem Cells **11**(4): 336-47.

The ability of Steel Factor (SF) to stimulate colony formation and progenitor cell generation by hemopoietic stem cells (HSCs) in vitro in the absence of interleukin 3 (IL-3) was investigated. IL-3 was required for HSC proliferation, and no or restricted proliferation occurred in the presence of SF, IL-6, IL-11, or IL-12 as single factors or in combination. Neutralizing concentrations of anti-transforming growth factor (TGF)-beta 1 antibodies enhanced progenitor cell generation 2-3-fold in the presence of IL-3, but 75 to over 300-fold when cultures contained at least SF in the absence of IL-3. Exogenous TGF-beta 1 fully abrogated the antibody effects. In the presence of antibodies to TGF-beta 1, SF alone stimulated the delayed formation of small blast cell colonies and SF synergized with IL-6, IL-11, or IL-12 to greatly hasten colony formation, enhance colony number and size, and increase colony forming unit-culture (CFU-C) output from suspension cultures of enriched HSC populations. Secondary CFU-C colonies were significantly larger when IL-3 was absent during the suspension culture phase. Single cell and limiting dilution analysis using a homogenous colony forming unit-spleen (CFU-S) day-12 population and an 800-fold enriched long-term repopulating HSC fraction, respectively, indicated that TGF-beta 1 was an autocrine product of these HSC subsets. Addition of nucleosides, insulin, extra glucose, or serum could not replace the effects of the anti-TGF-beta 1 antibody. While these data offer one possible explanation for reports on the inability of SF to stimulate HSC proliferation, they present the basis for a novel model of the regulation of HSC activation wherein: 1) close-range interactions of HSCs with mesenchymal stromal cells do not exclusively determine maintenance of HSC quiescence; 2) competence acquisition by dormant HSCs may involve the down-regulation or inactivation of autocrine TGF-beta 1; and 3) SF may act as a primary growth factor rather than exclusively as a synergistic cytokine.

Pode-Shakked, N., S. Metsuyanim, et al. (2009). "Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population." J Cell Mol Med **13**(8B): 1792-808.

During development, renal stem cells reside in the nephrogenic blastema. Wilms' tumour (WT), a common childhood malignancy, is suggested to arise from the nephrogenic blastema that undergoes partial differentiation and as such is an attractive model to study renal stem cells leading to cancer initiation and maintenance. Previously we have made use of

blastema-enriched WT stem-like xenografts propagated in vivo to define a 'WT-stem' signature set, which includes cell surface markers convenient for cell isolation (frizzled homolog 2 [Drosophila] - FZD2, FZD7, G-protein coupled receptor 39, activin receptor type 2B, neural cell adhesion molecule - NCAM). We show by fluorescenceactivated cell sorting analysis of sphere-forming heterogeneous primary WT cultures that most of these markers and other stem cell surface antigens (haematopoietic, CD133, CD34, c-Kit; mesenchymal, CD105, CD90, CD44; cancer, CD133, MDR1; hESC, CD24 and putative renal, cadherin 11), are expressed in WT cell sub-populations in varying levels. Of all markers, NCAM, CD133 and FZD7 were constantly detected in low-to-moderate portions likely to contain the stem cell fraction. Sorting according to FZD7 resulted in extensive cell death, while sorted NCAM and CD133 cell fractions were subjected to clonogenicity assays and quantitative RT-PCR analysis, exclusively demonstrating the NCAM fraction as highly clonogenic, overexpressing the WT 'stemness' genes and topoisomerase2A (TOP2A), a bad prognostic marker for WT. Moreover, treatment of WT cells with the topoisomerase inhibitors, Etoposide and Irinotecan resulted in down-regulation of TOP2A along with NCAM and WT1. Thus, we suggest NCAM as a marker for the WT progenitor cell population. These findings provide novel insights into the cellular hierarchy of WT, having possible implications for future therapeutic options.

Pollard, Y., M. J. Watts, et al. (1999). "Use of the haemopoietic progenitor cell count of the Sysmex SE-9500 to refine apheresis timing of peripheral blood stem cells." Br J Haematol **106**(2): 538-44.

The Sysmex SE-9500 automated cell counter provides an estimate of immature cells referred to as 'haemopoietic progenitor cells' (HPC). The aim of this study was to relate the HPC count to CD34+ cell levels in mobilized peripheral blood and to determine whether the HPC count was valuable in predicting apheresis yields of CD34+ cells. Studies were performed on 114 samples from 67 patients undergoing progenitor cell mobilization. HPC cells were undetectable in the steady state. On the day of apheresis the HPC and CD34 counts were weakly correlated, with the median HPC count being 2.3-fold greater than the CD34+ cell count. The HPC count did not include the CD34+ cells as immunomagnetic depletion of CD34+ cells did not significantly reduce the HPC count. CD34+ cell counts predicted for apheresis yield (r = 0.773) on that day as did the HPC count (r = 0. 623). The optimal strategy to prevent unnecessary harvesting while minimizing the risk of missing an adequate harvest, and minimizing laboratory investigations, was to screen all samples for HPC and limit CD34+ cell measurements to those with an HPC count <10 x 106/l (19/114 samples). If the CD34+ cell count was also <10 x 106/l then harvesting should not be carried out.

Ponti, D., A. Costa, et al. (2005). "Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties." Cancer Res **65**(13): 5506-11.

Breast cancer-initiating cells have been recently identified in breast carcinoma as CD44+/CD24(-/low) cells, which exclusively retain tumorigenic activity and display stem cell-like properties. However, at present, direct evidence that breast cancer-initiating cells can be propagated in vitro is still lacking. We report here the isolation and in vitro propagation of breast cancer-initiating cells from three breast cancer lesions and from an established breast carcinoma cell line. Our breast carcinoma-derived cultures encompassed undifferentiated cells capable of self-renewal, extensive proliferation as clonal nonadherent spherical clusters, and differentiation along different mammary epithelial lineages (ductal and myoepithelial). Interestingly, cultured cells were CD44+/CD24- and Cx43-, overexpressed neoangiogenic and cytoprotective factors, expressed the putative stem cell marker Oct-4, and gave rise to new tumors when as few as 10(3) cells were injected into the mammary fat pad of SCID mice. Long-term cultures of breast tumorigenic cells with stem/progenitor cell properties represent a suitable in vitro model to study breast cancer-initiating cells and to develop therapeutic strategies aimed at eradicating the tumorigenic subpopulation within breast cancer.

Porta, C., R. Caporali, et al. (2004). "Impaired bone marrow hematopoietic progenitor cell function in rheumatoid arthritis patients candidated to autologous hematopoietic stem cell transplantation." Bone Marrow Transplant **33**(7): 721-8.

We have evaluated bone marrow morphology, percentage of bone marrow CD34(+) cells, proliferative activity of bone marrow precursors, clonogenic assay (BFU-E and CFU-GM) in short-term bone marrow cultures, and bone marrow cell apoptosis, together with serum TNF-alpha and IL-6, in 16 chronic, refractory RA patients, as well as in five healthy controls. Of 16 RA patients (68.7%), 11 showed a reduced bone marrow cellularity, while it was normal in all the controls. In RA patients, the median percentage of CD34(+) bone marrow cells, the median percentage of proliferating bone marrow myeloid precursors, and the median number of both BFU-E and CFU-GM colonies were significantly

lower than observed in the controls. As far as TNF-alpha and IL-6 titers is concerned, the latter did not significantly differ from controls' values, while TNF-alpha titers were significantly lower in healthy controls. Finally, the median apoptotic index of early bone marrow myeloid cells of RA patients was significantly higher compared with controls. These observations may identify the biological risk factors for impaired mobilization and/or engraftment when RA patients are candidates for autologous hematopoietic stem cell grafting.

Preijers, F. W., P. B. van Hennik, et al. (1999). "Counterflow centrifugation allows addition of appropriate numbers of T cells to allogeneic marrow and blood stem cell grafts to prevent severe GVHD without substantial loss of mature and immature progenitor cells." Bone Marrow Transplant **23**(10): 1061-70.

Using counterflow centrifugation elutriation (CCE) lymphocytes can be separated from CD34+ populations based on size. Immature progenitors tend to be smaller than mature cells suggesting that CCE introduces loss of stem cells. We compared the separation of 12 PBSC with 16 BM transplants. Cells were separated in 12 fractions (3000-2200 r.p.m.) and the rotor off (RO) fraction. Separation patterns of BM and PBSC were comparable. B cells were collected in the high speed fractions followed by T and NK cells. In contrast, progenitor cells were collected in lower speed fractions. By adding successively T cell-depleted fractions to the RO fraction a BM transplant could be composed containing 0.7 x 10(6) T cells/kg and 90%, 89% and 68% recovery of CD34+, CFU-GM and BFU-E. PBSC were separated in four CCE runs inducing higher numbers of T cells in the graft (4.4 x 10(6)/kg) and 54% CD34+, 46% CFU-GM and 37% BFU-E recovery. Time of engraftment was not delayed and no graft failure was observed. The higher number of T cells was not associated with higher incidence of GVHD. Acute GVHD > or = grade III occurred in 0 of 16 BM and two of 12 PBSC recipients; extensive chronic GVHD was observed in four of 15 and three of nine recipients, respectively. To study immature cells in the graft, CD34 subpopulations and cells with long-term repopulating ability, determined using cobble-stone area formation (CAFC assay), were evaluated in each fraction. The separation patterns in BM and PBSC were comparable. Cells with mature and immature phenotype were enriched in lower speed fractions (mean recovery of 74% CD34+/CD13-/DR-). The CAFC week 2, 4 and 6 were also enriched in these fractions. These data show that the used CCE procedure is a reliable method to deplete T cells from stem cell transplants without substantial loss of immature and mature progenitors.

Preti, R. A., T. Ahmed, et al. (1992). "Hemopoietic stem cell processing: comparison of progenitor cell recovery using the Cobe 2991 cell washer and the Haemonetics V50 apheresis system." Bone Marrow Transplant **9**(5): 377-81.

Using 24 bone marrow (BM) harvests intended for cryopreservation and transplantation, we compared the use of the Cobe 2991 cell washer (2991) and the Haemonetics V50 apheresis system (HV50) for automated BM processing. Our in vitro data indicate that while the mononuclear cell (MNC) concentration of the HV50 product was significantly greater than that of the 2991, the overall MNC recovery of the two products was equivalent. In addition, although the concentration of CFU-GM and BFU-E in the products was equivalent, recovery of these progenitors in the 2991 product was significantly greater than those of the HV50 product. There was no significant difference in either the final product concentration or the overall recovery of cells bearing the primitive myeloid antigens, CD33 or CD34, between the two methods. The HV50 product volume, the red cell and the granulocyte mass were significantly lower than those of the 2991. We conclude that the advantages gained through the use of each machine should be evaluated within the context of the specific intention for the graft. Future advances in the identification and understanding of the primitive stem cell will aid in attempts to evaluate the methods used to isolate these cells.

Priestley, G. V., T. Ulyanova, et al. (2007). "Sustained alterations in biodistribution of stem/progenitor cells in Tie2Cre+ alpha4(f/f) mice are hematopoietic cell autonomous." Blood **109**(1): 109-11.

We have generated Tie2Cre+alpha4(f/f) mice with documented alpha4-integrin ablation in hematopoietic and endothelial cells. A prominent feature in this model is a sustained, significant increase in circulating progenitors at levels higher than the levels seen with Tie2Cre+VCAM-1(f/f) mice. To test whether phenotypic differences are due to contributions by ligands other than VCAM-1 in bone marrow, or to alpha4-deficient endothelial cells or pericytes, we carried out transplantation experiments using these mice as donors or as recipients. Changes in progenitor biodistribution after transplantation were seen only with alpha4-deficient donor cells, suggesting that these cells were necessary and sufficient to reproduce the phenotype with no discernible contribution by alpha4-deficient nonhematopoietic cells. Because several similarities are seen after transplantation between our results and

those with CXCR4-/- donor cells, the data suggest that VLA4/VCAM-1 and CXCR4/CXCL12 pathways contribute to a nonredundant, ongoing signaling required for bone marrow retention of progenitor cells during homeostasis.

Pruitt, S. C., K. J. Bailey, et al. (2007). "Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer." Stem Cells **25**(12): 3121-32.

Mcm2 is a component of the DNA replication licensing complex that marks DNA replication origins during G1 of the cell cycle for use in the subsequent S-phase. It is expressed in stem/progenitor cells in a variety of regenerative tissues in mammals. Here, we have used the Mcm2 gene to develop a transgenic mouse in which somatic stem/progenitor cells can be genetically modified in the adult. In these mice, a tamoxifen-inducible form of Cre recombinase is integrated 3' to the Mcm2 coding sequence and expressed via an internal ribosome entry site (IRES). Heterozygous Mcm2(IRES-CreERT2/wild-type (wt)) mice are phenotypically indistinguishable from wild-type at least through 1 year of age. In bigenic Mcm2(IRES-CreERT2/wt); Z/EG reporter mice, tamoxifen-dependent enhanced green fluorescence protein expression is inducible in a wide variety of somatic stem cells and their progeny. However, in Mcm2(IRES-CreERT2/IRES-CreERT2) homozygous embryos or mouse embryonic fibroblasts, Mcm2 is reduced to approximately one-third of wild-type levels. Despite the fact that these mice develop normally and are asymptomatic as young adults, life span is greatly reduced, with most surviving to only approximately 10-12 weeks of age. They demonstrate severe deficiencies in the proliferative cell compartments of a variety of tissues, including the subventricular zone of the brain, muscle, and intestinal crypts. However, the immediate cause of death in most of these animals is cancer, where the majority develop lymphomas. These studies directly demonstrate that deficiencies in the function of the core DNA replication machinery that are compatible with development and survival nonetheless result in a chronic phenotype leading to stem cell deficiency in multiple tissues and cancer. Disclosure of potential conflicts of interest is found at the end of this article.

Puglisi, M. A., L. Giuliani, et al. (2008). "Identification and characterization of a novel expandable adult stem/progenitor cell population in the human exocrine pancreas." J Endocrinol Invest **31**(6): 563-72.

It is a general opinion that tissue-specific stem cells are present in adult tissues but their specific properties remain elusive. They are rare in tissues and heterogeneous; in addition, their identification and the characterization of their progeny has encountered technical difficulties. In particular, the existence of pancreatic stem cells remains elusive because specific markers for their identification are not available. We established a method for the isolation of a population of stem/progenitor cells from the human exocrine pancreas, and propose it as a model for other human compact organs. We also used markers that identified and finally characterized these cells. Spheroids with self-replicative potential were obtained from all specimens. The isolated population contained a subset of CD34+ CD45- cells and was able to generate, in appropriate conditions, colonies that produce insulin. We obtained evidence that most freshly isolated spheroids, when co-cultured with the c-kit positive neuroblastoma cell line LAN 5, produced a c-kit positive progeny of cells larger in their cytoplasmic content than the original spheroid population, with elongated morphology resembling the neuronal phenotype. We identified a novel predominant functional type of stem/progenitor cell within the human exocrine pancreas, able to generate insulin-producing cells and potentially non-pancreatic cells.

Pyatt, D. W., W. S. Stillman, et al. (1996). "Reactive oxygen species mediate stem cell factor synergy with granulocyte/macrophage colony-stimulating factor in a subpopulation of primitive murine hematopoietic progenitor cells." Mol Pharmacol **49**(6): 1097-1103.

Reactive oxygen species (ROS) have been shown to stimulate proliferation and growth responses in a variety of mammalian cell types and to act as important mediators in many cellular processes, including hematolymphopoiesis. We examined the effect on primitive murine hematopoietic progenitor cells (HPC) of ROS generated by xanthine plus xanthine oxidase (xanthine/XO) and various antioxidants. Pretreatment of murine HPC (C57BL/6) with xanthine/XO produced a dose-dependent enhancement of clonogenic response to granulocyte/macrophage colony-stimulating factor (GM-CSF) but not to interleukin-3 or granulocyte colony-stimulating factor. Stem cell factor (SCF), a potent comitogen for many hematopoietic growth factors, also synergized with GM-CSF. However, the synergistic enhancement of GM-CSF with xanthine/XO and SCF was not additive, indicating that xanthine/XO and SCF may target the same subpopulation of HPC. Support for this conclusion came from experiments demonstrating that 1) mutant mice strains constitutively lacking a SCF-responsive population of HPC [White spotted (W/WV) and Steel (SI/SId)] are unresponsive to xanthine/XO- and SCF-induced enhancement of GM-CSF and 2) 3,4-epoxybutene, which selectively abrogates SCF

synergy with GM-CSF, inhibits xanthine/XO-induced enhancement. As xanthine/XO can mimic SCF in this population of HPC, the possibility exists that ROS also play a role in normal SCF-mediated proliferation of these cells. To test this hypothesis, we used the antioxidants N-tert-butyl-alpha-phenylnitrone, exogenous superoxide dismutase, and catalase. Both N-tert-butyl-alpha-phenylnitrone and superoxide dismutase effectively inhibited SCF and xanthine/XO synergism with GM-CSF, whereas catalase had no effect, indicating that the superoxide anion may be involved. Also, none of these compounds affected SCF synergism with other hematopoietic growth factors, such as interleukin-3 or granulocyte colony-stimulating factor, suggesting a population-specific phenomenon. These findings indicate that xanthine/XO mimics SCF in stimulating a subpopulation of murine HPC to proliferate and that SCF synergy with GM-CSF in this population is sensitive to antioxidant inhibition.

Qian, H., K. Tryggvason, et al. (2006). "Contribution of alpha6 integrins to hematopoietic stem and progenitor cell homing to bone marrow and collaboration with alpha4 integrins." Blood **107**(9): 3503-10.

The laminin receptor integrin alpha6 chain is ubiquitously expressed in human and mouse hematopoietic stem and progenitor cells. We have studied its role for homing of stem and progenitor cells to mouse hematopoietic tissues in vivo. A function-blocking anti-integrin alpha6 antibody significantly reduced progenitor cell homing to bone marrow (BM) of lethally irradiated mice, with a corresponding retention of progenitors in blood. Remarkably, the anti-integrin alpha6 antibody profoundly inhibited BM homing of long-term multilineage engrafting stem cells, studied by competitive repopulation assay and analysis of donor-derived lymphocytes and myeloid cells in blood 16 weeks after transplantation. A similar profound inhibition of long-term stem cell homing was obtained by using a function-blocking antibody against alpha4 integrin, studied in parallel. Furthermore, the anti-integrin alpha6 and alpha4 antibodies synergistically inhibited homing of short-term repopulating stem cells. Intravenous injection of anti-integrin alpha6 antibodies, in contrast to antibodies against alpha4 integrin, did not mobilize progenitors or enhance cytokine-induced mobilization by G-CSF. Our results provide the first evidence for a distinct functional role of integrin alpha6 receptor during hematopoietic stem and progenitor cell homing and collaboration of alpha6 integrin with alpha4 integrin receptors during homing of short-term stem cells.

Qian, Z., L. Chen, et al. (2008). "A critical role for Apc in hematopoietic stem and progenitor cell survival." J Exp Med **205**(9): 2163-75.

The adenomatous polyposis coli (Apc) tumor suppressor is involved in the initiation and progression of colorectal cancer via regulation of the Wnt signaling cascade. In addition, Apc plays an important role in multiple cellular functions, including cell migration and adhesion, spindle assembly, and chromosome segregation. However, its role during adult hematopoiesis is unknown. We show that conditional inactivation of Apc in vivo dramatically increases apoptosis and enhances cell cycle entry of hematopoietic stem cells (HSCs)/ hematopoietic progenitor cells (HPCs), leading to their rapid disappearance and bone marrow failure. The defect in HSCs/HPCs caused by Apc ablation is cell autonomous. In addition, we found that loss of Apc leads to exhaustion of the myeloid progenitor pool (common myeloid progenitor, granulocyte-monocyte progenitor, and megakaryocyte-erythroid progenitor), as well as the lymphoid-primed multipotent progenitor pool. Down-regulation of the genes encoding Cdkn1a, Cdkn1b, and Mcl1 occurs after acute Apc excision in candidate HSC populations. Together, our data demonstrate that Apc is essential for HSC and HPC maintenance and survival.

Rafii, S. and D. Lyden (2003). "Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration." Nat Med **9**(6): 702-12.

Emerging evidence suggests that bone marrow-derived endothelial, hematopoietic stem and progenitor cells contribute to tissue vascularization during both embryonic and postnatal physiological processes. Recent preclinical and pioneering clinical studies have shown that introduction of bone marrow-derived endothelial and hematopoietic progenitors can restore tissue vascularization after ischemic events in limbs, retina and myocardium. Corecruitment of angiocompetent hematopoietic cells delivering specific angiogenic factors facilitates incorporation of endothelial progenitor cells (EPCs) into newly sprouting blood vessels. Identification of cellular mediators and tissue-specific chemokines, which facilitate selective recruitment of bone marrow-derived stem and progenitor cells to specific organs, will open up new avenues of research to accelerate organ vascularization and regeneration. In addition, identification of factors that promote differentiation of the progenitor cells will permit functional incorporation into neo-vessels of specific tissues while diminishing potential toxicity to other organs. In this review, we discuss the clinical potential of vascular

progenitor and stem cells to restore long-lasting organ vascularization and function.

Raghavachar, A., O. Prummer, et al. (1983). "Progenitor cell (CFUc) reconstitution after autologous stem cell transfusion in lethally irradiated dogs: decreased CFUc populations in blood and bone marrow correlate with the fraction mobilizable by dextran sulphate." Exp Hematol **11**(10): 996-1004.

The present study in dogs concerns the functional potentials of the myeloid progenitor cell system during hemopoietic recovery after lethal total-body irradiation and autotransfusion of cryopreserved stem cells derived from peripheral blood and bone marrow. CFUc were assayed in peripheral blood and bone marrow before and at various intervals after grafting. In addition, the net increase of CFUc mobilizable from extravascular sites into the blood was determined after i.v. injection of 15 mg/kg body weight of dextran sulphate (DS). The data indicate a sustained deficiency of CFUc in otherwise hematologically normal recipients. The DS-response was subnormal even 225 days after transplantation, suggesting residual damage of the hemopoietic system is present for a prolonged period of time. A significant correlation was found between the actual blood CFUc concentration and the numbers of CFUc mobilizable by DS. Insight into progenitor cell kinetics and corresponding changes in the size of a mobilizable pool in extravascular sites may be helpful in estimating the marrow reserve of transplanted individuals suffering myelosuppressive effects of chemotherapy.

Ramirez, M., G. A. Rottman, et al. (1998). "Mature human hematopoietic cells in donor bone marrow complicate interpretation of stem/progenitor cell assays in xenogeneic hematopoietic chimeras." Exp Hematol **26**(4): 332-44.

Xenogeneic hematopoietic chimeras have been used to assay the growth and differentiation of human stem/progenitor cells. The presence of human hematopoietic cells in immunodeficient mice transplanted with human marrow cells may be caused by proliferation and differentiation of early stem/progenitor cells and/or proliferation of mature cells. Unpurified human marrow mononuclear cells, T cell-depleted, or stem/progenitor cell-enriched (CD34+ or CD34+CD38-) populations were injected into sublethally irradiated NOD/LtSz scid/scid (NOD/SCID) mice. High levels of human cells were detected in mice (hu/mu chimeras) transplanted with each of the above human marrow populations. Large numbers of mature human T lymphocytes were found in marrow, spleens, and thymuses from hu/mu chimeras that had been transplanted with unpurified human mononuclear marrow cells. Human immunoglobulin was detected in sera from these chimeras, and some exhibited a clinical syndrome suggestive of graft-versus-host disease. In contrast, in hu/mu chimeras that had received T cell-depleted or stem/progenitor cell-enriched populations, multilineage hematopoiesis (myeloid, B lymphoid, and progenitor cells by immunophenotype) was detected but T lymphocytes and human immunoglobulin were not; in addition, no human cells were detected in the thymuses. Thus, injection of adult human marrow cells into immunodeficient mice can result in hematopoietic chimerism for at least 3 months after transplant. However, the types of cells present in hu/mu chimeras differ depending on the human cell population transplanted. This should be taken into account when hematopoietic chimeras are used to assess human stem/progenitor cell function.

Reddy, G. P., C. Y. Tiarks, et al. (1997). "Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells." Blood **90**(6): 2293-9.

Hematopoietic stem cells purified from mouse bone marrow are quiescent with less than 2% of Lin- Hoechst(low)/Rhodamine(low) (Lin-Ho(low)/Rho(low)) and 10% to 15% of Lin-/Sca+ cells in S phase. These cells enter proliferative cycle and progress through G1 and into S phase in the presence of cytokines and 5% heat-inactivated fetal calf serum (HI-FCS). Cytokine-stimulated Lin-Ho(low)/Rho(low) cells took 36 to 40 hours to complete first division and only 12 hours to complete each of 5 subsequent divisions. These cells require 16 to 18 hours to transit through G0/G1 period and 28 to 30 hours to enter into mid-S phase during the first cycle. Up to 56% of Lin- Rho(low)/Ho(low) cells are high-proliferative potential (7 factor-responsive) colony-forming cells (HPP-CFC). At isolation, HPP-CFC are quiescent, but after 28 to 30 hours of culture, greater than 60% are in S phase. Isoleucine-deprivation of Lin- Ho(low)/Rho(low) cells in S phase of first cycle reversibly blocked them from entering into second cycle. After the release from isoleucine-block, these cells exhibited a G1 period of less than 2 hours and entered into mid-S phase by 12 hours. Thus, the duration of G1 phase of the cells in second cycle is 4 to 5 times shorter than that observed in their first cycle. Similar cell cycle kinetics are observed with Lin-/Sca+ population of bone marrow cells. Stem cell factor (SCF) alone, in the presence of HI-FCS, is as effective as a cocktail of 2 to 7 cytokines in inducing quiescent Lin-/Sca+ cells to enter into proliferative cycle. Aphidicolin treatment reversibly blocked cytokine-stimulated Lin-/Sca+ cells at G1/S boundary, allowing their tight synchrony as they progress

through first S phase and enter into second G1. For these cells also, SCF alone is sufficient for their progression through S phase. These studies indicate a very short G1 phase for stem cells induced to proliferate and offer experimental approaches to synchronize murine hematopoietic stem cells.

Reynolds, S. D., A. Giangreco, et al. (2004). "Airway injury in lung disease pathophysiology: selective depletion of airway stem and progenitor cell pools potentiates lung inflammation and alveolar dysfunction." Am J Physiol Lung Cell Mol Physiol **287**(6): L1256-65.

Identification of early events that contribute to the establishment of chronic lung disease has been complicated by the variable involvement of the airway and alveolar compartments in the complex physiology of end-stage disease. In particular, the impact of airway injury on alveolar integrity and function has not been addressed and would be facilitated by development of animal models of lung disease that specifically target a single cell type within the airway epithelium. We have previously demonstrated that ganciclovir treatment of CCtk transgenic mice, which express the herpes simplex thymidine kinase gene under regulation of the mouse Clara cell secretory protein (CCSP) promoter, results in elimination of the airway progenitor and stem cell pools and a consequent failure of airway regeneration that is associated with rapid morbidity and mortality. In this study, we used the CCtk model to test the hypothesis that selective airway injury initiates profound lung dysfunction through mechanisms that compromise alveolar integrity. Results demonstrate that elimination of the CCSP-expressing cell population results in secondary alveolar inflammation, edema, and depletion of the alveolar type II cell population. On the basis of these data we conclude that selective airway injury can serve as the inciting injury in diseases characterized by severely compromised alveolar function.

Robinson, S., R. van Os, et al. (2000). "Reduction of marrow hematopoietic progenitor and stem cell content is not sufficient for enhanced syngeneic engraftment." Stem Cells **18**(2): 93-101.

The mechanisms regulating long-term engraftment of primitive stem cells are largely unknown. Most conditioning strategies use myeloablative agents for experimental or clinical hematopoietic stem cell transplantation. Host conditioning regimens, in part, have been designed on the assumption that transplanted cells home to specific marrow sites and if these sites are occupied by host stem cells, engraftment will not take place. However, there is now evidence that stable long-term syngeneic engraftment may occur in the absence of host marrow stem cell depletion. To further study the association of engraftment with stem cell depletion, we investigated whether the marked egress of hematopoietic progenitor and stem cells from the marrow into the peripheral blood in C57BL6 mice following a single dose of cyclophosphamide (day 1) and four days of G-CSF (days 3-6) afforded an increased opportunity for long-term syngeneic donor engraftment. During and after mobilization, glucose phosphate isomerase (GPI)-1(b) mice received 30 x 10(6) GPI-1(a) marrow cells without further myeloablation. The level of donor/recipient chimerism was assessed in cell lysates after six months. Increased long-term syngeneic donor engraftment was observed prior to mobilization (before day 6), during a period of active hematopoietic regeneration following the administration of cyclophosphamide. Hematopoietic regeneration was evidenced by a reduced but rapidly increasing marrow cellularity and an increased proportion of hematopoietic progenitors in S-phase. In contrast, long-term syngeneic donor engraftment was not increased over controls during the period of maximum progenitor and stem cell mobilization (after day 5). At this time there were minimal numbers of progenitor and stem cells in the marrow. These data suggest that in the absence of host stem cell ablation, maximal engraftment does not occur during marrow progenitor or stem cell depletion, suggesting that the presence of "open" marrow sites is not a prerequisite for engraftment. The mechanisms for increased engraftment during progenitor cell regeneration following cyclophosphamide need further investigation. Understanding the mechanisms for engraftment without host stem cell ablation may allow strategies for improved long-term engraftment of syngeneic or autologous stem cells with reduced post-transplant toxicity.

Roessger, A., L. Denk, et al. (2009). "Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules." Biomaterials **30**(22): 3723-32.

The cell biological mechanism controlling the regeneration of renal tubules in renal failure after application of stem/progenitor cells is subject of actual research. Unsolved issues are the integration of stem/progenitor cells in a diseased organ environment, the differentiation into epithelial tissue and the formation of tubules in a spatial environment. Following this therapeutic strategy new biomaterials have to be found promoting spatial development of tubules. To obtain new information about the growth of tubules renal stem/progenitor cells from neonatal rabbit kidney were isolated and mounted in a tissue carrier between a selection of commercially available

polyester fleeces. This procedure replaces coating by extracellular matrix proteins and creates an artificial interstitium supporting development of tubules. Perfusion culture was performed with chemically defined IMDM containing aldosterone as tubulogenic factor. Polyester fleeces were investigated by scanning electron microscopy. The spatial development of tubules was registered on whole-mount specimens and on cryosections labeled with SBA and antibodies indicating tubule differentiation. It is found that some polyester fleeces promote the spatial development of tubules between the fibers, whereat each of them produces its individual growth pattern.

Roitbak, T., L. Li, et al. (2008). "Neural stem/progenitor cells promote endothelial cell morphogenesis and protect endothelial cells against ischemia via HIF-1alpha-regulated VEGF signaling." J Cereb Blood Flow Metab **28**(9): 1530-42.

Vascular cells provide a neural stem/progenitor cell (NSPC) niche that regulates expansion and differentiation of NSPCs within the germinal zones of the embryonic and adult brain under both physiologic and pathologic conditions. Here, we examined the NSPC-endothelial cell (NSPC/EC) interaction under conditions of ischemia, both in vitro and after intracerebral transplantation. In culture, embryonic mouse NSPCs supported capillary morphogenesis and protected ECs from cell death induced by serum starvation or by transient oxygen and glucose deprivation (OGD). Neural stem/progenitor cells constitutively expressed hypoxia-inducible factor 1alpha (HIF-1alpha) transcription factor and vascular endothelial growth factor (VEGF), both of which were increased approximately twofold after the exposure of NSPCs to OGD. The protective effects of NSPCs on ECs under conditions of serum starvation and hypoxia were blocked by pharmacological inhibitors of VEGF signaling, SU1498 and Flt-1-Fc. After intracerebral transplantation, NSPCs continued to express HIF-1alpha and VEGF, and promoted microvascular density after focal ischemia. These studies support a role for NSPCs in stabilization of vasculature during ischemia, mediated via HIF-1alpha-VEGF signaling pathways, and suggest therapeutic application of NSPCs to promote revascularization and repair after brain injury.

Roszko, I., P. Faure, et al. (2007). "Stem cell growth becomes predominant while neural plate progenitor pool decreases during spinal cord elongation." Dev Biol **304**(1): 232-45.

The antero-posterior dispersion of clonally related cells is a prominent feature of axis elongation in vertebrate embryos. Two major models have been proposed: (i) the intercalation of cells by convergent-extension and (ii) the sequential production of the forming axis by stem cells. The relative importance of both of these cell behaviors during the long period of elongation is poorly understood. Here, we use a combination of single cell lineage tracing in the mouse embryo, computer modeling and confocal video-microscopy of GFP labeled cells in the chick embryo to address the mechanisms involved in the antero-posterior dispersion of clones. In the mouse embryo, clones appear as clusters of labeled cells separated by intervals of non-labeled cells. The distribution of intervals between clonally related clusters correlates with a statistical model of a stem cell mode of growth only in the posterior spinal cord. A direct comparison with published data in zebrafish suggests that elongation of the anterior spinal cord involves similar intercalation processes in different vertebrate species. Time-lapse analyses of GFP labeled cells in cultured chick embryos suggest a decrease in the size of the neural progenitor pool and indicate that the dispersion of clones involves ordered changes of neighborhood relationships. We propose that a pre-existing stem zone of growth becomes predominant to form the posterior half of the axis. This temporal change in tissue-level motion is discussed in terms of the clonal and genetic continuities during axis elongation.

Rusten, L. S., E. B. Smeland, et al. (1994). "Tumor necrosis factor-alpha inhibits stem cell factor-induced proliferation of human bone marrow progenitor cells in vitro. Role of p55 and p75 tumor necrosis factor receptors." J Clin Invest **94**(1): 165-72.

Stem cell factor (SCF), a key regulator of hematopoiesis, potently synergizes with a number of hematopoietic growth factors. However, little is known about growth factors capable of inhibiting the actions of SCF. TNF-alpha has been shown to act as a bidirectional regulator of myeloid cell proliferation and differentiation. This study was designed to examine interactions between TNF-alpha and SCF. Here, we demonstrate that TNF-alpha potently and directly inhibits SCF-stimulated proliferation of CD34+ hematopoietic progenitor cells. Furthermore, TNF-alpha blocked all colony formation stimulated by SCF in combination with granulocyte colony-stimulating factor (CSF) or CSF-1. The synergistic effect of SCF observed in combination with GM-CSF or IL-3 was also inhibited by TNF-alpha, resulting in colony numbers similar to those obtained in the absence of SCF. These effects of TNF-alpha were mediated through the p55 TNF receptor, whereas little or no inhibition was signaled through the p75 TNF receptor. Finally, TNF-alpha downregulated c-kit cell-surface expression on CD34+ bone marrow cells, and

this was predominantly a p55 TNF receptor-mediated event as well.

Sakaguchi, D. S., S. J. Van Hoffelen, et al. (2004). "Transplantation of neural progenitor cells into the developing retina of the Brazilian opossum: an in vivo system for studying stem/progenitor cell plasticity." Dev Neurosci **26**(5-6): 336-45.

In developing cell transplant strategies to repair the diseased or injured retina is essential to consider host-graft interactions and how they may influence the outcome of the transplants. In the present study we evaluated the influence of the host microenvironment upon neural progenitor cells (NPCs) transplanted into the developing and mature retina of the Brazilian opossum, Monodelphis domestica. Monodelphis pups are born in an extremely immature state and the neonatal pups provide a fetal-like environment in which to study the interactions between host tissues and transplanted NPCs. Three different populations of GFP-expressing NPCs were transplanted by intraocular injection in hosts ranging in age from 5 days postnatal to adult. Extensive survival, differentiation and morphological integration of NPCs were observed within the developing retina. These results suggest that the age of the host environment can strongly influence NPC differentiation and integration.

Sales, V. L., B. A. Mettler, et al. (2007). "Endothelial progenitor and mesenchymal stem cell-derived cells persist in tissue-engineered patch in vivo: application of green and red fluorescent protein-expressing retroviral vector." Tissue Eng **13**(3): 525-35.

An unresolved question regarding tissue-engineered (TE) cardiac valves and vessels is the fate of the transplanted cells in vivo. We have developed a strategy to track the anatomic location of seeded cells within TE constructs and neighboring tissues using a retroviral vector system encoding green and red fluorescent proteins (GFPs and RFPs, respectively) in ovine circulating endothelial progenitor cells (EPCs) and bone marrow-derived mesenchymal stem cells (BMSCs). We demonstrate that stable transduction ex vivo with high-titer Moloney murine leukemia virus-based retroviral vector yields transduction efficiency of greater than 97% GFP(+) EPC- and RFP(+) mesenchymal stem cell (MSC)-derived cells. Cellular phenotype and transgene expression were also maintained through 25 subsequent passages. Using a retroviral vector system to distinguish our pre-seeded cells from tissue-resident progenitor cells and circulating endothelial and marrow-derived precursors, we simultaneously co-seeded 2 x 10(6) GFP(+) EPCs and 2 x 10(5) RFP(+) MSCs onto the TE patches. In a series of ovine pulmonary artery patch augmentation studies, transplanted GFP(+) EPC- and RFP(+) MSC-derived cells persisted within the TE patch 7 to 14 days after implantation, as identified using immunofluorescence. Analysis showed 81% luminal coverage of the TE patches before implantation with transduced cells, increasing to 96% at day 7 and decreasing to 67% at day 14 post-implantation. This suggests a temporal association between retroviral expression of progenitor cells and mediating effects of these cells on the physiological remodeling and maturation of the TE constructs. To our knowledge, this is the first cardiovascular tissue-engineering in vivo study using a double-labeling method to demonstrate a direct evidence of the source, persistence, and incorporation into a TE vascular patch of co-cultured and simultaneously pre-seeded adult progenitor cells.

Salter, A. B., S. K. Meadows, et al. (2009). "Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo." Blood **113**(9): 2104-7.

Hematopoietic stem cells (HSCs) reside in association with bone marrow (BM) sinusoidal vessels in vivo, but the function of BM endothelial cells (ECs) in regulating hematopoiesis is unclear. We hypothesized that hematopoietic regeneration following injury is regulated by BM ECs. BALB/c mice were treated with total body irradiation (TBI) and then infused with C57Bl6-derived endothelial progenitor cells (EPCs) to augment endogenous BM EC activity. TBI caused pronounced disruption of the BM vasculature, BM hypocellularity, ablation of HSCs, and pancytopenia in control mice, whereas irradiated, EPC-treated mice displayed accelerated recovery of BM sinusoidal vessels, BM cellularity, peripheral blood white blood cells (WBCs), neutrophils, and platelets, and a 4.4-fold increase in BM HSCs. Systemic administration of anti-VE-cadherin antibody significantly delayed hematologic recovery in both EPC-treated mice and irradiated, non-EPC-treated mice compared with irradiated controls. These data demonstrate that allogeneic EPC infusions can augment hematopoiesis and suggest a relationship between BM microvascular recovery and hematopoietic reconstitution in vivo.

Sandhu, J. S., P. M. Petkov, et al. (2001). "Stem cell properties and repopulation of the rat liver by fetal liver epithelial progenitor cells." Am J Pathol **159**(4): 1323-34.

The potential of embryonal day (ED) 14 fetal liver epithelial progenitor (FLEP) cells from Fischer (F)344 rats to repopulate the normal and retrorsine-treated liver was studied throughout a 6-month period in syngeneic dipeptidyl peptidase IV (DPPIV-) mutant

F344 rats. In normal liver, FLEP cells formed: 1) hepatocytic clusters ranging in size up to approximately 800 to 1000 cells; 2) bile duct structures connected to pre-existing host bile ducts; and 3) mixed clusters containing both hepatocytes and bile duct epithelial cells. Liver repopulation after 6 months was moderate (5 to 10%). In retrorsine-treated liver, transplanted cells formed large multilobular structures containing both parenchymal and bile duct cells and liver repopulation was extensive (60 to 80%). When the repopulating capacity of ED 14 FLEP cells transplanted into normal liver was compared to adult hepatocytes, three important differences were noted: 1) FLEP cells continued to proliferate at 6 months after transplantation, whereas adult hepatocytes ceased proliferation within the first month; 2) both the number and size of clusters derived from FLEP cells gradually increased throughout time but decreased throughout time with transplanted mature hepatocytes; and 3) FLEP cells differentiated into hepatocytes when engrafted into the liver parenchyma and into bile epithelial cells when engrafted in the vicinity of the host bile ducts, whereas adult hepatocytes did not form bile duct structures. Finally, after transplantation of ED 14 FLEP cells, new clusters of DPPIV+ cells appeared after 4 to 6 months, suggesting reseeding of the liver by transplanted cells. This study represents the first report with an isolated fetal liver epithelial cell fraction in which the cells exhibit properties of tissue-determined stem cells after their transplantation into normal adult liver; namely, bipotency and continued proliferation long after their transplantation.

Sartor, M. M., F. Garvin, et al. (2007). "Failure to achieve a threshold dose of CD34+ CD110+ progenitor cells in the graft predicts delayed platelet engraftment after autologous stem cell transplantation." Bone Marrow Transplant **40**(9): 851-7.

In this study, we retrospectively analysed the utility of CD110 expression on CD34(+) cells as a predictor of delayed platelet transfusion independence in 39 patients who underwent autologous peripheral blood stem cell transplantation. Absolute CD34(+) cells and CD34(+) subsets expressing CD110 were enumerated using flow cytometry. Of the 39 patients, 7 required 21 days or more to achieve platelet transfusion independence. Six of the seven patients received a dose of CD34(+)CD110(+) cells below 6.0 x 10(4)/kg while 30 of 32 patients who achieved platelet transfusion independence in <21 days received a dose of CD34(+)CD110(+) cells >6.0 x 10(4)/kg (P<0.001). Patients with delayed platelet engraftment received a median dose of 5.2 x 10(4) CD34(+)CD110(+) cells/kg compared with a median dose of 16.4 x 10(4) cells/kg for those engrafting within 21 days (P=0.003). Further analysis showed that >6.0 x 10(4) CD34(+)CD110(+) cells/kg was highly sensitive (93.8%) and highly specific (85.7%) for achieving platelet transfusion independence within 21 days. Delay in platelet transfusion independence translated into an increased requirement for platelet transfusion (median 6 vs 2 transfusions, P<0.0001). The dose of CD34(+)/CD110(+) cells/kg infused at time of transplantation appears to be an important factor identifying patients at risk of delayed platelet engraftment.

Satoh, M., H. Sugino, et al. (2000). "Activin promotes astrocytic differentiation of a multipotent neural stem cell line and an astrocyte progenitor cell line from murine central nervous system." Neurosci Lett **284**(3): 143-6.

The effects of activin A were investigated on the development of a multipotent neural stem cell line (MEB5) and an astrocyte progenitor cell line (AP-16) that were established from murine central nervous system (CNS). Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis demonstrated that each cell line expresses both type I and type II activin receptors and signaling molecules for activin, Smad2, Smad3, and Smad4. Activin A did not affect the proliferation of MEB5 and AP-16 cells. When each cell line was treated alone with activin A, glial fibrillary acidic protein (GFAP), a marker for astrocytes, was induced in AP-16 cells, but not in MEB5 cells. However, activin A accelerated the leukemia inhibitory factor (LIF)-induced astroglial differentiation of MEB5 cells. These results suggest that activin promotes astrocyte differentiation of CNS neural progenitors, and the competence to activin is different between multipotent stem cells and unipotent astrocyte progenitor cells.

Schaffer, P., J. A. Gleave, et al. (2008). "Isostructural fluorescent and radioactive probes for monitoring neural stem and progenitor cell transplants." Nucl Med Biol **35**(2): 159-69.

A construct for tagging neurospheres and monitoring cell transplantations was developed using a new technology for producing luminescent and radiolabeled probes that have identical structures. The HIV1-Tat basic domain derivatives NAcGRKKRRQRRR(SAACQ)G (SAACQ-1) and [NAcGRKKRRQRRR(Re(CO)3SAACQ)G]+ (ReSAACQ-1) were prepared in excellent yields using the single amino acid chelate-quinoline (SAACQ) ligand and its Re(I) complex and conventional automated peptide synthesis methods. The distribution of the luminescent Re probe, using epifluorescence microscopy, showed that it localized primarily in the

cell nucleus with a significant degree of association on the nuclear envelope. A smaller amount was found to be dispersed in the cytoplasm. The 99m Tc analogue was then prepared in 43+/-7% (n=12) yield and very high effective specific activity. Following incubation, average uptake of the probe in neurospheres ranged between 10 and 20 Bq/cell. As determined by colorimetric assays, viability for cells labeled with high effective specific activity 99m TcSAACQ-1 was 97+/-4% at 2 h postlabeling and 85+/-25% at 24 h postlabeling for incubation activities ranging from 245 to 8900 Bq/cell. DNA analysis showed that at these levels, there was no significant difference between the extent of DNA damage in the treated cells versus control cells. A series of preliminary SPECT/CT studies of transplants in mice were performed, which showed that the strategy is convenient and feasible and that it is possible to routinely assess procedures noninvasively and determine the number of cells transplanted.

Schaumburg, C., B. A. O'Hara, et al. (2008). "Human embryonic stem cell-derived oligodendrocyte progenitor cells express the serotonin receptor and are susceptible to JC virus infection." J Virol **82**(17): 8896-9.

We studied the susceptibility of human embryonic stem cell-derived oligodendrocyte progenitor cells to infection with JC virus, the causative agent of progressive multifocal leukoencephalopathy (PML). A human embryonic stem cell line, H7, was used to derive an enriched population of cells expressing the oligodendrocyte progenitor cell-specific marker NG2. These cells expressed the 5HT2a receptor (5HT2aR) for JC virus and were highly susceptible to infection. Infection was reduced by treatment with anti-5HT2aR antibodies and by the 5HT2aR antagonists ritanserin and ketanserin. This is the first demonstration that human embryonic stem cell-derived oligodendrocyte progenitor cells are susceptible to JC virus infection and indicates that cells poised to replenish mature oligodendrocytes in PML lesions may also be a target of viral infection.

Schreiber, T. D., C. Steinl, et al. (2009). "The integrin alpha9beta1 on hematopoietic stem and progenitor cells: involvement in cell adhesion, proliferation and differentiation." Haematologica **94**(11): 1493-501.

BACKGROUND: Hematopoietic stem and progenitor cells can interact with their microenvironment via integrins which are adhesion receptors consisting of alpha and beta subunits. Current knowledge suggests that the integrin subunits alpha4 and alpha6 expressed on hematopoietic stem and progenitor cells have distinct roles in retaining stem cells in the bone marrow. The aim of our study was to gain insight into the expression and functions of the integrin subunits alpha7-alpha11 within the endosteal stem cell niche. DESIGN AND METHODS: Human osteoblasts isolated from trabecular bone and hematopoietic stem and progenitor cells purified from umbilical cord blood or bone marrow aspirates were analyzed for the expression of integrin alpha7-alpha11 chains by reverse transcriptase polymerase chain reaction. The involvement of the integrin alpha9beta1 in hematopoietic stem and progenitor cell adhesion, proliferation and differentiation was analyzed in functional assays. RESULTS: Transcripts for all investigated integrin chains were found in primary osteoblasts. Highly purified hematopoietic stem and progenitor cells, however, expressed only transcripts encoding integrin subunits alpha7 and alpha9. Flow cytometric analysis verified extracellular expression of the integrin alpha9beta1 on hematopoietic stem and progenitor cells. Cell-cell adhesion assays with osteoblasts and dye-labeled CD34(+) hematopoietic stem and progenitor cells in the presence of function-blocking antibodies revealed a role of integrin alpha9 in hematopoietic stem and progenitor cell adhesion to osteoblasts. Furthermore, the addition of anti-integrin alpha9 antibodies significantly inhibited proliferation and in vitro differentiation of CD34(+) hematopoietic stem and progenitor cells. CONCLUSIONS: The integrin alpha9beta1 has been identified as a new member of the integrin beta1-subfamily expressed on human hematopoietic stem and progenitor cells. The functional studies strongly suggest that integrin alpha9beta1 contributes to adhesion and differentiation of hematopoietic stem and progenitor cells in the endosteal stem cell niche.

Shapiro, F., T. J. Yao, et al. (1997). "Effects of prior therapy on the in vitro proliferative potential of stem cell factor plus filgrastim-mobilized CD34-positive progenitor cells." Clin Cancer Res **3**(9): 1571-8.

The quantity of hematopoietic progenitors in an apheresis collection is defined by the number of CD34(+) cells or granulocyte macrophage colony-forming units present. These parameters are believed to give roughly equivalent information on graft quality. We here report that the in vitro proliferative potential of r-metHuSCF (stem cell factor) plus filgrastim (granulocyte colony-stimulating factor; r-metHuG-CSF) mobilized peripheral blood (PB) CD34(+) cells obtained from previously heavily treated non-Hodgkin's lymphoma patients inversely correlates with extent of prior therapy. CD34(+) cells were enriched using the CellPro Ceprate system and placed in liquid culture for 4 weeks in the presence of either r-metHuSCF, IL-3, IL-6, filgrastim (S36G), or

S36G plus erythropoietin (S36GE) with a weekly exchange of media and cytokines with reestablishment of culture at the starting cell concentration (Delta assay) and enumeration of progenitors. Starting with 4 x 10(4) CD34(+) cells from apheresis samples from patients who had received <10 cycles of prior chemotherapy, progenitors were detectable in culture at 4 weeks 81% of the time as compared to 14% with CD34(+) cells from patients who had received >10 cycles and 5% for >10 cycles plus radiotherapy. The total number of progenitors generated over the duration of culture (area under the curve) was calculated using the trapezoidal rule as a novel measure of the proliferative potential of the enriched PB CD34(+) cell population. The median area under the curve of CD34(+) cells from patients receiving <10 cycles of prior chemotherapy was 7.4 and 5.7 (x10(5)) using S36G or S36GE, respectively, 1.8 and 1.9 if the patients received >10 cycles of prior chemotherapy, and 1.4 and 1.2 if the patients received >10 cycles of prior chemotherapy plus radiotherapy ($P < 0.001$). These data show that prior therapy impacts on the quality of PB CD34(+) cells as measured by their ability to generate committed progenitors over a number of weeks in liquid culture.

Sharma, A. D., T. Cantz, et al. (2008). "Murine embryonic stem cell-derived hepatic progenitor cells engraft in recipient livers with limited capacity of liver tissue formation." Cell Transplant **17**(3): 313-23.

Directed endodermal differentiation of murine embryonic stem (ES) cells gives rise to a subset of cells with a hepatic phenotype. Such ES cell-derived hepatic progenitor cells (ES-HPC) can acquire features of hepatocytes in vitro, but fail to form substantial hepatocyte clusters in vivo. In this study, we investigated whether this is due to inefficient engraftment or an immature phenotype of ES-HPC. ES cells engrafted into recipient livers of NOD/SCID mice with a similar efficacy as adult hepatocytes after 28 days. Because transplanted unpurified ES-HPC formed teratomas in the spleen and liver, we applied an albumin promoter/enhancer-driven reporter system to purify ES-HPC by cell sorting. RT-PCR analyses for hepatocyte-specific genes showed that the cells exhibited a hepatic phenotype, lacking the expression of the pluripotency marker Oct4, comparable to cells of day 11.5 embryos. Sorted ES-HPC derived from beta-galactosidase transgenic ES cells were injected into fumaryl-acetoacetate-deficient (FAH(-/-)) SCID mice and analyzed after 8 to 12 weeks. Staining with X-gal solution revealed the presence of engrafted cells throughout the liver. However, immunostaining for the FAH protein indicated hepatocyte formation at a very low frequency, without evidence for large hepatocyte cluster formation. In conclusion, the limited repopulation capacity of ES-HPC is not caused by a failure of primary engraftment, but may be due to an immature hepatic phenotype of the transplanted ES-HPC.

Shen, H., M. Boyer, et al. (2008). "Flow cytometry-based cell cycle measurement of mouse hematopoietic stem and progenitor cells." Methods Mol Biol **430**: 77-86.

The balance between the quiescent hematopoietic stem cell (HSC) and the highly proliferative hematopoietic progenitor compartments maintains homeostasis in the hematopoietic system. Therefore, the entry of HSCs into the cell cycle and the rate of proliferation of hematopoietic progenitor cells are fundamental aspects in the field. This chapter describes two intracellular staining methods for DNA and RNA in conjunction with membrane staining for multiple hematopoietic cell-surface markers, and subsequent flow cytometric analysis to determine the cell cycle characteristics of primitive hematopoietic cells. First, the DNA stain Hoechst 33342 and the RNA dye Pyronin Y are used in combination with cell-surface markers to identify the proportion of cells in G(0) and G(1) in hematopoietic stem and progenitor cells. The second details the staining of bromodeoxyuridine incorporated into replicating DNA as a measure for the cycling cell fraction within a specific hematopoietic cell subset.

Shetty, A. K., B. Hattiangady, et al. (2005). "Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes." Glia **51**(3): 173-86.

Dentate neurogenesis, important for learning and memory, declines dramatically by middle age. Although studies have shown that this age-related decrease can be reversed to some extent by exogenous applications of mitogenic factors, it is unclear whether one or more of these factors exhibits decline by middle age. We hypothesize that multiple stem/progenitor cell proliferation factors exhibit early decline during the course of aging in the hippocampus, and some of these declines are linked to age-related alterations in hippocampal astrocytes. We measured the concentrations of fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) in the hippocampus of young, middle-aged, and aged F344 rats, using enzyme-linked immunosorbent assay (ELISA). In addition, we quantified the total number of FGF-2 immunopositive (FGF-2+) and glial fibrillary acidic protein immunopositive (GFAP+) cells in the dentate gyrus and the entire hippocampus. Our results provide new evidence that the

concentrations of FGF-2, IGF-1, and VEGF decline considerably by middle age but remain steady between middle age and old age. Further, decreased concentrations of FGF-2 during aging are associated with decreased numbers of FGF-2+ astrocytes. Quantification of GFAP+ cells, and GFAP and FGF-2 dual immunostaining analyses, reveal that aging does not decrease the total number of astrocytes but fractions of astrocytes that express FGF-2 decline considerably by middle age. Thus, dramatically decreased dentate neurogenesis by middle age is likely linked to reduced concentrations of FGF-2, IGF-1, and VEGF in the hippocampus, as each of these factors can individually influence the proliferation of stem/progenitor cells in the dentate gyrus. Additionally, the results demonstrate that decreased FGF-2 concentration during aging is a consequence of age-related impairment in FGF-2 synthesis by astrocytes.

Shitara, T., H. Ijima, et al. (1994). "Increased cytokine levels and abnormal response of myeloid progenitor cells to granulocyte colony-stimulating factor in a case of severe congenital neutropenia. In vitro effects of stem cell factor." Am J Pediatr Hematol Oncol **16**(2): 167-72.

PURPOSE: The cytokine levels and the in vitro granulopoiesis were studied to evaluate the mechanism of impaired granulopoiesis in severe congenital neutropenia (SCN). PATIENT AND METHODS: The patient was a 5-year-old boy with SCN. We assayed the colony-stimulating activity (CSA) produced by peripheral blood (PB) cells from the patient. The plasma levels of cytokines were measured using enzyme immunoassay. These included granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 1 alpha (IL-1 alpha), IL-1 beta, IL-2, IL-3, IL-4, IL-6, and tumor necrosis factor-alpha. The effects of IL-3 and stem cell factor (SCF) on the proliferation of granulocyte-macrophage colony-forming cells (GM-CFCs) were studied. RESULTS: CSA produced by PB cells from the patient was almost the same as in the healthy control. The level of endogenous G-CSF was elevated to 334 pg/ml, and GM-CSF, IL-2, IL-3, and IL-6 were slightly elevated. The numbers of GM-CFCs were markedly depressed in the presence of G-CSF alone and showed no increment on additional stimulation by IL-3. SCF in combination with G-CSF significantly augmented the proliferation of GM-CFCs. CONCLUSIONS: These findings suggest that some cytokines including G-CSF may be elevated in SCN patients and that CSF may play an important role in the pathogenesis of SCN.

Shlebak, A. A., I. A. Roberts, et al. (1998). "The impact of antenatal and perinatal variables on cord blood haemopoietic stem/progenitor cell yield available for transplantation." Br J Haematol **103**(4): 1167-71.

We investigated the impact of maternal and fetal variables on cord blood (CB) haemopoietic stem/progenitor cell content. These included maternal age, ethnic origin, parity, ABO and Rhesus D blood group, antenatal haemoglobin, alcohol and cigarette consumption at time of registration, mode of delivery, duration of the first and second stages of labour, gestational age, birth weight, cord pH and cord erythrocyte mean cell volume (MCV). Cord volumes and total nucleated cellularities (TNC) were recorded, the colony assay for granulocyte-macrophage colony-forming-cells (CFU-GM) was used to quantify the progenitor cells and the potential of CFU-GM to produce secondary colonies on replating was used as a measure of progenitor cell quality. We found: (1) significantly greater (P=0.04) volumes were collected from babies who weighed > or = 2.5kg versus babies with a birth weight <2.5kg; (2) significantly greater numbers of mononuclear cells (MNC) from mothers who drank 0-3 units versus those who drank > or = 4 units of alcohol weekly (P=0.03), and in babies with a cord pH < or = 7.1 v > 7.1 (P=0.02); (3) Significantly greater numbers of cord CFU-GM in mothers who drank 0-3 v > or = 4 units weekly (P=0.004) and smokers of > or = 10 v 0-9 cigarettes daily (P=0.02) and in spontaneous vaginal deliveries than assisted vaginal and caesarean deliveries (P=0.04), and (4) the potential of CFU-GM to produce secondary colonies was significantly greater in CB derived from Caucasians than from non-Caucasians (P=0.02); in assisted vaginal delivery v spontaneous vaginal (P=0.02) and in deliveries with prolonged first stage of labour v short first stage of labour (P=0.04). We conclude that antenatal and perinatal variables may influence the CB stem/progenitor cell yield available for transplantation.

Sirko, S., A. von Holst, et al. (2007). "Chondroitin sulfate glycosaminoglycans control proliferation, radial glia cell differentiation and neurogenesis in neural stem/progenitor cells." Development **134**(15): 2727-38.

Although the local environment is known to regulate neural stem cell (NSC) maintenance in the central nervous system, little is known about the molecular identity of the signals involved. Chondroitin sulfate proteoglycans (CSPGs) are enriched in the growth environment of NSCs both during development and in the adult NSC niche. In order to gather insight into potential biological roles of CSPGs for NSCs, the enzyme chondroitinase ABC

(ChABC) was used to selectively degrade the CSPG glycosaminoglycans. When NSCs from mouse E13 telencephalon were cultivated as neurospheres, treatment with ChABC resulted in diminished cell proliferation and impaired neuronal differentiation, with a converse increase in astrocytes. The intrauterine injection of ChABC into the telencephalic ventricle at midneurogenesis caused a reduction in cell proliferation in the ventricular zone and a diminution of self-renewing radial glia, as revealed by the neurosphere-formation assay, and a reduction in neurogenesis. These observations suggest that CSPGs regulate neural stem/progenitor cell proliferation and intervene in fate decisions between the neuronal and glial lineage.

Sitnicka, E., D. Bryder, et al. (2002). "Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool." Immunity **17**(4): 463-72.

The first lineage commitment step of hematopoietic stem cells (HSC) results in separation into distinct lymphoid and myeloid differentiation pathways, reflected in the generation of common lymphoid and myeloid progenitors (CLP and CMP, respectively). In this report we present the first evidence for a nonredundant regulator of this process, in that adult mice deficient in expression of the flt3 ligand (FL) have severely (10-fold) reduced levels of the CLP, accompanied by reductions in the earliest identifiable B and T cell progenitors. In contrast, CMP and HSC are unaffected in FL-deficient mice. Noteworthy, CLP express high levels of both the flt3 receptor and ligand, indicating a potential autocrine role of FL in regulation of the earliest lymphoid commitment step from HSC.

Slovak, M. L., S. T. Traweek, et al. (1995). "Trisomy 11: an association with stem/progenitor cell immunophenotype." Br J Haematol **90**(2): 266-73.

The clinicopathological features and the prognostic significance of acute myeloid leukaemia (AML) with trisomy 11 are currently unknown. In this study we describe 15 adult AML cases with trisomy 11. Trisomy 11 was the sole chromosomal anomaly in eight cases; the remaining seven cases were characterized by +11 in association with other karyotypic aberrations. Patients ages ranged from 34 to 79 years. 12 patients were male; three were female. Although there was no correlation of trisomy 11 with any specific FAB subgroup [M2 (n = 7), M1 (n = 5), M4/5 (n = 2), M3 (n = 1)] less mature forms predominated. Immunologically, the leukaemic blasts showed a strikingly consistent stem cell phenotype with expression of HLA-DR, CD34 and the myeloid antigens (CD15, CD33 and/or CD13). In addition, two cases expressed the B-cell associated antigen CD19. The presence of trilineage dysplasia, suggesting the presence of an underlying myelodysplasia (MDS), was observed at presentation in five cases; in another case MDS was evident at relapse only. Unexpectedly, MLL gene rearrangements were observed in two of four cases characterized by trisomy 11 as the sole karyotypic abnormality; however, MLL aberrations were not identified in three cases with trisomy 11 accompanied by other karyotypic anomalies. The majority of patients in each subgroup (i.e. those with and without additional cytogenetic abnormalities) achieved a short first complete remission (CR) (mean 8 months) and failed to obtain a second CR. Only one patient in each trisomy 11 subgroup is in a continuous CR for > 34 months. These findings suggest that trisomy 11 leukaemia is characterized by a stem/progenitor cell immunophenotype with poor response to standard chemotherapeutic regimens and an unfavourable prognosis.

Smalley, M. J. and R. B. Clarke (2005). "The mammary gland "side population": a putative stem/progenitor cell marker?" J Mammary Gland Biol Neoplasia **10**(1): 37-47.

Hematopoietic Stem Cells have been isolated by their ability to pump out Hoechst 33342 dye and form a distinct population definable by flow cytometry--the Side Population (SP). The membrane pump Bcrp has been identified as the molecular determinant of the SP phenotype. An SP population with Bcrp activity has been defined in a number of tissues, including mouse mammary and human breast epithelium, and it has been proposed that the SP phenotype is a universal stem cell marker. Studies of mouse and human breast SP suggest that the population is undifferentiated but capable of differentiating into epithelial structures of both luminal and myoepithelial lineages both in vitro and in vivo. However, evidence that the SP is enriched for stem cells is, at the moment, only correlative, and there are potentially confounding technical issues. We still await formal proof that the SP contains a stem cell population.

Smit, W. M., M. Rijnbeek, et al. (1998). "T cells recognizing leukemic CD34(+) progenitor cells mediate the antileukemic effect of donor lymphocyte infusions for relapsed chronic myeloid leukemia after allogeneic stem cell transplantation." Proc Natl Acad Sci U S A **95**(17): 10152-7.

Adoptive immunotherapy with donor lymphocyte infusions (DLI) is an effective treatment for relapsed chronic myeloid leukemia (CML) after allogeneic stem cell transplantation. To identify the

effector and target cell populations responsible for the elimination of the leukemic cells in vivo we developed an assay to measure the frequency of T lymphocyte precursor cells capable of suppressing leukemic progenitor cells. Target cells in this assay were CML cells that were cultured in the presence of stem cell factor, interleukin 3, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and erythropoietin. [3H]thymidine incorporation at day 7 represented the proliferation of the progeny of the CD34(+) CML progenitor cells, and not of the more mature CD34(-) CML cells. Effector cells were mononuclear cells, which were used in a limiting dilution analysis to measure the frequencies of CML progenitor cell-inhibitory lymphocyte precursors (PCILp) in peripheral blood of seven patients before and after DLI for relapsed CML. In the six patients who entered complete remission, a 5- to 100-fold increase of PCILp was found during the clinical response. In the patient with resistant relapse the frequency of PCILp was <10 per ml before and after DLI. Leukemia-reactive helper T lymphocyte precursor frequencies remained unchanged after DLI. A significant increase in cytotoxic T lymphocyte precursor frequency against more mature leukemic cells was found in only two responding patients. These results indicate that T cells specifically directed against CD34(+) CML progenitor cells mediate the antileukemic effect of DLI.

Snowden, J. A., V. Nink, et al. (1998). "Composition and function of peripheral blood stem and progenitor cell harvests from patients with severe active rheumatoid arthritis." Br J Haematol **103**(3): 601-9.

High-dose chemotherapy with autologous stem cell rescue has been proposed as an intensive therapy for severe rheumatoid arthritis (RA). In view of previous observations of abnormal haemopoiesis in RA patients, the composition and function of peripheral blood stem cell harvests (PBSCH) was investigated. Compared with PBSCH from healthy allogeneic donors mobilized with the same dose of G-CSF (filgrastim; 10 microg/kg/d, n = 14), RA PBSCH (n = 9) contained significantly fewer mononuclear cells (375 v 569 x 10(6)/kg, P = 0.03) and CD34+ cells (2.7 v 5.8 x 10(6)/kg, P = 0.003). However, there were increased proportions of CD14+ cells (P = 0.006) and CD14+ CD15+ cells (the phenotype of previously described 'abnormal' myeloid cells, P = 0.002) in the RA PBSCH which translated into 3.5- and 7-fold increases respectively on a per CD34+ cell basis. There were no differences in T-cell activation status as judged by proportions of CD4+ and CD8+ expressing CD45RA, CD45RO, HLA-DR and CD28 (RA PBSCH, n = 7, donor PBSCH, n = 5, P = 0.2-0.7). Phytohaemagglutinin responses determined fluorocytometrically with induction of CD69 expression were reduced in CD4+ and CD8+ cells following filgrastim administration in 3/3 RA patients tested. Compared with bone marrow as a potential source of CD34+ cells, PBSCH contained 11-fold more T cells (P < 0.0005), 8-fold more B cells (P < 0.0005) and 4-fold more monocytes (P = 0.02). In short-term methylcellulose culture there were no differences in colony counts (CFU-GM, CFU-GEMM, BFU-E) per CD34+ cell from PBSCH from RA patients (n = 11) and healthy donors (n = 10). Long-term culture initiator cells were cultured successfully from cryopreserved PBSCH from RA patients (n = 9). In conclusion, PBSCH from RA patients differed significantly in composition from normal individuals, but in vitro studies support normal stem and progenitor cell function. Changes in T-cell function occur during mobilization in RA patients. This work provides reassurance for the use of PBSCH as haematological rescue and baseline data for clinical trials of graft manipulation strategies in patients with RA.

Strohsnitter, W. C., T. M. Savarese, et al. (2008). "Correlation of umbilical cord blood haematopoietic stem and progenitor cell levels with birth weight: implications for a prenatal influence on cancer risk." Br J Cancer **98**(3): 660-3.

We examined the relation with birth weight and umbilical cord blood concentrations of haematopoietic stem and progenitor populations in 288 singleton infants. Across the whole range of birth weight, there was a positive relation between birth weight and CD34+CD38(-) cells, with each 500 g increase in birth weight being associated with a 15.5% higher (95% confidence interval: 1.6-31.3%) cell concentration. CD34+ and CD34+c-kit+ cells had J-shaped relations and CFU-GM cells had a U-shaped relation with birth weight. Among newborns with >or=3000 g birth weights, concentrations of these cells increased with birth weight, while those below 3000 g had higher stem cell concentrations than the reference category of 3000-3499 g. Adjustment for cord blood plasma insulin-like growth factor-1 levels weakened the stem and progenitor cell-birth weight associations. The positive associations between birth weight and stem cell measurements for term newborns with a normal-to-high birth weight support the stem cell burden hypothesis of cancer risk.

Stuart, S. A., Y. Minami, et al. (2009). "The CML stem cell: evolution of the progenitor." Cell Cycle **8**(9): 1338-43.

The success of imatinib mesylate (STI571, Gleevec) in treating chronic myeloid leukemia (CML) is, to date, the crowning achievement of targeted

molecular therapy in cancer. Nearly 90% of newly diagnosed patients treated with imatinib in the chronic phase of the disease achieve a complete cytogenetic response. However, more than 95% of these patients retain detectable levels of BCR-ABL mRNA and patients discontinuing imatinib therapy almost invariably relapse, demonstrating that an imatinib insensitive population of leukemia-initiating cells (LICs) persists in nearly all patients. These findings underscore the need for treatments specifically targeting the leukemia-initiating population of CML cells. While mounting evidence suggests that the LIC in the chronic phase of CML is the BCR-ABL positive hematopoietic stem cell, several recent publications suggest that during CML blast crisis, a granulocyte-macrophage progenitor (GMP) population also acquires LIC properties through activation of the beta-catenin pathway. Characterization of these cells and evaluation of their sensitivity to imatinib is critical to our understanding and treatment of CML blast crisis.

Suetake, K., S. S. Liour, et al. (2003). "Expression of gangliosides in an immortalized neural progenitor/stem cell line." J Neurosci Res **74**(5): 769-76.

Glycosphingolipids (GSLs) are known to play important roles in cellular growth and differentiation in the nervous system. The change in expression of gangliosides is correlated with crucial developmental events and is evolutionarily conserved among many vertebrate species. The emergence of neural progenitors represents a crucial step in neural development, but little is known about the exact composition and subcellular localization of gangliosides in neural progenitor cells. The C17.2 cell line was derived after v-myc transformation of neural progenitor cells isolated from neonatal mouse cerebellar cortex. The developmental potential of C17.2 cells is similar to that of endogenous neural progenitor/stem cells in that they are multipotential and capable of differentiating into all neural cell types. We characterized the GSL composition of C17.2 cells and found the presence of only a-series gangliosides. Subcellular localization studies revealed that GM1 and GD1a are localized mainly on the plasma membrane and partly in the cytoplasm, both as punctate clusters. Reverse transcription-polymerase chain reaction revealed the absence of ST-II transcripts in C17 cells, which most likely accounts for the lack of expression of b- and c-series complex gangliosides in this cell line. These data suggest that the divergence in ganglioside expression in C17.2 cells is regulated at the transcriptional level.

Sui, X., S. B. Krantz, et al. (2000). "Stem cell factor and erythropoietin inhibit apoptosis of human erythroid progenitor cells through different signalling pathways." Br J Haematol **110**(1): 63-70.

Erythropoietin (EPO) and stem cell factor (SCF) are two important factors in human erythropoiesis. We have recently demonstrated that SCF and EPO synergistically activate mitogen-activated protein (MAP) kinase, thereby promoting growth of human erythroid colony-forming cells (ECFCs). In the present study, we have examined the intracellular mechanisms by which SCF and EPO maintain survival of these cells. In the absence of SCF and EPO, human ECFCs underwent rapid apoptosis. The process was significantly inhibited by addition of a single factor and was totally prevented in the presence of both factors. Treatment of ECFCs with wortmannin, a specific inhibitor of phosphoinositide 3-kinase (PI3K), inhibited the antiapoptotic effect of SCF but had no effect on that of EPO, indicating that SCF but not EPO inhibits apoptosis through the PI3K pathway. In contrast, treatment of ECFCs with PD98059, a specific inhibitor of MAP kinase/ERK kinase (MEK), inhibited cell growth but had no effect on the antiapoptotic activity of either SCF or EPO, suggesting that SCF and EPO prevent apoptosis of human ECFCs independent of the extracellular signal-regulated kinase (ERK) pathway. Interestingly, both EPO and SCF induced activation of PI3K. However, through PI3K, SCF caused activation of protein kinase B (PKB), an anti-apoptosis signal, whereas EPO led to activation of ERKs. Furthermore, the SCF- and EPO-maintained expression of antiapoptotic protein Bcl-XL was correlated with the activation of ERKs and was inhibited by PD98059, suggesting that Bcl-XL may not have a major role in preventing apoptosis of human ECFCs. Phosphorylated BAD was not affected by SCF, EPO or wortmannin. Taken together with our previous results, the present study indicates that SCF and EPO support survival and growth of human ECFCs through different signalling pathways and that they transduce distinctly different signals through activation of PI3K.

Summerfield, A., M. P. Horn, et al. (2003). "C-kit positive porcine bone marrow progenitor cells identified and enriched using recombinant stem cell factor." J Immunol Methods **280**(1-2): 113-23.

Porcine haematological studies have been hampered by the lack of monoclonal antibodies against porcine CD34 or CD117 expressed on haematological progenitors. The present report describes the enumeration, phenotyping and isolation of porcine haematopoietic progenitor cells expressing stem cell factor (SCF, c-kit ligand) receptor (c-kit, CD117). Recombinant porcine (rp) SCF and granulocyte-macrophage colony-stimulating factor (GM-CSF) were expressed in the mammalian

HEK293 cell-based expression system. Both were biologically active and induced the proliferation of the human erythroleukemic cell line TF-1, as well as of porcine bone marrow haematopoietic cells (BMHC), in a concentration-dependent manner. The effect of rpSCF on BMHC proliferation was synergistic with rpGM-CSF. Furthermore, rpSCF had a synergistic effect on the generation of BMHC-derived dendritic cells (DC) induced by GM-CSF and TNF-alpha. RpSCF was expressed with a 6-histidine epitope, permitting both its purification and immunological detection. Binding studies with BMHC demonstrated ligation of SCF to 4-11% of BMHC. These cells represented the SWC3(low/-)SWC8- BMHC subset, with characteristics of immature proliferative progenitor BMHC. In contrast, no expression was noted on the SWC3+SWC8- monocytic, the SWC3+SWC8+ granulocytic or the SWC3-SWC8+ B cell lineage cells. Using magnetic or fluorescence-activated cell sorting, SCF-ligating BMHC were enriched for pluripotent progenitor cells. In this manner, porcine haematological studies can be pursued in a detailed manner not before possible.

Sussman, M. (2007). ""AKT"ing lessons for stem cells: regulation of cardiac myocyte and progenitor cell proliferation." Trends Cardiovasc Med **17**(7): 235-40.

Cardiac development and postnatal growth depend on activation of AKT, but initial strategies to improve myocardial repair using AKT were stymied by undesirable corollary alterations in myocardial structure and function. These unfortunate precedents were based on high-level expression of constitutively activated AKT, predominantly in the cytoplasm of the cell. Based on subsequent studies establishing that activated AKT accumulates in the nucleus, we reasoned that the location of AKT, not simply the activity level, would be a critical determinant of the phenotypic outcome resulting from AKT activation. Using myocardial-specific expression of nuclear-targeted AKT (AKT/nuc), the proliferation of myocardial stem and progenitor cell populations is enhanced, casting new light on the implementation of AKT activity as a molecular interventional approach for treatment of cardiomyopathic damage resulting from acute injury, chronic stress, or the debilitating changes of aging.

Sutton, R. E., M. J. Reitsma, et al. (1999). "Transduction of human progenitor hematopoietic stem cells by human immunodeficiency virus type 1-based vectors is cell cycle dependent." J Virol **73**(5): 3649-60.

Human immunodeficiency virus (HIV) type 1 vectors are highly efficient in their ability to transduce human progenitor hematopoietic stem cells (PHSC). Although mitosis was not required for transduction of these cells, transduction rates were much greater once cells had been cultured in the presence of cytokines. Transduction rates, however, rarely exceeded 70%. We demonstrate here that there is a distinct subpopulation that is more easily transduced by HIV vectors. These cells were distinguished by a disproportionate population in the S/G2/M phases of the cell cycle. By sorting them prior to transduction, we found that those cells in either the G1 or S/G2/M fraction were more readily transduced than G0 cells. Maintaining the cells in G0 by omitting cytokines from the medium reduced transduction rates by up to 10-fold. Addition of cytokines to the medium immediately after transduction did not improve the transduction efficiency as measured by expression of the transgene. Analysis of replication intermediates indicated that the block to transduction of G0 cells operated near the time of initiation of reverse transcription. These results suggest that although lentivirus vectors can transduce nondividing PHSC, transduction efficiency is severalfold greater once the cells exit G0 and enter G1. Further characterization of these more transducible cells and identification of the cellular factors responsible may enhance transduction while maintaining the pluripotentiality of the PHSC.

Suzuki, A., Y. W. Zheng, et al. (2001). "Clonal expansion of hepatic stem/progenitor cells following flow cytometric cell sorting." Cell Transplant **10**(4-5): 393-6.

Although hepatic stem cells are believed to exist and play a critical role in developing and regenerating liver, little is known about their cell surface specificity or differentiation capabilities. To make prospective studies of hepatic stem cells possible, we established an in vitro culture system for identification and characterization of hepatic stem/progenitor cells. By combining this culture system with fluorescence activated cell sorting (FACS), a population of cells that were capable of forming large colonies and providing their descendants for relative longer period was isolated from fetal mouse livers. These data suggest that hepatic stem/progenitor cells with high proliferative potential are existent in the developing mouse liver, and that they are enriched by using flow cytometry.

Suzuki, H., T. Watabe, et al. (2005). "Roles of vascular endothelial growth factor receptor 3 signaling in differentiation of mouse embryonic stem cell-derived vascular progenitor cells into endothelial cells." Blood **105**(6): 2372-9.

Vascular endothelial growth factor receptor 2 (VEGFR2/Flk-1)-positive cells derived from

embryonic stem (ES) cells serve as vascular progenitors, which differentiate into endothelial cells (ECs) in the presence of VEGF-A. VEGFR3/Flt-4 (fms-like tyrosine kinase 4) signaling is known to be important for the development of lymphatic endothelial cells (LECs). To elucidate the roles of VEGFR3 signaling in the differentiation of vascular progenitor cells into ECs, we introduced various types of VEGFR3 cDNAs into mouse ES cells. VEGF-C, a ligand for VEGFR2 and VEGFR3, stimulated the endothelial differentiation of the VEGFR2+ cells transfected with the VEGFR3 cDNA but not those transfected with kinase-negative mutants of VEGFR3. The VEGFR3-transfected ECs exhibited high expression levels of lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), one of the markers of LECs, and showed efficient binding of hyaluronan. VEGF-C(C152S), which is able to activate VEGFR3 but not VEGFR2, failed to induce the endothelial differentiation of mock- and VEGFR3-transfected VEGFR2(+) cells, suggesting the essential role of VEGFR2 signaling for endothelial differentiation. Furthermore, kinase-negative mutants of VEGFR3 prevented the VEGF-C-mediated endothelial differentiation of the vascular progenitor cells. Thus, VEGFR2 signaling is required for the endothelial differentiation of mouse ES cells induced by VEGF-C, and VEGFR3 signaling may confer lymphatic endothelial-like phenotypes to ECs.

Szilvassy, S. J. (2006). "Haematopoietic stem and progenitor cell-targeted therapies for thrombocytopenia." Expert Opin Biol Ther **6**(10): 983-92.

This review discusses the present outlook for new thrombocytopenia therapies that induce haematopoietic stem and progenitor cells to proliferate, differentiate and produce functional platelets. A brief overview of megakaryopoiesis and its regulation by thrombopoietin (TPO) is followed by a discussion of how early experience with recombinant TPO therapies stimulated the search for novel TPO receptor ligands. A summary is then provided of the results of Phase I clinical trials with the new small molecule and peptide TPO mimetics that are in development at present. Finally, recent developments in the ex vivo expansion of primitive haematopoietic cells and the potential enhancement of cell-based therapies by haematopoietic growth factors in vivo are briefly summarised as part of a look towards the future.

Szotek, P. P., H. L. Chang, et al. (2008). "Normal ovarian surface epithelial label-retaining cells exhibit stem/progenitor cell characteristics." Proc Natl Acad Sci U S A **105**(34): 12469-73.

Ovulation induces cyclic rupture and regenerative repair of the ovarian coelomic epithelium. This process of repeated disruption and repair accompanied by complex remodeling typifies a somatic stem/progenitor cell-mediated process. Using BrdU incorporation and doxycycline inducible histone2B-green fluorescent protein pulse-chase techniques, we identify a label-retaining cell population in the coelomic epithelium of the adult mouse ovary as candidate somatic stem/progenitor cells. The identified population exhibits quiescence with asymmetric label retention, functional response to estrous cycling in vivo by proliferation, enhanced growth characteristics by in vitro colony formation, and cytoprotective mechanisms by enrichment for the side population. Together, these characteristics identify the label-retaining cell population as a candidate for the putative somatic stem/progenitor cells of the coelomic epithelium of the mouse ovary.

Takami, A., S. Nakao, et al. (1999). "Impaired response of granulocyte-committed progenitor cells to stem cell factor and granulocyte colony-stimulating factor in human cyclic neutropenia." Ann Hematol **78**(4): 197-9.

Although cyclic neutropenia (CN) has been the subject of extensive studies due to its striking clinical picture, the abnormality of hematopoietic progenitor cells in patients with CN has been poorly defined. We studied the sensitivity of progenitor cells of a CN patient to colony-stimulating factors (CSF) including G-CSF, interleukin-3 (IL-3), and stem cell factor (SCF). Peripheral blood progenitor cells of the patient required a significantly higher dose of G-CSF to give rise to colonies than those of normal controls. While the presence of SCF enhanced the number of G-CSF-induced colonies regardless of the concentration of G-CSF in normal controls, this synergistic effect of SCF was limited to the high concentration of G-CSF in the patient, indicating that the abnormality in hematopoiesis in CN involved more immature progenitor cells responsive to SCF.

Tang, B., N. Yoo, et al. (2007). "Transforming growth factor-beta can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model." Cancer Res **67**(18): 8643-52.

The transforming growth factor-beta (TGF-beta) pathway has tumor-suppressor activity in many epithelial tissues. Because TGF-beta is a potent inhibitor of epithelial cell proliferation, it has been widely assumed that this property underlies the tumor-suppressor effect. Here, we have used a xenograft model of breast cancer to show that endogenous TGF-beta has the potential to suppress tumorigenesis

through a novel mechanism, involving effects at two distinct levels in the hierarchy of cellular progeny that make up the epithelial component of the tumor. First, TGF-beta reduces the size of the putative cancer stem or early progenitor cell population, and second it promotes differentiation of a more committed, but highly proliferative, progenitor cell population to an intrinsically less proliferative state. We further show that reduced expression of the type II TGF-beta receptor correlates with loss of luminal differentiation in a clinical breast cancer cohort, suggesting that this mechanism may be clinically relevant. At a molecular level, the induction of differentiation by TGF-beta involves down-regulation of Id1, and forced overexpression of Id1 can promote tumorigenesis despite persistence of the antiproliferative effect of TGF-beta. These data suggest new roles for the TGF-beta pathway in regulating tumor cell dynamics that are independent of direct effects on proliferation.

Toda, H., M. Tsuji, et al. (2003). "Stem cell-derived neural stem/progenitor cell supporting factor is an autocrine/paracrine survival factor for adult neural stem/progenitor cells." J Biol Chem **278**(37): 35491-500.

Recent evidence suggests that adult neural stem/progenitor cells (ANSCs) secrete autocrine/paracrine factors and that these intrinsic factors are involved in the maintenance of adult neurogenesis. We identified a novel secretory molecule, stem cell-derived neural stem/progenitor cell supporting factor (SDNSF), from adult hippocampal neural stem/progenitor cells by using the signal sequence trap method. The expression of SDNSF in adult central nervous system was localized to hippocampus including dentate gyrus, where the neurogenesis persists throughout life. In induced neurogenesis status seen in ischemically treated hippocampus, the expression of SDNSF was up-regulated. As functional aspects, SDNSF protein provided a dose-dependent survival effect for ANSC following basic fibroblast growth factor 2 (FGF-2) withdrawal. ANSCs treated by SDNSF also retain self-renewal potential and multipotency in the absence of FGF-2. However, SDNSF did not have mitogenic activity, nor was it a cofactor that promoted the mitogenic effects of FGF-2. These data suggested an important role of SDNSF as an autocrine/paracrine factor in maintaining stem cell potential and lifelong neurogenesis in adult central nervous system.

Tokar, E. J., B. B. Ancrile, et al. (2005). "Stem/progenitor and intermediate cell types and the origin of human prostate cancer." Differentiation **73**(9-10): 463-73.

Theories of cell lineage in human prostatic epithelium, based on protein expression, propose that basal and luminal cells: 1) are either independently capable of self-renewal or 2) arise from stem cells expressing a full spectrum of proteins (p63, cytokeratins CK5/14, CK8/18, and glutathione-S-transferase-pi [GST-pi]) similar to cells of the embryonic urogenital sinus (UGS). Such embryonic-like stem cells are thought to give rise to mature basal cells and secretory luminal cells. By single cell cloning of an immortalized, normal human prostate-derived, non-tumorigenic RWPE-1 cell line, we isolated and characterized two epithelial cell types, WPE-stem and WPE-int. WPE-stem cells show: i) strong, sixfold greater nuclear expression of p63; ii) nearly twofold greater expression of CK14; iii) threefold less CK18, and iv) low androgen receptor (AR) expression as compared with WPE-int cells. WPE-stem cells are androgen-independent for growth and survival. WPE-int cells express very low p63 and CK5/14, and high CK18. WPE-int cells are androgen-independent for growth and survival but are highly responsive as shown by androgen induction of AR and prostate specific antigen (PSA). Compared with WPE-int cells, WPE-stem cells are smaller and show more rapid growth. WPE-stem cells can grow in an anchorage-independent manner in agar with 4.5-fold greater cloning efficiency and as free floating "prostaspheres" in liquid medium; and express over 40-fold higher matrix metalloproteinase-2 activity. These results indicate that WPE-stem cells express several features characteristic of stem/progenitor cells present in the UGS and in adult prostatic epithelium. In contrast, WPE-int cells have an intermediate, committed phenotype on the pathway to luminal cell differentiation. We propose that in normal prostatic epithelium, cells exist at many stages in a continuum of differentiation progressing from stem cells to definitive basal and luminal cells. Establishment and characterization of clones of human prostatic epithelial cells provide novel models for determining cell lineages, the origin of prostate cancer, and for developing new strategies for tumor prevention and treatment.

Torella, D., G. M. Ellison, et al. (2005). "Cardiac stem and progenitor cell biology for regenerative medicine." Trends Cardiovasc Med **15**(6): 229-36.

Stem cell therapy is a new and promising treatment of heart disease. However, the race is still on to find the "best" cell to reconstitute the myocardium and improve function after myocardial damage. The recent discovery in the adult mammalian myocardium of a small cell population with the phenotype, behavior, and regenerative potential of cardiac stem and progenitor cells has proposed these

cells as the most appropriate for cell therapy. The existence of these cells has provided an explanation for the hitherto unexplained existence of a subpopulation of immature cycling myocytes in the adult myocardium. These findings have placed the heart squarely among other organs with regenerative potential despite the fact that the working myocardium is mainly constituted of terminally differentiated cells. Although CSCs (cardiac cells proven to have stem and/or progenitor characteristics) can be isolated and amplified in vitro or stimulated to differentiate in situ, it has become reasonable to exploit this endogenous regenerative potential to replace the lost muscle with autologous functional myocardium. Therefore, it is imperative to obtain a better understanding of the biology and regenerative potential of the endogenous CSCs. This will enable us to design better protocols for the regeneration of functional contractile mass after myocardial injury.

Torres, A., J. Sanchez, et al. (2001). "Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation." Haematologica **86**(1): 24-9.

BACKGROUND AND OBJECTIVES: Hematopoietic restoration after marrow ablation is initiated by the erythroid compartment. However, the absolute microscope counts or corrected percentage of reticulocytes have proven to be poor markers of hematopoietic engraftment. Some reports have highlighted the usefulness of automatic flow cytometry methods to determine highly fluorescent reticulocytes, or mean fluorescence index. In this series of 60 hematopoietic stem cell transplants, we sought the normal kinetics throughout the post-transplant period of the following reticulocyte maturing parameters: highly fluorescent reticulocytes (RETH), immature reticulocyte fraction (IRF), mean fluorescence index (MFI) and also mean reticulocyte volume (MRV). DESIGN AND METHODS: Sixty consecutive patients undergoing allogeneic bone marrow (30 cases) and autologous mobilized stem cell transplantation (30 cases) were studied. Parameters of reticulocyte maturation were measured every other day from the beginning of the conditioning regimen until myeloid engraftment. RESULTS: Nadir values for the analyzed reticulocyte parameters were found between days +4 and +7 and thereafter, increases in these reticulocyte parameters appeared earlier than the rise in neutrophils. We considered erythroid engraftment to have occurred on the day when RETH reached 3%, IRF 10%, MFI 10 and MRV 110 fL. These cut-offs were assigned considering the 25% quartile for each parameter on the day that the myeloid engraftment occurred. The median engraftment days for RETH were +9 and +16, for IRF +9 and +13, for MFI +9 and +13 and for MRV +11 and +13 in autologous and allogeneic procedures, respectively. When compared to standard neutrophil engraftment, IRF and MFI engraftment occurred significantly earlier in all patients. Remarkably, we found a statistical correlation between the day a reticulocyte parameter reached its cut-off and the subsequent day of absolute neutrophil count (ANC) recovery for MFI after allogeneic transplants and for MRV after autologous procedures ($p < 0.001$ and $p= 0.02$, respectively). Of all the clinical parameters tested, only the number of infused CD34 cells showed a statistical influence on erythroid engraftment in autologous transplant. INTERPRETATION AND CONCLUSIONS: Early reticulocytes appear sooner than neutrophils after both autologous and allogeneic transplants, and any determined reticulocyte parameter can reliably measure this fraction. Nevertheless, our results show that MRV and MFI cut-offs are useful for determining subsequent myeloid engraftment. These findings could be relevant to decision-making in those patients with primary graft failure heralded by an absence of increasing values of MFI and MRV, indicating very low production of reticulocytes from the graft, who could, therefore, benefit from earlier rescue therapy.

Tsatalas, C., P. Chalkia, et al. (1995). "Increased peripheral blood normal myeloid progenitor cells (CFU-GM) in chronic lymphocytic leukemia: a perspective for autologous peripheral blood stem cell transplantation." Eur J Haematol **54**(4): 235-40.

We assayed granulocyte-macrophage committed progenitor cells (CFU-GM) in the peripheral blood of 34 patients with chronic lymphocytic leukemia (CLL) and 12 normal individuals. The patients were divided into separate groups on the basis of previous therapy (i.e. analysis performed at diagnosis, during and after chemotherapy) and clinical features of the disease (i.e. disease stage, pattern of bone marrow infiltration, peripheral blood lymphocytosis). The mean CFU-GM colony count of the patients was 30 times higher than that of the controls (206.4 +/- 197.8 (SD) CFU-GM per 5 x 10(5) cells plated versus 6.5 +/- 3.6). There was no statistical difference in the numbers of circulating CFU-GM between the patients studied at diagnosis (257 +/- 215.4 CFU-GM/5 x 10(5) cells) and those studied during (117.6 +/- 169.2 CFU-GM/5 x 10(5) cells) or after chemotherapy (207.5 +/- 105.9 CFU-GM/5 x 10(5) cells), although a trend towards a higher recovery of myeloid progenitors was observed as a function of time elapsing from the last treatment. In addition, we found no significant difference in the in vitro CFU-GM growth of patients grouped

according to their disease stage, pattern of bone marrow infiltration and degree of peripheral blood lymphocytosis. In conclusion, our data indicate that intensification with peripheral blood stem cell support may be feasible in CLL, since progenitor cells of myeloid-monocytic series are markedly increased in the peripheral blood of these patients. Moreover, it is possible to extend this kind of therapy to patients who have undergone previous extensive chemotherapy and who might have persisting bone marrow infiltration.

Tsuzuki, S., D. Hong, et al. (2007). "Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1." PLoS Med **4**(5): e172.

BACKGROUND: AML1/RUNX1 is the most frequently mutated gene in leukaemia and is central to the normal biology of hematopoietic stem and progenitor cells. However, the role of different AML1 isoforms within these primitive compartments is unclear. Here we investigate whether altering relative expression of AML1 isoforms impacts the balance between cell self-renewal and differentiation in vitro and in vivo. METHODS AND FINDINGS: The human AML1a isoform encodes a truncated molecule with DNA-binding but no transactivation capacity. We used a retrovirus-based approach to transduce AML1a into primitive haematopoietic cells isolated from the mouse. We observed that enforced AML1a expression increased the competitive engraftment potential of murine long-term reconstituting stem cells with the proportion of AML1a-expressing cells increasing over time in both primary and secondary recipients. Furthermore, AML1a expression dramatically increased primitive and committed progenitor activity in engrafted animals as assessed by long-term culture, cobblestone formation, and colony assays. In contrast, expression of the full-length isoform AML1b abrogated engraftment potential. In vitro, AML1b promoted differentiation while AML1a promoted proliferation of progenitors capable of short-term lymphomyeloid engraftment. Consistent with these findings, the relative abundance of AML1a was highest in the primitive stem/progenitor compartment of human cord blood, and forced expression of AML1a in these cells enhanced maintenance of primitive potential both in vitro and in vivo. CONCLUSIONS: These data demonstrate that the "a" isoform of AML1 has the capacity to potentiate stem and progenitor cell engraftment, both of which are required for successful clinical transplantation. This activity is consistent with its expression pattern in both normal and leukaemic cells. Manipulating the balance of AML1 isoform expression may offer novel therapeutic strategies, exploitable in the contexts of leukaemia and also in cord blood transplantation in adults, in whom stem and progenitor cell numbers are often limiting.

Tycko, B., J. Ritz, et al. (1992). "Changing antigen receptor gene rearrangements in a case of early pre-B cell leukemia: evidence for a tumor progenitor cell with stem cell features and implications for monitoring residual disease." Blood **79**(2): 481-8.

A case of acute lymphoblastic leukemia (ALL) was encountered in which the two clonal gamma T-cell receptor gene (TCR gamma) rearrangements found in bone marrow (BM) samples at relapse both differed from the single clonal TCR gamma rearrangement present in BM obtained at diagnosis 5 years previously. In contrast, two clonal Ig heavy chain gene (IgH) rearrangements present at relapse were identical to those present at diagnosis. Comparison of the DNA sequences of the relapse TCR gamma rearrangements with that of the diagnostic TCR gamma rearrangement indicated that they must have been generated de novo from TCR gamma loci in germline configuration. By polymerase chain reaction using clonotypic N-region oligonucleotide primers (N-PCR), cells bearing the diagnosis or relapse TCR gamma rearrangements were undetectable in the sample from the opposite time point. Two BM samples obtained at different times in clinical remission were both devoid of detectable residual tumor when analyzed by N-PCR, indicating a depth of remission of less than 1 tumor cell per 4 x 10(5) BM mononuclear cells. The tumor cells showed a primitive phenotype: T-cell antigen-negative, CALLA/CD10-negative, CD20-negative, CD19-positive, and positive for the myeloid marker My9. This case, which appears to represent a tumor arising from a progenitor cell with both early B-lineage and certain stem cell features, has implications for monitoring residual ALL and possibly also for treatment of the disease.

Ural, S. H., M. D. Sammel, et al. (2005). "Determination of engraftment potential of human cord blood stem-progenitor cells as a function of donor cell dosage and gestational age in the NOD/SCID mouse model." Am J Obstet Gynecol **193**(3 Pt 2): 990-4.

OBJECTIVE: The purpose of this study was to determine cell dosage parameters for successful engraftment of human cord blood hematopoietic stem cells (HSC) using an in vivo assay system, and to determine if there are differences with donor gestational age. STUDY DESIGN: HSCs were transplanted into nonobese diabetic-severe combined immunodeficient (NOD/SCID) mice. Donor cell dosage and gestational age ranges were 1 to 40 x 10(6) CD34+ cells per mouse, and 23 to 40 weeks,

respectively. Recipient bone marrow was assessed for engraftment capacity of the HSCs. RESULTS: There was increasing engraftment levels with increasing dosages of transplanted HSCs. When controlled for donor HSC dosage, engraftment levels using donor cord blood from earlier gestational ages were not different from that seen using later gestational ages. CONCLUSION: Similar dose responses are seen using HSCs derived from the late second trimester until term in engraftment potential in the NOD/SCID mouse model. Results from this study may be applicable to human postnatal and in utero transplantation studies.

Ushio-Fukai, M. and N. Urao (2009). "Novel role of NADPH oxidase in angiogenesis and stem/progenitor cell function." Antioxid Redox Signal **11**(10): 2517-33.

Neovascularization is involved in normal development and wound repair as well as ischemic heart disease and peripheral artery disease. Both angiogenesis and vasculogenesis [de novo new vessel formation through mobilization of stem/progenitor cells from bone marrow (BM) and their homing to the ischemic sites] contribute to the formation of new blood vessels after tissue ischemia. Angiogenesis is dependent on cell proliferation, migration, and capillary tube formation in endothelial cells (ECs). Stem/progenitor cells have been used for cell-based therapy to promote revascularization after peripheral or myocardial ischemia. Excess amounts of reactive oxygen species (ROS) are involved in senescence and apoptosis of ECs and stem/progenitor cells, causing defective neovascularization. ROS at low levels function as signaling molecules to mediate cell proliferation, migration, differentiation, and gene expression. NADPH oxidase is one of the major sources of ROS in ECs and stem/progenitor cells, and is activated by various growth factors, cytokines, hypoxia, and ischemia. ROS derived from NADPH oxidase play an important role in redox signaling linked to angiogenesis ECs, as well as stem/progenitor cell mobilization, homing, and differentiation, thereby promoting neovascularization. Understanding these mechanisms may provide insight into NADPH oxidase and its mediators as potential therapeutic targets for ischemic heart and limb disease.

van Beusechem, V. W., J. A. Bart-Baumeister, et al. (1995). "Influence of interleukin-3, interleukin-6, and stem cell factor on retroviral transduction of rhesus monkey CD34+ hematopoietic progenitor cells measured in vitro and in vivo." Gene Ther **2**(4): 245-55.

As a preclinical test for bone marrow gene therapy, we transduced Rhesus monkey CD34+CD11b- hematopoietic progenitor cells with recombinant retroviruses. We investigated the effects of the recombinant hematopoietic growth factors interleukin-3 (IL-3), interleukin-6 (IL-6) and stem cell factor (SCF) on the susceptibility of in vitro clonogenic progenitor cells and in vivo repopulating stem cells to retroviral transduction. IL-6 did not contribute to transduction of progenitor cells, whereas IL-3 and SCF supported expansion and transduction of progenitors. The combination of IL-3 and IL-6 was most efficient at promoting transduction of more mature progenitor cell types. Cultures containing IL-6+SCF yielded optimal maintenance of CD34+CD11b- cells without evidence for lineage-restricted maturation. Autologous transplantation of transduced grafts cultured in the presence of SCF, with or without IL-3 or IL-6, into lethally irradiated Rhesus monkeys resulted in a severely delayed hematopoietic reconstitution as compared with grafts transduced in the presence of IL-3 alone. After in vivo repopulation, transduced cells were found among peripheral blood mononuclear cells, granulocytes and CD34+CD11b- progenitor cells in the bone marrow of engrafted animals. However, no significant difference in transduction efficiency on in vivo repopulating stem cells could be demonstrated among the tested growth factor conditions.

Van Hennik, P. B., D. A. Breems, et al. (2000). "Stroma-supported progenitor production as a prognostic tool for graft failure following autologous stem cell transplantation." Br J Haematol **111**(2): 674-84.

To analyse the involvement of a possible numerical or qualitative stem cell defect in the development of sustained graft failure after autologous transplantation, we have determined the graft content of CD34+ nucleated cells, colony-forming cells and cobblestone area-forming cell subsets, as well as transplant ability to produce progenitors using the long-term culture colony-forming cell (LTC-CFC) assay. We evaluated material from the graft reference ampoules of 13 graft failure patients after bone marrow transplantation (BMT), four graft failure patients and four isolated thrombocytopenia patients after peripheral blood stem cell transplantation (PBSCT). We compared these data with those from six successfully engrafted BMT patients and 20 engrafted PBSCT patients respectively. In the BMT setting, the LTC-CFC 6-week assay represented a highly significant graft failure predictor. In the PBSCT setting, the total number of 2-week and 6-week LTC-CFCs transplanted per kg bodyweight (BW) showed the highest significant difference between the engrafted and the graft failure patients, as well as between the engrafted patients and the patients

suffering from isolated thrombocytopenia after transplantation. These data show that the ability of a graft to generate progenitors in vitro rather than the number of primitive progenitors transplanted can have prognostic value for post-transplant haematological reconstitution.

van Pel, M., H. Hagoort, et al. (2008). "Differential role of CD97 in interleukin-8-induced and granulocyte-colony stimulating factor-induced hematopoietic stem and progenitor cell mobilization." Haematologica **93**(4): 601-4.

CD97 is broadly expressed on hematopoietic cells and is involved in neutrophil migration. Since neutrophils are key regulators in HSC/HPC mobilization, we studied a possible role for CD97 in interleukin-8 and granulocyte-colony stimulating factor-induced HSC/HPC mobilization. Mobilization was absent in mice receiving CD97 mAb followed by interleukin-8, while granulocyte-colony stimulating factor-induced mobilization remained unaltered following anti-CD97 administration. Furthermore, combined administration of CD97 mAb and IL-8 induced a significant reduction in the neutrophilic compartment. We hypothesize that the absence of interleukin-8-induced HSC/HPC mobilization after CD97 mAb administration is due to its effect on neutrophil function.

Vascotto, S. G. and M. Griffith (2006). "Localization of candidate stem and progenitor cell markers within the human cornea, limbus, and bulbar conjunctiva in vivo and in cell culture." Anat Rec A Discov Mol Cell Evol Biol **288**(8): 921-31.

Corneal diseases are some of the most prevalent causes of blindness worldwide. While the most common treatment for corneal blindness is the transplantation of cadaver corneas, expanded limbal stem cells are finding recent application. Unknown, however, is the identity of the actual repopulating stem cell fraction utilized in both treatments and the critical factors governing successful engraftment and repopulation. In order to localize potential stem cell populations in vivo, we have immunohistochemically mapped a battery of candidate stem and progenitor cell markers including c-Kit and other growth factor receptors, nuclear markers including DeltaNp63, as well as adhesion factors across the cornea and distal sclera. Cell populations that differentially and specifically stained for some of these markers include the basal and superficial limbal/conjunctival epithelium and scattered cells within the substantia propria of the bulbar conjunctiva. We have also determined that the culture of differentiated cornea epithelial cells as dissociated and explant cultures induces the expression of several markers previously characterized as candidate limbal stem cell markers. This study provides a foundation to explore candidate corneal stem cell populations. As well, we show that expression of traditional stem cell markers may not be reliable indicator of stem cell content during limbal stem cell expansion in vitro and could contribute to the variable success rates of corneal stem cell transplantation.

Ventura, J. J., S. Tenbaum, et al. (2007). "p38alpha MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation." Nat Genet **39**(6): 750-8.

Stem cell function is central for the maintenance of normal tissue homeostasis. Here we show that deletion of p38alpha mitogen-activated protein (MAP) kinase in adult mice results in increased proliferation and defective differentiation of lung stem and progenitor cells both in vivo and in vitro. We found that p38alpha positively regulates factors such as CCAAT/enhancer-binding protein that are required for lung cell differentiation. In addition, p38alpha controls self-renewal of the lung stem and progenitor cell population by inhibiting proliferation-inducing signals, most notably epidermal growth factor receptor. As a consequence, the inactivation of p38alpha leads to an immature and hyperproliferative lung epithelium that is highly sensitized to K-Ras(G12V)-induced tumorigenesis. Our results indicate that by coordinating proliferation and differentiation signals in lung stem and progenitor cells, p38alpha has a key role in the regulation of lung cell renewal and tumorigenesis.

Viallard, J. F., C. Grosset, et al. (1996). "Effect of stem cell factor on leukemic progenitor cell growth and sensitivity to cytosine-arabinoside." Leuk Res **20**(11-12): 915-23.

Recruitment of quiescent, clonogenic blasts from patients with acute myeloid leukemia (AML) by hematopoietic growth factors (HGFs) may improve the cytotoxic effects of cell-cycle-specific drugs like cytosine-arabinoside (Ara-C). Using the culture methods described by Nara and McCulloch and making a distinction between self-renewing and post-deterministic mitoses, we analyzed the effects of stem cell factor (SCF), a growth factor acting on early hematopoietic progenitor and stem cells. First, we demonstrated that SCF, used in combination with other HGFs included in fetal calf serum (FCS) and/or in 5637 cell line supernatant (5637-CM), stimulated both colony formation and self-renewal of blast progenitors from 10 patients, unlike SCF alone. We tested the effects of SCF on the recruitment of cells in the S-phase by using a bromodeoxyuridine/DNA (BrdUrd/DNA) staining method in flow cytometry

(FCM). We showed that SCF stimulated proliferation of AML cells significantly in 9/18 patients with AML. Second, we tested the influence of SCF on the sensitivity to Ara-C of self-renewing leukemic cells from 18 patients with AML. We showed that SCF was efficient in increasing the toxicity of Ara-C on the self-renewing blast progenitors, especially with high concentrations of Ara-C. However, a large patient-to-patient heterogeneity was found and the activity of SCF was not correlated with its effect on the cell cycle. These data indicate that SCF can enhance sensitivity to Ara-C of some leukemic cells with self-renewing capacity.

Wagemaker, G. and T. P. Visser (1986). "Enumeration of stem cells and progenitor cells in alpha-thalassemic mice reveals lack of specific regulation of stem cell differentiation." Exp Hematol **14**(4): 303-6.

Heterozygous alpha-thalassemic (Hbath/+) female mice were investigated for the effect of persistent erythropoietic stress on the number of stem cells and progenitor cells along the the erythroid (E), granulocyte-macrophage (GM), and megakaryocyte (Meg) pathways. At the progenitor cell level, compensatory erythropoiesis was demonstrated in the spleen but not in the bone marrow. In the spleen, developmentally early progenitor cells (BFU-E) were expanded two- to threefold and late progenitor cells (CFU-E) five- to sixfold. A comparable expansion of progenitor cells was observed along the GM and Meg pathways. CFU-S numbers were increased in the spleen, but not in the bone marrow. The increases in GM and Meg progenitor cells appeared to result in an inappropriate hemopoiesis: peripheral thrombocyte and monocyte numbers were elevated. However, granulocyte numbers were not significantly increased. It is concluded that the persistently increased erythropoietic demand results in inappropriate production of other hemopoietic cells, most likely because pathway-specific regulatory mechanisms do not influence differentiation at the stem cell level.

Wagner, W., A. Ansorge, et al. (2004). "Molecular evidence for stem cell function of the slow-dividing fraction among human hematopoietic progenitor cells by genome-wide analysis." Blood **104**(3): 675-86.

The molecular mechanisms that regulate asymmetric divisions of hematopoietic progenitor cells (HPCs) are not yet understood. The slow-dividing fraction (SDF) of HPCs is associated with primitive function and self-renewal, whereas the fast-dividing fraction (FDF) predominantly proceeds to differentiation. CD34+/CD38- cells of human umbilical cord blood were separated into the SDF and FDF. Genomewide gene expression analysis of these populations was determined using the newly developed Human Transcriptome Microarray containing 51 145 cDNA clones of the Unigene Set-RZPD3. In addition, gene expression profiles of CD34+/CD38- cells were compared with those of CD34+/CD38+ cells. Among the genes showing the highest expression levels in the SDF were the following: CD133, ERG, cyclin G2, MDR1, osteopontin, CLQR1, IFI16, JAK3, FZD6, and HOXA9, a pattern compatible with their primitive function and self-renewal capacity. Furthermore, morphologic differences between the SDF and FDF were determined. Cells in the SDF have more membrane protrusions and CD133 is located on these lamellipodia. The majority of cells in the SDF are rhodamine-123dull. These results provide molecular evidence that the SDF is associated with primitive function and serves as basis for a detailed understanding of asymmetric division of stem cells.

Wagner, W., F. Wein, et al. (2007). "Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction." Exp Hematol **35**(2): 314-25.

OBJECTIVE: The significant role of direct contact between hematopoietic progenitor cells (HPC) and the cellular microenvironment for maintaining "stemness" has been demonstrated. Human mesenchymal stem cell (MSC) feeder layers represent a surrogate model for this interaction. Specific adhesion molecules are responsible for this cell-cell contact. METHODS: To define cell-cell contact between HPC and MSC, we have studied adhesive interaction of various fractions of HPC by using a novel assay based on gravitational force upon inversion. Adherent and nonadherent cells were separated and further analyzed with regard to gene expression and long-term hematopoietic culture initiating cell (LTC-IC) frequency. RESULTS: HPC subsets with higher self-renewing capacity demonstrated significantly higher adherence to human MSC (CD34(+) vs CD34(-), CD34(+)/CD38(-) vs CD34(+)/CD38(+), slow dividing fraction vs fast dividing fraction). LTC-IC frequency was significantly higher in the adherent fraction than in the nonadherent fraction. Furthermore, genes coding for adhesion proteins and extracellular matrix were higher expressed in the adherent subsets of CD34(+) cells (fibronectin 1, cadherin 11, vascular cell adhesion molecule-1, connexin 43, integrin beta-like 1, and TGFBI). CONCLUSION: In this study we have demonstrated that primitive subsets of HPC have higher affinity to human MSC. The essential role of specific junction proteins for stabilization of cell-cell contact is indicated by their significant higher expression.

Wallington-Beddoe, C. T., D. J. Gottlieb, et al. (2009). "Failure to achieve a threshold dose of CD34+CD110+ progenitor cells in the graft predicts delayed platelet engraftment after autologous stem cell transplantation for multiple myeloma." Biol Blood Marrow Transplant **15**(11): 1386-93.

To predict platelet engraftment more accurately post autologous stem cell transplantation (SCT), we retrospectively analyzed the CD34(+)CD110(+) (CD110 or c-mpl, thrombopoietin receptor) content in the grafts of 70 patients undergoing transplantation for multiple myeloma (MM) with an in-house flow cytometric assay. We found that infusing at least 3.0 x 10(4) CD34(+)CD110(+) cells/kg clearly separated the cohort into those who achieved platelet engraftment before or after 21 days. This early megakaryocyte cell marker correlated more closely with early versus delayed platelet engraftment than CD34(+) measurements. Of the 70 patients, 4 required > or = 21 days to achieve platelet transfusion independence. Three of the 4 received a CD34(+)CD110(+) cell dose of <3.0 x 10(4) cells/kg, whereas 66 of 70 patients who received >3.0 x 10(4) CD34(+)CD110(+) cells/kg achieved platelet transfusion independence in <21 days (P < .001). Infusing >3.0 x 10(4) CD34(+)CD110(+) cells/kg was sensitive (100%) and specific (75%) for achieving platelet engraftment within 21 days. Patients with delayed platelet engraftment received a median of 2.28 x 10(4) CD34(+)CD110(+) cells/kg versus 12.1 x 10(4) cells/kg in those without this complication (P = .033). No effect was seen with neutrophil engraftment. Patients with early engraftment required a median of 1 platelet transfusion post transplant versus 2.5 in those with late engraftment (P = .009). A subthreshold absolute CD34(+)CD110(+) cell dose in the graft is a reliable predictor of delayed platelet engraftment, and could be used to guide the timing and number of peripheral blood stem cell (PBSC) collections for patients with MM undergoing an SCT.

Wan, H., M. Yuan, et al. (2007). "Stem/progenitor cell-like properties of desmoglein 3dim cells in primary and immortalized keratinocyte lines." Stem Cells **25**(5): 1286-97.

We showed previously that primary keratinocytes selected for low desmoglein 3 (Dsg3) expression levels exhibited increased colony-forming efficiency and heightened proliferative potential relative to cells with higher Dsg3 expression levels, characteristics consistent with a more "stem/progenitor cell-like" phenotype. Here, we have confirmed that Dsg3(dim) cells derived from cultured primary human adult keratinocytes have comparability with alpha(6)(bri)/CD71(dim) stem cells in terms of colony-forming efficiency. Moreover, these Dsg3(dim) cells exhibit increased reconstituting ability in in vitro organotypic culture on de-epidermalized dermis (DED); they are small, actively cycling cells, and they express elevated levels of various p63 isoforms. In parallel, using the two immortalized keratinocyte cell lines HaCaT and NTERT, we obtained essentially similar though occasionally different findings. Thus, reduced colony-forming efficiency by Dsg3(bri) cells consistently was observed in both cell lines even though the cell cycle profile and levels of p63 isoforms in the bri and dim populations differed between these two cell lines. Dsg3(dim) cells from both immortalized lines produced thicker and better ordered hierarchical structural organization of reconstituted epidermis relative to Dsg3(bri) and sorted control cells. Dsg3(dim) HaCaT cells also show sebocyte-like differentiation in the basal compartment of skin reconstituted after a 4-week organotypic culture. No differences in percentages of side population cells (also a putative marker of stem cells) were detected between Dsg3(dim) and Dsg3(bri) populations. Taken together our data indicate that Dsg3(dim) populations from primary human adult keratinocytes and long-term established keratinocyte lines possess certain stem/progenitor cell-like properties, although the side population characteristic is not one of these features. Disclosure of potential conflicts of interest is found at the end of this article.

Wandzioch, E., C. E. Edling, et al. (2004). "Activation of the MAP kinase pathway by c-Kit is PI-3 kinase dependent in hematopoietic progenitor/stem cell lines." Blood **104**(1): 51-7.

The Steel factor (SF) and its receptor c-Kit play a critical role for various cell types at different levels in the hematopoietic hierarchy. Whether similar or distinct signaling pathways are used upon c-Kit activation in different cell types within the hematopoietic hierarchy is not known. To study c-Kit signaling pathways in the hematopoietic system we have compared c-Kit downstream signaling events in SF-dependent hematopoietic stem cell (HSC)-like cell lines to those of mast cells. Both Erk and protein kinase B (PKB)/Akt are activated by ligand-induced activation of the c-Kit receptor in the HSC-like cell lines. Surprisingly, phosphoinositide-3 (PI-3) kinase inhibitors block not only PKB/Akt activation but also activation of Raf and Erk. SF-induced activation of Ras is not affected by inhibition of PI-3 kinase. In mast cells and other more committed hematopoietic precursors, the activation of Erk by SF is not PI-3 kinase dependent. Our results suggest that a molecular signaling switch occurs during differentiation in the hematopoietic system whereby immature

hematopoietic progenitor/stem cells use a PI-3 kinase-sensitive pathway in the activation of both Erk and PKB/Akt, which is then switched upon differentiation to the more commonly described PI-3 kinase-independent mitogen-activated protein (MAP) kinase pathway.

Wang, L., L. Yang, et al. (2006). "Genetic deletion of Cdc42GAP reveals a role of Cdc42 in erythropoiesis and hematopoietic stem/progenitor cell survival, adhesion, and engraftment." Blood **107**(1): 98-105.

Rho family GTPases are key signal transducers in cell regulation. Although a body of literature has implicated the Rho family members Rac1 and Rac2 in multiple hematopoietic-cell functions, the role of Cdc42 in hematopoiesis remains unclear. Here we have examined the hematopoietic properties and the hematopoietic stem/progenitor cell (HSP) functions of gene-targeted mice carrying null alleles of cdc42gap, a negative regulator of Cdc42. The Cdc42GAP-/- fetal liver and bone marrow cells showed a 3-fold increase in Cdc42 activity but normal Rac and RhoA activities, indicating that Cdc42GAP knockout resulted in a gain of Cdc42 activity in the hematopoietic tissues. Cdc42GAP-/- mice were anemic. The cellularity of fetal liver and bone marrow, the number and composition percentage of HSPs, and the erythroid blast-forming unit and colony-forming unit (BFU-E/CFU-E) activities were significantly reduced in the homozygous mice. The decrease in HSP number was associated with increased apoptosis of the Cdc42GAP-/- HSPs and the activation of JNK-mediated apoptotic machinery. Moreover, homozygous HSPs showed impaired cortical F-actin assembly, deficiency in adhesion and migration, and defective engraftment. These results provide evidence that Cdc42 activity is important for erythropoiesis and for multiple HSP functions, including survival, adhesion, and engraftment.

Wiesmann, A., M. Kim, et al. (2000). "Modulation of hematopoietic stem/progenitor cell engraftment by transforming growth factor beta." Exp Hematol **28**(2): 128-39.

OBJECTIVE: To investigate if cell cycle progression plays a role in modulating the engraftment potential of mouse hematopoietic stem and progenitor cells (HSPC). MATERIALS AND METHODS: HSPC were isolated from adult mouse bone marrow, cultured in vitro under conditions promoting cell cycle arrest, and subsequently were evaluated for cell cycle status, clonogenic activity, and transplant potential. RESULTS In the presence of steel factor (STL) as a survival cytokine, transforming growth factor beta (TGF-beta) increased the G0/G1 fraction of cycling progenitor cells (Rh(high)) after a 20-hour culture. Clonogenic activity of quiescent long-term repopulating (Rh(low)) HSPC was unaffected by this culture, whereas clonogenic potential of Rh(high) cells decreased by about 30%. In competitive repopulation assays, Rh(low) cells cultured in STL + TGF-beta engrafted better than cells cultured in STL alone. However, culture in STL + TGF-beta did not overcome the failure of Rh(high) cells to engraft after transplant. We also utilized a two-stage culture system to first induce proliferation of Rh(low) HSPC by a 48-hour culture in STL + interleukin 6 + Flt-3 ligand, followed by shifting the culture to STL + TGF-beta for 24 hours to induce cycle arrest. A competitive repopulation assay demonstrated a relative decrease in repopulating potential in cultures that were cycle arrested compared to those that were not. CONCLUSION: Cell cycle progression by itself cannot account for the decrease in repopulating potential that is observed after ex vivo expansion. Other determinants of engraftment must be identified to facilitate the transplantation of cultured HSPC.

Xie, Z. and L. S. Chin (2008). "Molecular and cell biology of brain tumor stem cells: lessons from neural progenitor/stem cells." Neurosurg Focus **24**(3-4): E25.

The results of studies conducted in the past several years have suggested that malignant brain tumors may harbor a small fraction of tumor-initiating cells that are likely to cause tumor recurrence. These cells are known as brain tumor stem cells (BTSCs) because of their multilineage potential and their ability to self-renew in vitro and to recapitulate original tumors in vivo. The understanding of BTSCs has been greatly advanced by knowledge of neural progenitor/stem cells (NPSCs), which are multipotent and self-renewing precursor cells for neurons and glia. In this article, the authors summarize evidence that genetic mutations that deregulate asymmetric cell division by affecting cell polarity, spindle orientation, or cell fate determinants may result in the conversion of NPSCs to BTSCs. In addition, they review evidence that BTSCs and normal NPSCs may reside in similar vascularized microenvironments, where similar evolutionarily conserved signaling pathways control their proliferation. Finally, they discuss preliminary evidence that mechanisms of BTSC-associated infiltrativeness may be similar to those underlying the migration of NPSCs and neurons.

Xu, Q., S. Wang, et al. (2007). "Hypoxia-induced astrocytes promote the migration of neural progenitor cells via vascular endothelial factor, stem cell factor, stromal-derived factor-1alpha and monocyte chemoattractant protein-1 upregulation in vitro." Clin Exp Pharmacol Physiol **34**(7): 624-31.

1. The aim of the present study was to examine if and how rat hypoxia-induced astrocytes affect the migration of neural progenitor cells (NPC) and to investigate the expression patterns of some chemokines, such as vascular endothelial growth factor (VEGF), stem cell factor (SCF), stromal-derived factor-1alpha (SDF-1alpha), fractalkine and monocyte chemoattractant protein-1 (MCP-1) in hypoxia-induced astrocytes and their contribution to NPC migration in vitro. 2. Costar Transwell inserts were used for the chemotaxis assay and quantified changes in the chemokines mRNA for between 0 h and 24 h posthypoxia were tested using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. The results showed that the chemotaxis of astrocyte cells exposed to hypoxia for 18 h reached a peak value, whereas the chemotaxis of astrocytes exposed to hypoxia for 24 h began to decrease compared with those exposed to hypoxia for 18 h. Hypoxia upregulated chemokine VEGF, SCF, SDF-1alpha and MCP-1 expression in a time-dependent manner but downregulated fractalkine expression in astrocytes. In addition, the time points of the peak expressions for VEGF, SCF, SDF-1alpha and MCP-1 were similar to the time point of maximum NPC migration. 3. Specific inhibitors that block the binding of specific chemokines to its receptors were used for analysing the contribution of the chemokine to NPC migration. When VEGF, SCF, SDF-1alpha and MCP-1 were each inhibited independently, NPC migration was reduced. When they were inhibited together, NPC migration was obviously inhibited compared with both the control and single-block cultures, which implies that the migratory effect of hypoxia-induced astrocytes was synergetic by several chemokines. 4. In conclusion, we demonstrated the time-dependent manner of NPC migration promotion by hypoxia-induced astrocytes. We also provide evidence that soluble factors, such as VEGF, SCF, SDF-1alpha and MCP-1, released from astrocytes, direct the migration of NPC under hypoxic circumstances. Given that astrocytes were activated to all hypoxia-ischaemia diseases, these results indicate an important role for astrocytes in directing NPC replacement therapy in the central nervous system.

Yamaguchi, H., E. Ishii, et al. (1996). "Umbilical vein endothelial cells are an important source of c-kit and stem cell factor which regulate the proliferation of haemopoietic progenitor cells." Br J Haematol **94**(4): 606-11.

The expression and production of c-kit and its ligand, stem cell factor (SCF), in cord blood and neonates were studied. Serum SCF levels were significantly higher in cord blood, neonates aged 1-30 d, and in 4-month-old infants than in the maternal serum ($P < 0.01$). SCF levels decreased in children from 7 months to 15 years of age ($P < 0.01$). The serum soluble c-kit levels were significantly higher in cord ($P < 0.01$) and neonatal blood ($P < 0.05$) than in the maternal blood. SCF and c-kit levels in placental tissue homogenates and the culture media of decidual cells and trophoblasts were low. To determine the sites of high SCF and c-kit production in cord blood and in early neonates. SCF and c-kit mRNA expression was analysed in various tissues by polymerase chain reaction. High SCF mRNA expression was observed in human umbilical vein endothelial cells (HUVEC). Moderate c-kit mRNA expression was detected in HUVEC, the bone marrow, and cord blood. These findings suggest that endothelial cells mainly produce the SCF in cord blood and in early neonates. To confirm the role of endothelial cells in haemopoiesis, colony-forming assays were performed in the presence of HUVEC culture media, which induced the formation of high numbers of granulocyte and erythroid colonies in cord blood. IL-3, IL-6 and SCF levels were elevated in the media. Our findings suggest that endothelial cells have an important role in the maintenance and proliferation of progenitor cells in neonatal blood via the interaction of c-kit and SCF with other factors. The ex vivo expansion of cord progenitor cells in the presence of endothelial cells needs to be investigated further.

Yamamoto, K., T. Kondo, et al. (2004). "Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: therapeutic effect by stem cell transplantation." Arterioscler Thromb Vasc Biol **24**(12): e192-6.

OBJECTIVE: Although some patients with limb ischemia have recently undergone therapeutic angiogenesis by cell transplantation, their angiogenic potential has not been well characterized. It is also important to evaluate endothelial progenitor cell (EPC) contents in different stem cell sources to choose the best material for therapeutic angiogenesis. METHODS AND RESULTS: We quantitated the mRNA expression of EPC-specific molecules (eg, Flk-1, Flt-1, CD133, VE-cadherin, etc) in bone marrow-derived or peripheral blood-derived mononuclear cells obtained from patients with ischemic limbs, using real-time reverse-transcription polymerase chain reaction technique. The mRNA expression level of EPC markers was significantly lower in the patients than in healthy controls, which was consistent with results of flow cytometric analysis. However, the implantation of autologous bone marrow mononuclear cells increased the circulating EPCs in the peripheral blood of patients. We furthermore revealed the different expression pattern of EPC markers in possible sources for stem

cell transplantation, including normal bone marrow, peripheral blood obtained from recombinant granulocyte colony-stimulating factor-treated donor, and umbilical cord blood. CONCLUSIONS: Patients with peripheral obstructive arterial diseases may have lower angiogenic potential because of decreased expression of EPC specific molecules in their marrow and blood. Therapeutic angiogenesis by transplantation of autologous marrow mononuclear cells increased circulating EPCs in the patients and improved ischemic symptoms.

Yamashita, T., J. Ji, et al. (2009). "EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features." Gastroenterology **136**(3): 1012-24.

BACKGROUND & AIMS: Cancer progression/metastases and embryonic development share many properties including cellular plasticity, dynamic cell motility, and integral interaction with the microenvironment. We hypothesized that the heterogeneous nature of hepatocellular carcinoma (HCC), in part, may be owing to the presence of hepatic cancer cells with stem/progenitor features. METHODS: Gene expression profiling and immunohistochemistry analyses were used to analyze 235 tumor specimens derived from 2 recently identified HCC subtypes (EpCAM(+) alpha-fetoprotein [AFP(+)] HCC and EpCAM(-) AFP(-) HCC). These subtypes differed in their expression of AFP, a molecule produced in the developing embryo, and EpCAM, a cell surface hepatic stem cell marker. Fluorescence-activated cell sorting was used to isolate EpCAM(+) HCC cells, which were tested for hepatic stem/progenitor cell properties. RESULTS: Gene expression and pathway analyses revealed that the EpCAM(+) AFP(+) HCC subtype had features of hepatic stem/progenitor cells. Indeed, the fluorescence-activated cell sorting-isolated EpCAM(+) HCC cells displayed hepatic cancer stem cell-like traits including the abilities to self-renew and differentiate. Moreover, these cells were capable of initiating highly invasive HCC in nonobese diabetic, severe combined immunodeficient mice. Activation of Wnt/beta-catenin signaling enriched the EpCAM(+) cell population, whereas RNA interference-based blockage of EpCAM, a Wnt/beta-catenin signaling target, attenuated the activities of these cells. CONCLUSIONS: Taken together, our results suggest that HCC growth and invasiveness is dictated by a subset of EpCAM(+) cells, opening a new avenue for HCC cancer cell eradication by targeting Wnt/beta-catenin signaling components such as EpCAM.

Yan, X. Q., C. Hartley, et al. (1995). "Peripheral blood progenitor cells mobilized by recombinant human granulocyte colony-stimulating factor plus recombinant rat stem cell factor contain long-term engrafting cells capable of cellular proliferation for more than two years as shown by serial transplantation in mice." Blood **85**(9): 2303-7.

Mobilized peripheral blood progenitor cells (PBPC) have been shown to provide rapid engraftment in patients given high-dose chemotherapy. PBPC contain cells with long-term engraftment potential as shown in animal models. In this study we have further analyzed mobilized PBPC for their ability to support serial transplantation of irradiated mice. Transplantation of recombinant human granulocyte colony-stimulating factor (rhG-CSF) plus recombinant rat stem cell factor (rrSCF) mobilized PBPC resulted in 98% donor engraftment of primary recipients at 12 to 14 months post-transplantation. Bone marrow (BM) cells from these primary recipients were harvested and transplanted into secondary recipients. At 6 months posttransplantation, all surviving secondary recipients had donor engraftment. Polymerase chain reaction (PCR) analysis showed greater than 90% male cells in spleens, thymuses, and lymph nodes. Myeloid colonies from BM cells of secondary recipients demonstrated granulocyte/macrophage colony-forming cells (GM-CFC) of male origin in all animals. In comparison, transplantation of rhG-CSF mobilized PBPC resulted in decreased male engraftment in secondary recipients. BM cells from secondary recipients, who originally received PBPC mobilized by the combination of rrSCF and rhG-CSF, were further passaged to tertiary female recipients. At 6 months posttransplantation, 90% of animals had male-derived hematopoiesis by whole-blood PCR analysis. These data showed that PBPC mobilized with rhG-CSF plus rrSCF contained cells that are transplantable and able to maintain hematopoiesis for more than 26 months, suggesting that the mobilized long-term reconstituting stem cells (LTRC) have extensive proliferative potential and resemble those that reside in the BM. In addition, the data demonstrated increased mobilization of LTRC with rhG-CSF plus rrSCF compared to rhG-CSF alone.

Yang, L., M. H. Soonpaa, et al. (2008). "Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population." Nature **453**(7194): 524-8.

The functional heart is comprised of distinct mesoderm-derived lineages including cardiomyocytes, endothelial cells and vascular smooth muscle cells. Studies in the mouse embryo and the mouse embryonic stem cell differentiation model have provided evidence indicating that these three lineages develop from a common Flk-1(+) (kinase insert

domain protein receptor, also known as Kdr) cardiovascular progenitor that represents one of the earliest stages in mesoderm specification to the cardiovascular lineages. To determine whether a comparable progenitor is present during human cardiogenesis, we analysed the development of the cardiovascular lineages in human embryonic stem cell differentiation cultures. Here we show that after induction with combinations of activin A, bone morphogenetic protein 4 (BMP4), basic fibroblast growth factor (bFGF, also known as FGF2), vascular endothelial growth factor (VEGF, also known as VEGFA) and dickkopf homolog 1 (DKK1) in serum-free media, human embryonic-stem-cell-derived embryoid bodies generate a KDR(low)/C-KIT(CD117)(neg) population that displays cardiac, endothelial and vascular smooth muscle potential in vitro and, after transplantation, in vivo. When plated in monolayer cultures, these KDR(low)/C-KIT(neg) cells differentiate to generate populations consisting of greater than 50% contracting cardiomyocytes. Populations derived from the KDR(low)/C-KIT(neg) fraction give rise to colonies that contain all three lineages when plated in methylcellulose cultures. Results from limiting dilution studies and cell-mixing experiments support the interpretation that these colonies are clones, indicating that they develop from a cardiovascular colony-forming cell. Together, these findings identify a human cardiovascular progenitor that defines one of the earliest stages of human cardiac development.

Yasutake, M., Y. Zheng, et al. (2005). "SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem and/or progenitor cells in a nonobese diabetic/Shi-SCID mice serial xenotransplantation model and immune cell activities in vitro: a comparative study of the filter method and the hydroxyethyl starch method." Transfusion **45**(12): 1899-908.

BACKGROUND: A novel filter system was developed for umbilical cord blood (UCB) volume reduction. The aim of this study was to compare the functions of cryopreserved UCB cells processed by the filter and by the hydroxyethyl starch (HES) sedimentation method from the aspect of the graft quality. STUDY DESIGN AND METHODS: UCB specimens were divided into two portions, processed in parallel by the filter or HES, and then cryopreserved in the clinical setting. The thawed UCB specimens containing 1 x 10(5) CD34+ cells were injected into nonobese diabetic/Shi-SCID mice, and the engraftment capacity in primary and secondary transplants was assessed. The functions of natural killer (NK) cells and monocyte-derived dendritic cells (DCs) were also assayed in vitro. RESULTS: The percentage of recovery of CD34+ cells by both methods was equivalent. In the marrow of the primary transplant recipients, the percentage of hCD45+ cells in the filter group and HES group was 58.2 +/- 31.6 and 46.5 +/- 28.4 percent, respectively ($p = 0.016$). The engraftment capacity and multilineage differentiation in the secondary transplantations were equal in both groups. The cytotoxic activity of the NK cells and phagocytosis activity of the DCs from both the groups were similar. CONCLUSION: The filter yielded a desirable percentage of recovery of hematopoietic cells with engraftment ability in the clinical setting. Thus, it is considered that the filter system may be useful for UCB banking for cord blood transplantation.

Yoder, M. C., L. E. Mead, et al. (2007). "Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals." Blood **109**(5): 1801-9.

The limited vessel-forming capacity of infused endothelial progenitor cells (EPCs) into patients with cardiovascular dysfunction may be related to a misunderstanding of the biologic potential of the cells. EPCs are generally identified by cell surface antigen expression or counting in a commercially available kit that identifies "endothelial cell colony-forming units" (CFU-ECs). However, the origin, proliferative potential, and differentiation capacity of CFU-ECs is controversial. In contrast, other EPCs with blood vessel-forming ability, termed endothelial colony-forming cells (ECFCs), have been isolated from human peripheral blood. We compared the function of CFU-ECs and ECFCs and determined that CFU-ECs are derived from the hematopoietic system using progenitor assays, and analysis of donor cells from polycythemia vera patients harboring a Janus kinase 2 V617F mutation in hematopoietic stem cell clones. Further, CFU-ECs possess myeloid progenitor cell activity, differentiate into phagocytic macrophages, and fail to form perfused vessels in vivo. In contrast, ECFCs are clonally distinct from CFU-ECs, display robust proliferative potential, and form perfused vessels in vivo. Thus, these studies establish that CFU-ECs are not EPCs and the role of these cells in angiogenesis must be re-examined prior to further clinical trials, whereas ECFCs may serve as a potential therapy for vascular regeneration.

Yokota, T., K. Oritani, et al. (1998). "Growth-supporting activities of fibronectin on hematopoietic stem/progenitor cells in vitro and in vivo: structural requirement for fibronectin activities of CS1 and cell-binding domains." Blood **91**(9): 3263-72.

Fibronectin (FN) is supposed to play important roles in various aspects of hematopoiesis

through binding to very late antigen 4 (VLA4) and VLA5. However, effects of FN on hematopoietic stem cells are largely unknown. In an effort to determine if FN had a growth-supporting activity on hematopoietic stem cells, human CD34(+)/VLA4(bright)/VLA5(dull) hematopoietic stem cells and a murine stem cell factor (SCF)-dependent multipotent cell line, EML-C1, were treated with or without FN in a serum and growth-factor-deprived medium, and then subjected to clonogenic assay in the presence of hematopoietic growth factors. The pretreatment of the CD34(+) cells with FN gave rise to significantly increased numbers of granulocyte-macrophage colony-forming units (CFU-GM), erythroid burst colony-forming units, and mixed erythroid-myeloid colony-forming units. In addition, the numbers of blast colony-forming units and CFU-GM that developed after culture of EML-C1 cells with SCF and the combination of SCF and interleukin-3, respectively, were augmented by the pretreatment with FN. The augmented colony formation by FN was completely abrogated by the addition of CS1 fragment, but not of GRGDSP peptide, suggesting an essential role of FN-VLA4 interaction in the FN effects. Furthermore, the effects of various FN fragments consisting of RGDS-containing cell-binding domain (CBD), heparin-binding domain (HBD), and/or CS1 portion were tested on clonogenic growth of CD34(+) cells. Increased colony formation was induced by CBD-CS1 and CBD-HBD-CS1 fragments, but not with other fragments lacking CBD or CS1 domains, suggesting that both CS1 and CBD of FN were required for the augmentation of clonogenic growth of hematopoietic stem/progenitor cells in vitro. In addition to the in vitro effects, the in vivo administration of CBD-CS1 fragment into mice was found to increase the numbers of hematopoietic progenitor cells in bone marrow and spleen in a dose-dependent manner. Thus, FN may function on hematopoietic stem/progenitor cells as a growth-supporting factor in vitro and in vivo.

Yuan, Q., M. F. Gurish, et al. (1998). "Generation of a novel stem cell factor-dependent mast cell progenitor." J Immunol **161**(10): 5143-6.

Tissue mast cell development requires stem cell factor (SCF), whereas helminth-induced intestinal mucosal mast cell hyperplasia also requires T cell-derived factors such as IL-3. We generated progenitor mast cells (PrMC) from mouse bone marrow cells (BMC) in vitro with a triad of SCF, IL-6, and IL-10 that exhibit IL-3-mediated mitogenic and maturation responses. SCF/IL-6/IL-10 transiently elicited a cell subpopulation with the phenotype (c-kit(high)Thy-1(low)) of fetal blood promastocytes at 3 wk of culture that progressed within 1 wk to FcepsilonRI-bearing PrMC, designated PrMCTriad. PrMCTriad lacked mouse mast cell carboxypeptidase A (mMC-CPA) protein, required SCF for IL-3-driven thymidine incorporation, and responded to SCF plus IL-3 with strong mMc-CPA immunoreactivity, clarifying distinct sequential roles for SCF and IL-3 in mast cell development. PrMCTriad, arising from BMC through promastocytes, are metamastocytes that acquire microenvironmentally determined phenotypic features.

Zahir, T., H. Nomura, et al. (2008). "Bioengineering neural stem/progenitor cell-coated tubes for spinal cord injury repair." Cell Transplant **17**(3): 245-54.

The aim of this study was to understand the survival and differentiation of neural stem/progenitor cells (NSPCs) cultured on chitosan matrices in vivo in a complete transection model of spinal cord injury. NSPCs were isolated from the subependyma of lateral ventricles of adult GFP transgenic rat forebrains. The GFP-positive neurospheres were seeded onto the inner lumen of chitosan tubes to generate multicellular sheets ex vivo. These bioengineered neurosphere tubes were implanted into a completely transected spinal cord and assessed after 5 weeks for survival and differentiation. The in vivo study showed excellent survival of NSPCs, as well as differentiation into astrocytes and oligodendrocytes. Importantly, host neurons were identified in the tissue bridge that formed within the chitosan tubes and bridged the transected cord stumps. The excellent in vivo survival of the NSPCs coupled with their differentiation and maintenance of host neurons in the regenerated tissue bridge demonstrates the promise of the chitosan tubes for stem cell delivery and tissue regeneration.

Zangiacomi, V., N. Balon, et al. (2008). "Cord blood-derived neurons are originated from CD133+/CD34 stem/progenitor cells in a cell-to-cell contact dependent manner." Stem Cells Dev **17**(5): 1005-16.

Previous studies described that neurons could be generated in vitro from human umbilical cord blood cells. However, there are few data concerning their origin. Notably, cells generating neurons are not well characterized. The present study deals with the origin of cord blood cells generating neurons and mechanisms allowing the neuronal differentiation. We studied neuronal markers of both total fractions of cord blood and stem/progenitor cord blood cells before and after selections and cultures. We also compared neuronal commitment of cord blood cells to that observed for the neuronal cell line SK-N-BE(2). Before cultures, neuronal markers are found within the total fraction of cord blood cells. In CD133+ stem/progenitor cell fraction only immature neuronal markers are detected. However, CD133+ cells are

unable to give rise to neurons in cultures, whereas this is achieved when total fraction of cord blood cells is used. In fact, mature functional neurons can be generated from CD133+ cells only in cell-to-cell close contact with either CD133- fraction or a neurogenic epithelium. Furthermore, since CD133+ fraction is heterogenous, we used several selections to precisely identify the phenotype of cord blood-derived neuronal stem/progenitor cells. Results reveal that only CD34- cells from CD133+ fraction possess neuronal potential. These data show the phenotype of cord blood neuronal stem/progenitor cells and the crucial role of direct cell-to-cell contact to achieve their commitment. Identifying the neuron supporting factors may be beneficial to the use of cord blood neuronal stem/progenitor cells for regenerative medicine.

Zardo, G., G. Cimino, et al. (2008). "Epigenetic plasticity of chromatin in embryonic and hematopoietic stem/progenitor cells: therapeutic potential of cell reprogramming." Leukemia **22**(8): 1503-18.

During embryonic development and adult life, the plasticity and reversibility of modifications that affect the chromatin structure is important in the expression of genes involved in cell fate decisions and the maintenance of cell-differentiated state. Epigenetic changes in DNA and chromatin, which must occur to allow the accessibility of transcriptional factors at specific DNA-binding sites, are regarded as emerging major players for embryonic and hematopoietic stem cell (HSC) development and lineage differentiation. Epigenetic deregulation of gene expression, whether it be in conjunction with chromosomal alterations and gene mutations or not, is a newly recognized mechanism that leads to several diseases, including leukemia. The reversibility of epigenetic modifications makes DNA and chromatin changes attractive targets for therapeutic intervention. Here we review some of the epigenetic mechanisms that regulate gene expression in pluripotent embryonic and multipotent HSCs but may be deregulated in leukemia, and the clinical approaches designed to target the chromatin structure in leukemic cells.

Zenzmaier, C., G. Untergasser, et al. (2008). "Aging of the prostate epithelial stem/progenitor cell." Exp Gerontol **43**(11): 981-5.

Maintenance of the prostatic epithelial cell compartment is ensured by proliferation of adult epithelial progenitor or stem cells. These cells are characterized by an undifferentiated state, high proliferative capacity and long life span. Prostate progenitor/stem cells are localized in their stem cell-niche in the basal cell compartment in close contact to the basement membrane and the stromal cell compartment and are characterized by expression of the basal cytokeratins 5 and 14, high levels of integrins, CD44, the stem cell markers CD133 and ABCG2, and AR negativity. They give rise to secretory luminal (cytokeratins 8/18, CD57, AR, p27, PSA, PAP) and neuroendocrine cells (cytokeratins 8/18, CD57, CgA, NSE, NEPs), the two major cell types observed in the glandular epithelium. A growing body of experimental evidence has identified the amplifying progenitor/stem cell (CD44(+), alpha(2)beta(1)(hi), CD133(+)), as a putative origin of prostate cancer. Differentiation of this cell type can be affected by mutations in the intrinsic genetic program, by age-related changes in stromal-epithelial interactions or in the basement membrane/ECM composition. All these stochastic events occur during aging and can transform a normal prostate progenitor/stem cell into a cancer stem cell, a source of androgen-dependent and independent tumor cell clones. Thus, the heterogeneous and multifocal nature of prostatic cancer with a pleora of different tumor cell clones clearly reflects the differentiation capacity of the prostatic epithelial progenitor cells.

Zhang, J., X. C. He, et al. (2006). "Bone morphogenetic protein signaling inhibits hair follicle anagen induction by restricting epithelial stem/progenitor cell activation and expansion." Stem Cells **24**(12): 2826-39.

Epithelial stem cells (EP-SCs) located in the bulge region of a hair follicle (HF) have the potential to give rise to hair follicle stem/progenitor cells that migrate down to regenerate HFs. Bone morphogenetic protein (BMP) signaling has been shown to regulate the HF cycle by inhibiting anagen induction. Here we show that active BMP signaling functions to prevent EP-SC activation and expansion. Dynamic expression of Noggin, a BMP antagonist, releases EP-SCs from BMP-mediated restriction, leading to EP-SC activation and initiation of the anagen phase. Experimentally induced conditional inactivation of the BMP type IA receptor (Bmpr1a) in EP-SCs leads to overproduction of HF stem/progenitor cells and the eventual formation of matricomas. This genetic manipulation of the BMP signaling pathway also reveals unexpected activation of beta-catenin, a major mediator of Wnt signaling. We propose that BMP activity controls the HF cycle by antagonizing Wnt/beta-catenin activity. This is at least partially achieved by BMP-mediated enhancement of transforming growth factor-beta-regulated epithelial cell-specific phosphatase (PTEN) function. Subsequently, PTEN, through phosphatidyl inositol 3-kinase-Akt, inhibits the activity of beta-catenin, the

convergence point of the BMP and Wnt signaling pathways.

Zhang, Y., N. Mukaida, et al. (1997). "Induction of dendritic cell differentiation by granulocyte-macrophage colony-stimulating factor, stem cell factor, and tumor necrosis factor alpha in vitro from lineage phenotypes-negative c-kit+ murine hematopoietic progenitor cells." Blood **90**(12): 4842-53.

To elucidate the capacity of murine early hematopoietic progenitor cells (HPCs) to differentiate into dendritic cells (DCs), lineage phenotypes (Lin)-c-kit+ HPCs were highly purified from either wild-type or tumor necrosis factor (TNF) receptor p55 (TNF-Rp55)-deficient mice. Upon culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SCF) for 14 days, wild-type mouse Lin-c-kit+ HPCs did not exhibit characteristic features of DC such as sheet-like projections and veil processes. Moreover, these cells expressed a marginal level of DC markers such as DEC-205, CD86, and barely supported allogenic MLR. However, the addition of mouse TNFalpha generated a large number of cells with typical DC morphology, expression of high levels of Ia, DEC-205, CD86, and function of stimulating allogenic MLR. Moreover, a proportion of these mature DCs and thymic DCs expressed Thy-1 mRNA as well as Thy-1 antigen, whereas freshly isolated splenic DCs did not. These results suggested that DCs generated in our culture system phenotypically resemble thymic ones. In contrast, mouse TNFalpha failed to induce TNF-Rp55-deficient mice-derived Lin-c-kit+ HPCs to generate DCs with characteristic morphology, immunophenotype, and accessory function for T cells under the same culture conditions, suggesting a crucial role of TNF-Rp55 in TNFalpha-mediated DC differentiation from HPCs. Interestingly, human TNFalpha, which can bind to mouse TNF-Rp55 but not TNF-Rp75, was incapable to augment DC generation from wild-type mouse Lin-c-kit+ HPCs. Collectively, these results suggest that TNFalpha has a pivotal role in DC generation from murine early HPCs in collaboration with GM-CSF and SCF through the interaction of TNF-Rp55 and TNF-Rp75.

Zhao, B., S. L. Allinson, et al. (2008). "Targeted cornea limbal stem/progenitor cell transfection in an organ culture model." Invest Ophthalmol Vis Sci **49**(8): 3395-401.

PURPOSE: To optimize a nonviral gene transfection system targeting the corneal limbal stem/progenitor cells. METHODS: A plasmid containing LacZ gene coding for beta-galactosidase (beta-gal) was transfected into human corneal epithelial cells (HCECs) and multilineage progenitor cells (MLPCs) with different transfection reagents, to determine the optimal transfection reagent. In an ex vivo study, the bovine corneal epithelium and limbal stem/progenitor cells were transfected with a microinjection system with a 36-gauge needle that delivered plasmid/transfection reagent (Lipofectamine 2000; Invitrogen, Carlsbad, CA) complexes. The transfected corneoscleral discs were cultured in an air-interface culture system. The expression of beta-gal was determined with an X-gal staining assay, and images were acquired with light microscopy and transmission electron microscopy. The expression of cytokeratin K5/14 and K3/K12 in corneal and limbal epithelium was determined by immunohistochemistry. RESULTS: The highest percentages of beta-gal expression in HCECs and MLPCs were achieved when the transfection reagent Lipofectamine 2000 was used. Corneal epithelial and limbal basal cells were successfully transfected with the reporter gene by targeted microinjection of plasmid/liposomal complexes. The location of the bovine limbal stem/progenitor cells was confirmed by positive K5/K14 labeling and negative K3/12 labeling. CONCLUSIONS: Targeted microinjection of plasmid/liposomal complexes resulted in limbal stem/progenitor cell transfection. This technique has potential for the short-term treatment of corneal diseases.

Zhao, M., S. A. Amiel, et al. (2007). "Evidence for the presence of stem cell-like progenitor cells in human adult pancreas." J Endocrinol **195**(3): 407-14.

The origin of cells replacing ageing beta-cells in adult life is unknown. This study assessed the expression of classic stem cell markers: Oct4, Sox2 and CD34 in islet-enriched fractions versus exocrine cell-enriched fractions from 25 adult human pancreases following human islet isolation. Expression of Oct4, Sox2 and CD34 mRNAs was found in all cell samples, with no significant differences between endocrine and exocrine cell fractions. Immunohistochemical staining for Oct4, Sox2, CD133, CD34, CK19, insulin and nestin on human pancreas sections showed that the majority of Oct4(+ve) cells were found in the walls of small ducts. Similar localisations were observed for Sox2(+ve) cells. The majority of Sox2(+ve) cells were found to co-express Oct4 proteins, but not vice versa. Cells positive for Oct4 and Sox2 appeared to be a unique cell population in the adult human pancreases without co-expression for CK19, CD34, CD133, insulin and nestin proteins. The numbers of Oct4(+ve) and Sox2(+ve) cells varied among donors and were approximately 1-200 and 1-30 per 100 000 pancreatic cells respectively.

Zheng, H., H. Ying, et al. (2008). "p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation." Nature **455**(7216): 1129-33.

Glioblastoma (GBM) is a highly lethal brain tumour presenting as one of two subtypes with distinct clinical histories and molecular profiles. The primary GBM subtype presents acutely as a high-grade disease that typically harbours mutations in EGFR, PTEN and INK4A/ARF (also known as CDKN2A), and the secondary GBM subtype evolves from the slow progression of a low-grade disease that classically possesses PDGF and TP53 events. Here we show that concomitant central nervous system (CNS)-specific deletion of p53 and Pten in the mouse CNS generates a penetrant acute-onset high-grade malignant glioma phenotype with notable clinical, pathological and molecular resemblance to primary GBM in humans. This genetic observation prompted TP53 and PTEN mutational analysis in human primary GBM, demonstrating unexpectedly frequent inactivating mutations of TP53 as well as the expected PTEN mutations. Integrated transcriptomic profiling, in silico promoter analysis and functional studies of murine neural stem cells (NSCs) established that dual, but not singular, inactivation of p53 and Pten promotes an undifferentiated state with high renewal potential and drives increased Myc protein levels and its associated signature. Functional studies validated increased Myc activity as a potent contributor to the impaired differentiation and enhanced renewal of NSCs doubly null for p53 and Pten (p53(-/-) Pten(-/-)) as well as tumour neurospheres (TNSs) derived from this model. Myc also serves to maintain robust tumorigenic potential of p53(-/-) Pten(-/-) TNSs. These murine modelling studies, together with confirmatory transcriptomic/promoter studies in human primary GBM, validate a pathogenetic role of a common tumour suppressor mutation profile in human primary GBM and establish Myc as an important target for cooperative actions of p53 and Pten in the regulation of normal and malignant stem/progenitor cell differentiation, self-renewal and tumorigenic potential.

Zheng, Y., A. Sun, et al. (2005). "Stem cell factor improves SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem/progenitor cells in xenotransplanted NOD/SCID mouse model." Bone Marrow Transplant **35**(2): 137-42.

Poor in vivo homing capacity of hematopoietic stem/progenitor cells (HS/PCs) from umbilical cord blood (UCB) can be reversed by short-term ex vivo manipulation with recombinant human stem cell factor (rHuSCF). This study was designed to evaluate the effect of ex vivo manipulation of UCB-derived HS/PCs with rHuSCF on human cell engraftment rates in xenotransplanted NOD/SCID mouse model. The human cell engraftment rates in xenotransplanted primary and secondary NOD/SCID mice were characterized using four-color flow cytometric analysis and progenitor assay. Grafts of rHuSCF-treated UCB CD34(+) cells resulted in significantly higher levels of human cell engraftment than that of nontreated ones in both xenotransplanted primary and secondary NOD/SCID recipients. Fresh UCB CD34(+) cells did not express either of the matrix metalloproteinase (MMP) family members MMP-2 or MMP-9. rHuSCF-treated UCB CD34(+) cells expressed significant levels of MMP-2 and MMP-9. Pretreatment of UCB CD34(+) cells with the specific MMP inhibitor completely blocked human cell engraftment in xenotransplanted NOD/SCID recipients. Our results indicate that ex vivo manipulation of human HS/PCs with rHuSCF might provide an optimal approach to develop more effective stem cell-based therapies in situations where engraftment is delayed due to limiting HS/PCs number, for example, UCB transplantation.

Zheng, Y., N. Watanabe, et al. (2003). "Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their transmigratory potential." Exp Hematol **31**(12): 1237-46.

OBJECTIVE: The cause of delayed hematopoietic reconstitution after umbilical cord blood transplantation (UCBT) remains controversial. We hypothesized that hematopoietic stem/progenitor cells (HS/PCs) from UCB have some defects of the homing-related molecules responsible for their slow engraftment. MATERIALS AND METHODS: A homing-related molecule repertoire expressed on HS/PCs from fresh and cryopreserved UCB, mobilized peripheral blood (mPB), and bone marrow (BM) were compared using sensitive, four-color fluorescence-activated cell sorting analysis. Purified CD34+ cells were subjected to ex vivo transmigration through double-coated transwell filter inserts, and an in vivo homing assay was performed in xenotransplanted NOD/SCID mice. RESULTS: UCB-derived CD34(bright) cells expressed significantly lower levels of CD49e, CD49f, and CXCR-4 than their mPB and BM counterparts. CD34+ cells from UCB (and BM) exhibited significantly lower ex vivo transmigration than those from mPB, which were largely blocked by neutralizing antibodies to CD49e or CD49f. Recombinant human tumor necrosis factor-

alpha treatment enhanced ex vivo transmigration of CD34+ cells from UCB and BM by inducing expression of the matrix metalloproteinases MMP-2/MMP-9. Short-term treatment of UCB-derived CD34+ cells with rHu-stem cell factor (rHuSCF) up-regulated levels of the homing-related molecules with their increased ex vivo transmigratory and in vivo homing potential. CONCLUSION: Our results indicate that disadvantageous transmigratory behavior of HS/PCs from UCB, which might partly explain the delayed reconstitution after UCBT, can be reversed by ex vivo manipulation with rHuSCF.

Zhong, L., W. Li, et al. (2006). "Evaluation of primitive murine hematopoietic stem and progenitor cell transduction in vitro and in vivo by recombinant adeno-associated virus vector serotypes 1 through 5." Hum Gene Ther **17**(3): 321-33.

Conflicting data exist on hematopoietic cell transduction by AAV serotype 2 (AAV2) vectors, and additional AAV serotype vectors have not been evaluated for their efficacy in hematopoietic stem/progenitor cell transduction. We evaluated the efficacy of conventional, single-stranded AAV serotype vectors 1 through 5 in primitive murine hematopoietic stem/progenitor cells in vitro as well as in vivo. In progenitor cell assays using Sca1+ c-kit+ Lin- hematopoietic cells, 9% of the colonies in cultures infected with AAV1 expressed the transgene. Coinfection of AAV1 with self-complementary AAV vectors carrying the gene for T cell protein tyrosine phosphatase (scAAV-TC-PTP) increased the transduction efficiency to 24%, indicating that viral secondstrand DNA synthesis is a rate-limiting step. This was further corroborated by the use of scAAV vectors, which bypass this requirement. In bone marrow transplantation studies involving lethally irradiated syngeneic mice, Sca1+ c-kit+ Lin- cells coinfected with AAV1 +/- scAAV-TC-PTP vectors led to transgene expression in 2 and 7.5% of peripheral blood (PB) cells, respectively, 6 months posttransplantation. In secondary transplantation experiments, 7% of PB cells and 3% of bone marrow (BM) cells expressed the transgene 6 months posttransplantation. Approximately 21% of BM-derived colonies harbored the proviral DNA sequences in integrated forms. These results document that AAV1 is thus far the most efficient vector in transducing primitive murine hematopoietic stem/progenitor cells. Further studies involving scAAV genomes and hematopoietic cell-specific promoters should further augment the transduction efficiency of AAV1 vectors, which should have implications in the optimal use of these vectors in hematopoietic stem cell gene therapy.

Zhou, Z., A. Flesken-Nikitin, et al. (2007). "Prostate cancer associated with p53 and Rb deficiency arises from the stem/progenitor cell-enriched proximal region of prostatic ducts." Cancer Res **67**(12): 5683-90.

Recently, we have shown that prostate epithelium-specific deficiency for p53 and Rb tumor suppressors leads to metastatic cancer, exhibiting features of both luminal and neuroendocrine differentiation. Using stage-by-stage evaluation of carcinogenesis in this model, we report that all malignant neoplasms arise from the proximal region of the prostatic ducts, the compartment highly enriched for prostatic stem/progenitor cells. In close similarity to reported properties of prostatic stem cells, the cells of the earliest neoplastic lesions express stem cell marker stem cell antigen 1 and are not sensitive to androgen withdrawal. Like a subset of normal cells located in the proximal region of prostatic ducts, the early neoplastic cells coexpress luminal epithelium markers cytokeratin 8, androgen receptor, and neuroendocrine markers synaptophysin and chromogranin A. Inactivation of p53 and Rb also takes place in the lineage-committed transit-amplifying and/or differentiated cells of the distal region of the prostatic ducts. However, the resulting prostatic intraepithelial neoplasms never progress to carcinoma by the time of mouse death. Interestingly, in an ectopic transplantation assay, early mutant cells derived from either region of the prostatic ducts are capable of forming neoplasms within 3 months. These findings indicate that p53 and Rb are critically important for the regulation of the prostatic stem cell compartment, the transformation in which may lead to particularly aggressive cancers in the context of microenvironment.

References

1. Aboody, K. S., J. Najbauer, et al. (2008). "Stem and progenitor cell-mediated tumor selective gene therapy." Gene Ther **15**(10): 739-52.
2. Adler, E. D., A. Bystrup, et al. (2009). "In vivo detection of embryonic stem cell-derived cardiovascular progenitor cells using Cy3-labeled Gadofluorine M in murine myocardium." JACC Cardiovasc Imaging **2**(9): 1114-22.
3. Aicher, A., O. Kollet, et al. (2008). "The Wnt antagonist Dickkopf-1 mobilizes vasculogenic progenitor cells via activation of the bone marrow endosteal stem cell niche." Circ Res **103**(8): 796-803.
4. Aiuti, A., C. Friedrich, et al. (1998). "Identification of distinct elements of the stromal microenvironment that control human hematopoietic stem/progenitor cell growth and differentiation." Exp Hematol **26**(2): 143-57.
5. Aizawa, Y., N. Leipzig, et al. (2008). "The effect of immobilized platelet derived growth factor AA on neural stem/progenitor cell differentiation on cell-adhesive hydrogels." Biomaterials **29**(35): 4676-83.
6. Akel, S., C. Petrow-Sadowski, et al. (2003). "Neutralization of autocrine transforming growth factor-beta in human cord blood CD34(+)CD38(-)Lin(-) cells promotes stem-cell-

factor-mediated erythropoietin-independent early erythroid progenitor development and reduces terminal differentiation." Stem Cells **21**(5): 557-67.
7. Alam, S., A. Sen, et al. (2004). "Cell cycle kinetics of expanding populations of neural stem and progenitor cells in vitro." Biotechnol Bioeng **88**(3): 332-47.
8. Albo, C., J. de la Fuente, et al. (2004). "Kinetics and immunophenotypic characterization of circulating hematopoietic progenitor cells after peripheral blood stem cell transplantation." Haematologica **89**(7): 845-51.
9. Al-Shaibi, N. and S. K. Ghosh (2009). "A novel cell-surface protein CSP82 on bone marrow stem cells and a cytosolic phosphoprotein DP58 (ankyrinRD 34B) are involved in promyeloid progenitor induction." Cell Immunol **258**(2): 172-80.
10. Andrews, R. G., R. A. Briddell, et al. (1994). "In vivo synergy between recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in baboons enhanced circulation of progenitor cells." Blood **84**(3): 800-10.
11. Andrews, R. G., R. A. Briddell, et al. (1995). "Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in nonhuman primates." Blood **85**(1): 15-20.
12. Aoyama, K., K. Oritani, et al. (1999). "Stromal cell CD9 regulates differentiation of hematopoietic stem/progenitor cells." Blood **93**(8): 2586-94.
13. Askenasy, N., J. Stein, et al. (2007). "Imaging approaches to hematopoietic stem and progenitor cell function and engraftment." Immunol Invest **36**(5-6): 713-38.
14. Astori, G., W. Malangone, et al. (2001). "A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use." Blood Cells Mol Dis **27**(4): 715-24; discussion 725-7.
15. Ayach, B. B., M. Yoshimitsu, et al. (2006). "Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction." Proc Natl Acad Sci U S A **103**(7): 2304-9.
16. Bakondi, B., I. S. Shimada, et al. (2009). "CD133 identifies a human bone marrow stem/progenitor cell sub-population with a repertoire of secreted factors that protect against stroke." Mol Ther **17**(11): 1938-47.
17. Barkho, B. Z., H. Song, et al. (2006). "Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation." Stem Cells Dev **15**(3): 407-21.
18. Bartkowiak, K., M. Wieczorek, et al. (2009). "Two-dimensional differential gel electrophoresis of a cell line derived from a breast cancer micrometastasis revealed a stem/ progenitor cell protein profile." J Proteome Res **8**(4): 2004-14.
19. Basser, R. L., L. B. To, et al. (1998). "Rapid hematopoietic recovery after multicycle high-dose chemotherapy: enhancement of filgrastim-induced progenitor-cell mobilization by recombinant human stem-cell factor." J Clin Oncol **16**(5): 1899-908.
20. Baumann, U., H. A. Crosby, et al. (1999). "Expression of the stem cell factor receptor c-kit in normal and diseased pediatric liver: identification of a human hepatic progenitor cell?" Hepatology **30**(1): 112-7.
21. Baumert, B., K. Grymula, et al. (2008). "An optimization of hematopoietic stem and progenitor cell isolation for scientific and clinical purposes by the application of a new parameter determining the hematopoietic graft efficacy." Folia Histochem Cytobiol **46**(3): 299-305.
22. Beckmann, J., S. Scheitza, et al. (2007). "Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment: identification of asymmetrically segregating proteins." Blood **109**(12): 5494-501.
23. Begley, C. G., R. Basser, et al. (1997). "Enhanced levels and enhanced clonogenic capacity of blood progenitor cells following administration of stem cell factor plus granulocyte colony-stimulating factor to humans." Blood **90**(9): 3378-89.
24. Bello, B. C., N. Izergina, et al. (2008). "Amplification of neural stem cell proliferation by intermediate progenitor cells in Drosophila brain development." Neural Dev **3**: 5.
25. Bender, J. G., S. F. Williams, et al. (1992). "Characterization of chemotherapy mobilized peripheral blood progenitor cells for use in autologous stem cell transplantation." Bone Marrow Transplant **10**(3): 281-5.
26. Benet, I., B. F. Prosper, et al. (1999). "Mobilization of peripheral blood progenitor cells (PBPC) in patients undergoing chemotherapy followed by autologous peripheral blood stem cell transplant (SCT) for high risk breast cancer (HRBC)." Bone Marrow Transplant **23**(11): 1101-7.
27. Beretta, F., S. van den Bosch, et al. (1998). "Intrapatient comparison of an intermittent and a continuous flow cell separator for the collection of progenitor and stem cells from the blood." Vox Sang **75**(2): 149-53.
28. Boecker, W. and H. Buerger (2003). "Evidence of progenitor cells of glandular and myoepithelial cell lineages in the human adult female breast epithelium: a new progenitor (adult stem) cell concept." Cell Prolif **36 Suppl 1**: 73-84.
29. Boecker, W., R. Moll, et al. (2002). "Usual ductal hyperplasia of the breast is a committed stem (progenitor) cell lesion distinct from atypical ductal hyperplasia and ductal carcinoma in situ." J Pathol **198**(4): 458-67.
30. Bongiorno, M. R., S. Doukaki, et al. (2008). "Identification of progenitor cancer stem cell in lentigo maligna melanoma." Dermatol Ther **21 Suppl 1**: S1-5.
31. Bonig, H., K. L. Watts, et al. (2009). "Concurrent blockade of alpha4-integrin and CXCR4 in hematopoietic stem/progenitor cell mobilization." Stem Cells **27**(4): 836-7.
32. Borgs, L., P. Beukelaers, et al. (2009). "Period 2 regulates neural stem/progenitor cell proliferation in the adult hippocampus." BMC Neurosci **10**: 30.
33. Boswell, H. S., P. M. Wade, Jr., et al. (1984). "Thy-1 antigen expression by murine high-proliferative capacity hematopoietic progenitor cells. I. Relation between sensitivity to depletion by Thy-1 antibody and stem cell generation potential." J Immunol **133**(6): 2940-9.
34. Boyd, N. L., K. R. Robbins, et al. (2009). "Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells." Tissue Eng Part A **15**(8): 1897-907.
35. Brice, P., J. P. Marolleau, et al. (1996). "Hematologic recovery and survival of lymphoma patients after autologous stem-cell transplantation: comparison of bone marrow and peripheral blood progenitor cells." Leuk Lymphoma **22**(5-6): 449-56.
36. Briddell, R. A., C. A. Hartley, et al. (1993). "Recombinant rat stem cell factor synergizes with recombinant human granulocyte colony-stimulating factor in vivo in mice to mobilize peripheral blood progenitor cells that have enhanced repopulating potential." Blood **82**(6): 1720-3.
37. Brooks, Y. S., G. Wang, et al. (2009). "Functional pre-mRNA trans-splicing of coactivator CoAA and corepressor RBM4 during stem/progenitor cell differentiation." J Biol Chem **284**(27): 18033-46.
38. Brown, J., M. F. Greaves, et al. (1991). "The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts." Int Immunol **3**(2): 175-84.
39. Broxmeyer, H. E. (1995). "Cord blood as an alternative source for stem and progenitor cell transplantation." Curr Opin Pediatr **7**(1): 47-55.

40. Broxmeyer, H. E., L. Kohli, et al. (2003). "Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells." J Leukoc Biol **73**(5): 630-8.
41. Brugger, W., W. Mocklin, et al. (1993). "Ex vivo expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, interleukin-1 beta (IL-1 beta), IL-6, IL-3, interferon-gamma, and erythropoietin." Blood **81**(10): 2579-84.
42. Bruns, I., A. Czibere, et al. (2009). "The hematopoietic stem cell in chronic phase CML is characterized by a transcriptional profile resembling normal myeloid progenitor cells and reflecting loss of quiescence." Leukemia **23**(5): 892-9.
43. Brunt, K. R., S. R. Hall, et al. (2007). "Endothelial progenitor cell and mesenchymal stem cell isolation, characterization, viral transduction." Methods Mol Med **139**: 197-210.
44. Buhring, H. J., S. Kuci, et al. (2004). "CDCP1 identifies a broad spectrum of normal and malignant stem/progenitor cell subsets of hematopoietic and nonhematopoietic origin." Stem Cells **22**(3): 334-43.
45. Bull, N. D. and P. F. Bartlett (2005). "The adult mouse hippocampal progenitor is neurogenic but not a stem cell." J Neurosci **25**(47): 10815-21.
46. Burchfield, J. S. and S. Dimmeler (2008). "Role of paracrine factors in stem and progenitor cell mediated cardiac repair and tissue fibrosis." Fibrogenesis Tissue Repair **1**(1): 4.
47. Bussolati, B., C. Grange, et al. (2009). "Endothelial cell differentiation of human breast tumour stem/progenitor cells." J Cell Mol Med **13**(2): 309-319.
48. Cairo, M. S., P. Law, et al. (1992). "The in vitro effects of stem cell factor and PIXY321 on myeloid progenitor formation (CFU-GM) from immunomagnetic separated CD34+ cord blood." Pediatr Res **32**(3): 277-81.
49. Campbell, T. B., S. Basu, et al. (2009). "Overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation." Blood **114**(16): 3392-401.
50. Capuco, A. V., C. M. Evock-Clover, et al. (2009). "In vivo expansion of the mammary stem/ progenitor cell population by xanthosine infusion." Exp Biol Med (Maywood) **234**(4): 475-82.
51. Care, A., M. Valtieri, et al. (1999). "Enforced expression of HOXB7 promotes hematopoietic stem cell proliferation and myeloid-restricted progenitor differentiation." Oncogene **18**(11): 1993-2001.
52. Carpentino, J. E., N. W. Hartman, et al. (2008). "Region-specific differentiation of embryonic stem cell-derived neural progenitor transplants into the adult mouse hippocampus following seizures." J Neurosci Res **86**(3): 512-24.
53. Cartron, G., O. Herault, et al. (2002). "Quantitative and qualitative analysis of the human primitive progenitor cell compartment after autologous stem cell transplantation." J Hematother Stem Cell Res **11**(2): 359-68.
54. Cesana, C., C. Carlo-Stella, et al. (1997). "In vitro growth of mobilized peripheral blood progenitor cells is significantly enhanced by stem cell factor." Stem Cells **15**(3): 207-13.
55. Chan, K. M., S. Bonde, et al. (2008). "Hematopoiesis and immunity of HOXB4-transduced embryonic stem cell-derived hematopoietic progenitor cells." Blood **111**(6): 2953-61.
56. Chaussain, C., A. S. Eapen, et al. (2009). "MMP2-cleavage of DMP1 generates a bioactive peptide promoting differentiation of dental pulp stem/progenitor cell." Eur Cell Mater **18**: 84-95.
57. Chen, J., N. Hersmus, et al. (2005). "The adult pituitary contains a cell population displaying stem/progenitor cell and early embryonic characteristics." Endocrinology **146**(9): 3985-98.
58. Cheng, T. and D. T. Scadden (2002). "Cell cycle entry of hematopoietic stem and progenitor cells controlled by distinct cyclin-dependent kinase inhibitors." Int J Hematol **75**(5): 460-5.
59. Christophersen, N. S., X. Meijer, et al. (2006). "Induction of dopaminergic neurons from growth factor expanded neural stem/progenitor cell cultures derived from human first trimester forebrain." Brain Res Bull **70**(4-6): 457-66.
60. Chung, I. J., C. Dai, et al. (2003). "Stem cell factor increases the expression of FLIP that inhibits IFNgamma -induced apoptosis in human erythroid progenitor cells." Blood **101**(4): 1324-8.
61. Cicuttini, F. M., C. G. Begley, et al. (1992). "The effect of recombinant stem cell factor (SCF) on purified CD34-positive human umbilical cord blood progenitor cells." Growth Factors **6**(1): 31-9.
62. Clore, J. N., A. R. Sharpe, et al. (1988). "Thyrotropin-induced hyperthyroidism: evidence for a common progenitor stem cell." Am J Med Sci **295**(1): 3-5.
63. Coles, B. L., D. J. Horsford, et al. (2006). "Loss of retinal progenitor cells leads to an increase in the retinal stem cell population in vivo." Eur J Neurosci **23**(1): 75-82.
64. Collis, S. J., S. Neutzel, et al. (2004). "Hematopoietic progenitor stem cell homing in mice lethally irradiated with ionizing radiation at differing dose rates." Radiat Res **162**(1): 48-55.
65. Colvin, G. A., J. F. Lambert, et al. (2004). "Intrinsic hematopoietic stem cell/progenitor plasticity: Inversions." J Cell Physiol **199**(1): 20-31.
66. Corrales, C. E., L. Pan, et al. (2006). "Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti." J Neurobiol **66**(13): 1489-500.
67. Couillard, M. and M. Trudel (2009). "C-myc as a modulator of renal stem/progenitor cell population." Dev Dyn **238**(2): 405-14.
68. da Silva, C. L., R. Goncalves, et al. (2009). "Differences amid bone marrow and cord blood hematopoietic stem/progenitor cell division kinetics." J Cell Physiol **220**(1): 102-11.
69. da Silva, M. G., P. Pimentel, et al. (2004). "Ancestim (recombinant human stem cell factor, SCF) in association with filgrastim does not enhance chemotherapy and/or growth factor-induced peripheral blood progenitor cell (PBPC) mobilization in patients with a prior insufficient PBPC collection." Bone Marrow Transplant **34**(8): 683-91.
70. Dahl, L., K. Richter, et al. (2008). "Lhx2 expression promotes self-renewal of a distinct multipotential hematopoietic progenitor cell in embryonic stem cell-derived embryoid bodies." PLoS One **3**(4): e2025.
71. Dai, C. H., S. B. Krantz, et al. (1994). "Polycythaemia vera. IV. Specific binding of stem cell factor to normal and polycythaemia vera highly purified erythroid progenitor cells." Br J Haematol **88**(3): 497-505.
72. D'Ascenzo, M., R. Piacentini, et al. (2006). "Role of L-type Ca2+ channels in neural stem/progenitor cell differentiation." Eur J Neurosci **23**(4): 935-44.
73. David, R., H. Theiss, et al. (2008). "Connexin 40 promoter-based enrichment of embryonic stem cell-derived cardiovascular progenitor cells." Cells Tissues Organs **188**(1-2): 62-9.
74. de Boer, F., A. M. Drager, et al. (2002). "Extensive early apoptosis in frozen-thawed CD34-positive stem cells decreases threshold doses for haematological recovery after autologous peripheral blood progenitor cell transplantation." Bone Marrow Transplant **29**(3): 249-55.

75. De Bruyn, C., A. Delforge, et al. (1994). "Modulation of human cord blood progenitor cell growth by recombinant human interleukin 3 (IL-3), IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF) in serum-supplemented and serum-free medium." Stem Cells **12**(6): 616-25.
76. de Kruijf, E. J., M. van Pel, et al. (2007). "Repeated hematopoietic stem and progenitor cell mobilization without depletion of the bone marrow stem and progenitor cell pool in mice after repeated administration of recombinant murine G-CSF." Hum Immunol **68**(5): 368-74.
77. De Propris, M. S., G. Meloni, et al. (2003). "Possibility of progenitor cell mobilization during the hematological recovery following peripheral blood stem cell autograft." Acta Haematol **109**(2): 57-63.
78. del Rosario, M. L. and K. I. Kao (1997). "Determination of the rate of reduction in platelet counts in recipients of hematopoietic stem and progenitor cell transplant: clinical implications for platelet transfusion therapy." Transfusion **37**(11-12): 1163-8.
79. Demirer, T., O. Ilhan, et al. (2001). "CD41+ and CD42+ hematopoietic progenitor cells may predict platelet engraftment after allogeneic peripheral blood stem cell transplantation." J Clin Apher **16**(2): 67-73.
80. Demirkazik, A., A. Kessinger, et al. (2001). "Progenitor and lymphoma cells in blood stem cell harvests: impact on survival following transplantation." Bone Marrow Transplant **28**(2): 207-12.
81. Demirkazik, A., A. Kessinger, et al. (2002). "Effect of prior therapy and bone marrow metastases on progenitor cell content of blood stem cell harvests in breast cancer patients." Biol Blood Marrow Transplant **8**(5): 268-72.
82. Di Rosa, P., J. C. Villaescusa, et al. (2007). "The homeodomain transcription factor Prep1 (pKnox1) is required for hematopoietic stem and progenitor cell activity." Dev Biol **311**(2): 324-34.
83. Donahue, R. E., K. Kuramoto, et al. (2005). "Large animal models for stem and progenitor cell analysis." Curr Protoc Immunol **Chapter 22**: Unit 22A 1.
84. Dontu, G., K. W. Jackson, et al. (2004). "Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells." Breast Cancer Res **6**(6): R605-15.
85. Douay, L., M. C. Giarratana, et al. (1995). "Characterization of late and early hematopoietic progenitor/stem cell sensitivity to mafosfamide." Bone Marrow Transplant **15**(5): 769-75.
86. Drachman, J. G. (2000). "Role of thrombopoietin in hematopoietic stem cell and progenitor regulation." Curr Opin Hematol **7**(3): 183-90.
87. Dreger, P., M. Kloss, et al. (1995). "Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts." Blood **86**(10): 3970-8.
88. Drize, N., J. Chertkov, et al. (1995). "Hematopoietic progenitor cell mobilization into the peripheral blood of mice using a combination of recombinant rat stem cell factor (rrSCF) and recombinant human granulocyte colony-stimulating factor (rhG-CSF)." Exp Hematol **23**(11): 1180-6.
89. Drouet, M., F. Herodin, et al. (2001). "Cell cycle activation of peripheral blood stem and progenitor cells expanded ex vivo with SCF, FLT-3 ligand, TPO, and IL-3 results in accelerated granulocyte recovery in a baboon model of autologous transplantation but G0/G1 and S/G2/M graft cell content does not correlate with transplantability." Stem Cells **19**(5): 436-42.
90. Easterday, M. C., J. D. Dougherty, et al. (2003). "Neural progenitor genes. Germinal zone expression and analysis of genetic overlap in stem cell populations." Dev Biol **264**(2): 309-22.
91. Era, T. and O. N. Witte (2000). "Regulated expression of P210 Bcr-Abl during embryonic stem cell differentiation stimulates multipotential progenitor expansion and myeloid cell fate." Proc Natl Acad Sci U S A **97**(4): 1737-42.
92. Erlich, S., S. R. Miranda, et al. (1999). "Fluorescence-based selection of gene-corrected hematopoietic stem and progenitor cells from acid sphingomyelinase-deficient mice: implications for Niemann-Pick disease gene therapy and the development of improved stem cell gene transfer procedures." Blood **93**(1): 80-6.
93. Ezoe, S., I. Matsumura, et al. (2004). "Cell cycle regulation in hematopoietic stem/progenitor cells." Cell Cycle **3**(3): 314-8.
94. Fabian, I., D. Douer, et al. (1985). "Human spleen cell generation of factors stimulating human pluripotent stem cell, erythroid, and myeloid progenitor cell growth." Blood **65**(4): 990-6.
95. Facon, T., J. L. Harousseau, et al. (1999). "Stem cell factor in combination with filgrastim after chemotherapy improves peripheral blood progenitor cell yield and reduces apheresis requirements in multiple myeloma patients: a randomized, controlled trial." Blood **94**(4): 1218-25.
96. Fahlman, C., F. W. Jacobsen, et al. (1994). "Tumor necrosis factor-alpha (TNF-alpha) potently enhances in vitro macrophage production from primitive murine hematopoietic progenitor cells in combination with stem cell factor and interleukin-7: novel stimulatory role of p55 TNF receptors." Blood **84**(5): 1528-33.
97. Farkas, L. M. and W. B. Huttner (2008). "The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development." Curr Opin Cell Biol **20**(6): 707-15.
98. Feng, Y., Y. L. Gao, et al. (2007). "Expression change of stem cell-derived neural stem/progenitor cell supporting factor gene in injured spinal cord of rats." Neurosci Bull **23**(3): 165-9.
99. Ferrero, D., C. Cherasco, et al. (1999). "In vitro growth and quantification of early (CD33-/CD38-) myeloid progenitor cells: stem cell factor requirement and effects of previous chemotherapy." Haematologica **84**(5): 390-6.
100. Ferret-Bernard, S., P. Sai, et al. (2004). "In vitro induction of inhibitory macrophage differentiation by granulocyte-macrophage colony-stimulating factor, stem cell factor and interferon-gamma from lineage phenotypes-negative c-kit-positive murine hematopoietic progenitor cells." Immunol Lett **91**(2-3): 221-7.
101. Fierabracci, A., M. A. Puglisi, et al. (2008). "Identification of an adult stem/progenitor cell-like population in the human thyroid." J Endocrinol **198**(3): 471-87.
102. Filshie, R. J. (2002). "Cytokines in haemopoietic progenitor mobilisation for peripheral blood stem cell transplantation." Curr Pharm Des **8**(5): 379-94.
103. Fischer, J. C., M. Frick, et al. (2005). "Superior mobilisation of haematopoietic progenitor cells with glycosylated G-CSF in male but not female unrelated stem cell donors." Br J Haematol **130**(5): 740-6.
104. Fois, E., M. Desmartin, et al. (2007). "Recovery, viability and clinical toxicity of thawed and washed haematopoietic progenitor cells: analysis of 952 autologous peripheral blood stem cell transplantations." Bone Marrow Transplant **40**(9): 831-5.
105. Forraz, N., R. Pettengell, et al. (2004). "Characterization of a lineage-negative stem-progenitor cell population optimized for ex vivo expansion and enriched for LTC-IC." Stem Cells **22**(1): 100-8.
106. Forsyth, N. R. and J. McWhir (2008). "Human embryonic stem cell telomere length impacts directly on clonal progenitor isolation frequency." Rejuvenation Res **11**(1): 5-17.

107. Fortunel, N. O., J. A. Hatzfeld, et al. (2003). "Control of hematopoietic stem/progenitor cell fate by transforming growth factor-beta." Oncol Res **13**(6-10): 445-53.
108. Fortunel, N., J. Hatzfeld, et al. (2000). "Release from quiescence of primitive human hematopoietic stem/progenitor cells by blocking their cell-surface TGF-beta type II receptor in a short-term in vitro assay." Stem Cells **18**(2): 102-11.
109. Foster, G. A. and B. M. Stringer (1999). "Genetic regulatory elements introduced into neural stem and progenitor cell populations." Brain Pathol **9**(3): 547-67.
110. Fraser, J., I. Wulur, et al. (2007). "Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots." Cytotherapy **9**(5): 459-67.
111. Froberg, M. K., U. C. Garg, et al. (1999). "Changes in serum osteocalcin and bone-specific alkaline phosphatase are associated with bone pain in donors receiving granulocyte-colony-stimulating factor for peripheral blood stem and progenitor cell collection." Transfusion **39**(4): 410-4.
112. Fu, S. Q., C. N. Abboud, et al. (2002). "Impact of mobilized blood progenitor cell quality determined by the CFU-GM/CD34+ ratio on rapid engraftment after blood stem cell transplantation." Blood Cells Mol Dis **28**(3): 315-21.
113. Fujii, S., H. Maeda, et al. (2008). "Investigating a clonal human periodontal ligament progenitor/stem cell line in vitro and in vivo." J Cell Physiol **215**(3): 743-9.
114. Fujii, T., Y. Zen, et al. (2008). "Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma--human and cell culture study." Hum Pathol **39**(8): 1185-96.
115. Fujimori, Y., K. Izumi, et al. (2009). "Isolation of small-sized human epidermal progenitor/stem cells by Gravity Assisted Cell Sorting (GACS)." J Dermatol Sci **56**(3): 181-7.
116. Fujita, A., M. Kobayashi, et al. (1996). "Synergistic response of cord blood myeloid progenitor cells to the combined administration of human granulocyte colony-stimulating factor and human stem cell factor in vitro." Pediatr Res **40**(3): 388-92.
117. Fukuchi, Y., H. Nakajima, et al. (2004). "Human placenta-derived cells have mesenchymal stem/progenitor cell potential." Stem Cells **22**(5): 649-58.
118. Fukuda, S. and L. M. Pelus (2002). "Elevation of Survivin levels by hematopoietic growth factors occurs in quiescent CD34+ hematopoietic stem and progenitor cells before cell cycle entry." Cell Cycle **1**(5): 322-6.
119. Gabrilove, J. L., K. White, et al. (1994). "Stem cell factor and basic fibroblast growth factor are synergistic in augmenting committed myeloid progenitor cell growth." Blood **83**(4): 907-10.
120. Gastens, M. H., K. Goltry, et al. (2007). "Good manufacturing practice-compliant expansion of marrow-derived stem and progenitor cells for cell therapy." Cell Transplant **16**(7): 685-96.
121. Gaudron, S., C. Grillon, et al. (1999). "In vitro effect of acetyl-N-Ser-Asp-Lys-Pro (AcSDKP) analogs resistant to angiotensin I-converting enzyme on hematopoietic stem cell and progenitor cell proliferation." Stem Cells **17**(2): 100-6.
122. Georgantas, R. W., 3rd, R. Hildreth, et al. (2007). "CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control." Proc Natl Acad Sci U S A **104**(8): 2750-5.
123. Gersdorff Korsgaard, M. P., P. Christophersen, et al. (2001). "Identification of a novel voltage-gated Na+ channel rNa(v)1.5a in the rat hippocampal progenitor stem cell line HiB5." Pflugers Arch **443**(1): 18-30.
124. Ghiaur, G., A. Lee, et al. (2006). "Inhibition of RhoA GTPase activity enhances hematopoietic stem and progenitor cell proliferation and engraftment." Blood **108**(6): 2087-94.
125. Gibbons, A. E., P. Price, et al. (1995). "Analysis of hematopoietic stem and progenitor cell populations in cytomegalovirus-infected mice." Blood **86**(2): 473-81.
126. Giebel, B. (2008). "Cell polarity and asymmetric cell division within human hematopoietic stem and progenitor cells." Cells Tissues Organs **188**(1-2): 116-26.
127. Giebel, B. and I. Bruns (2008). "Self-renewal versus differentiation in hematopoietic stem and progenitor cells: a focus on asymmetric cell divisions." Curr Stem Cell Res Ther **3**(1): 9-16.
128. Giebel, B. and J. Beckmann (2007). "Asymmetric cell divisions of human hematopoietic stem and progenitor cells meet endosomes." Cell Cycle **6**(18): 2201-4.
129. Ginsburg, H., T. Jehuda-Cohen, et al. (1986). "Does stem cell self renewal and progenitor cell commitment operate through an effector-memory cell mechanism?" Immunol Lett **13**(3): 107-19.
130. Glaspy, J. A., E. J. Shpall, et al. (1997). "Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients." Blood **90**(8): 2939-51.
131. Goldschneider, I., D. Metcalf, et al. (1980). "Analysis of rat hemopoietic cells on the fluorescence-activated cell sorter. I. Isolation of pluripotent hemopoietic stem cells and granulocyte-macrophage progenitor cells." J Exp Med **152**(2): 419-37.
132. Gonzalez-Barca, E., A. Fernandez de Sevilla, et al. (2000). "Autologous stem cell transplantation (ASCT) with immunologically purged progenitor cells in patients with advanced stage follicular lymphoma after early partial or complete remission: toxicity, follow-up of minimal residual disease and survival." Bone Marrow Transplant **26**(10): 1051-6.
133. Gonzalez-Requejo, A., L. Madero, et al. (1998). "Progenitor cell subsets and engraftment kinetics in children undergoing autologous peripheral blood stem cell transplantation." Br J Haematol **101**(1): 104-10.
134. Gordon, M. Y., N. Levicar, et al. (2006). "Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor." Stem Cells **24**(7): 1822-30.
135. Gordon, M. Y., S. B. Marley, et al. (2000). "Contact-mediated inhibition of human haematopoietic progenitor cell proliferation may be conferred by stem cell antigen, CD34." Hematol J **1**(2): 77-86.
136. Goussetis, E., M. Theodosaki, et al. (2003). "Kinetics of quiescent cord blood stem/progenitor cells with high proliferative potential in stem-cell expansion culture." Cytotherapy **5**(6): 500-8.
137. Gouti, M. and A. Gavalas (2008). "Hoxb1 controls cell fate specification and proliferative capacity of neural stem and progenitor cells." Stem Cells **26**(8): 1985-97.
138. Grassinger, J., D. N. Haylock, et al. (2009). "Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins." Blood **114**(1): 49-59.
139. Gratama, J. W., A. Orfao, et al. (1998). "Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis." Cytometry **34**(3): 128-42.
140. Gratama, J. W., J. Kraan, et al. (2003). "Validation of the single-platform ISHAGE method for CD34(+) hematopoietic stem and progenitor cell enumeration in an international multicenter study." Cytotherapy **5**(1): 55-65.
141. Guardiola, P., V. Runde, et al. (2002). "Retrospective comparison of bone marrow and granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for allogeneic stem cell transplantation using HLA

identical sibling donors in myelodysplastic syndromes." Blood **99**(12): 4370-8.
142. Gudmundsson, K. O., L. Thorsteinsson, et al. (2007). "Gene expression analysis of hematopoietic progenitor cells identifies Dlg7 as a potential stem cell gene." Stem Cells **25**(6): 1498-506.
143. Guo, Y., B. Graham-Evans, et al. (2006). "Murine embryonic stem cells secrete cytokines/growth modulators that enhance cell survival/anti-apoptosis and stimulate colony formation of murine hematopoietic progenitor cells." Stem Cells **24**(4): 850-6.
144. Hacein-Bey, S., G. D. Basile, et al. (1998). "gammac gene transfer in the presence of stem cell factor, FLT-3L, interleukin-7 (IL-7), IL-1, and IL-15 cytokines restores T-cell differentiation from gammac(-) X-linked severe combined immunodeficiency hematopoietic progenitor cells in murine fetal thymic organ cultures." Blood **92**(11): 4090-7.
145. Han, L., A. T. Wierenga, et al. (2009). "Single-cell STAT5 signal transduction profiling in normal and leukemic stem and progenitor cell populations reveals highly distinct cytokine responses." PLoS One **4**(11): e7989.
146. Han, M. (1992). "Synergistic effects of murine stem cell factor in combination with a variety of cytokines on the expansion of murine hematopoietic progenitor cells in short-term suspension cultures." Hokkaido Igaku Zasshi **67**(5): 674-83.
147. Haridass, D., Q. Yuan, et al. (2009). "Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice." Am J Pathol **175**(4): 1483-92.
148. Hart, C., D. Drewel, et al. (2004). "Expression and function of homing-essential molecules and enhanced in vivo homing ability of human peripheral blood-derived hematopoietic progenitor cells after stimulation with stem cell factor." Stem Cells **22**(4): 580-9.
149. Hartung, G., L. Uharek, et al. (1998). "Superior antileukemic activity of murine peripheral blood progenitor cell (PBPC) grafts mobilized by G-CSF and stem cell factor (SCF) as compared to G-CSF alone." Bone Marrow Transplant **21 Suppl 3**: S16-20.
150. Hartung, G., M. Zeis, et al. (2003). "Enhanced antileukemic activity of allogeneic peripheral blood progenitor cell transplants following donor treatment with the combination of granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) in a murine transplantation model." Bone Marrow Transplant **32**(1): 49-56.
151. Haslauer, M., K. Baltensperger, et al. (1999). "Erythropoietin- and stem cell factor-induced DNA synthesis in normal human erythroid progenitor cells requires activation of protein kinase Calpha and is strongly inhibited by thrombin." Blood **94**(1): 114-26.
152. Hattori, K., B. Heissig, et al. (2001). "Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells." Blood **97**(11): 3354-60.
153. Hayashi, R., M. Yamato, et al. (2007). "N-Cadherin is expressed by putative stem/progenitor cells and melanocytes in the human limbal epithelial stem cell niche." Stem Cells **25**(2): 289-96.
154. Hayashi, R., M. Yamato, et al. (2008). "Enrichment of corneal epithelial stem/progenitor cells using cell surface markers, integrin alpha6 and CD71." Biochem Biophys Res Commun **367**(2): 256-63.
155. Heng, B. C., H. K. Haider, et al. (2005). "Combining transfusion of stem/progenitor cells into the peripheral circulation with localized transplantation in situ at the site of tissue/organ damage: a possible strategy to optimize the efficacy of stem cell transplantation therapy." Med Hypotheses **65**(3): 494-7.
156. Henriksson, H., M. Thornemo, et al. (2009). "Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species." Spine (Phila Pa 1976) **34**(21): 2278-87.
157. Herbert, K. E., J. P. Levesque, et al. (2008). "The use of experimental murine models to assess novel agents of hematopoietic stem and progenitor cell mobilization." Biol Blood Marrow Transplant **14**(6): 603-21.
158. Hicks, C., R. Wong, et al. (2007). "Viable CD34+/CD133+ blood progenitor cell dose as a predictor of haematopoietic engraftment in multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation." Ann Hematol **86**(8): 591-8.
159. Hildrestrand, G. A., D. B. Diep, et al. (2007). "The capacity to remove 8-oxoG is enhanced in newborn neural stem/progenitor cells and decreases in juvenile mice and upon cell differentiation." DNA Repair (Amst) **6**(6): 723-32.
160. Hiraoka, A., K. Yano Ki, et al. (2001). "Stem cell growth factor: in situ hybridization analysis on the gene expression, molecular characterization and in vitro proliferative activity of a recombinant preparation on primitive hematopoietic progenitor cells." Hematol J **2**(5): 307-15.
161. Hiraoka, A., T. Nagasawa, et al. (1998). "A human myeloid cell line producing stem cell growth factor, KPB-M15, secretes another growth factor active on murine hematopoietic progenitor cells." Acta Haematol **100**(4): 174-80.
162. Hirohata, S., T. Yanagida, et al. (2002). "Bone marrow CD34+ progenitor cells stimulated with stem cell factor and GM-CSF have the capacity to activate IgD- B cells through direct cellular interaction." J Leukoc Biol **71**(6): 987-95.
163. Hiroyama, T., K. Miharada, et al. (2008). "Establishment of mouse embryonic stem cell-derived erythroid progenitor cell lines able to produce functional red blood cells." PLoS One **3**(2): e1544.
164. Ho, Y. C., H. P. Lee, et al. (2006). "Baculovirus transduction of human mesenchymal stem cell-derived progenitor cells: variation of transgene expression with cellular differentiation states." Gene Ther **13**(20): 1471-9.
165. Hoffmann, M., H. H. Chang, et al. (2008). "Noise-driven stem cell and progenitor population dynamics." PLoS One **3**(8): e2922.
166. Horn, P. A., B. M. Thomasson, et al. (2003). "Distinct hematopoietic stem/progenitor cell populations are responsible for repopulating NOD/SCID mice compared with nonhuman primates." Blood **102**(13): 4329-35.
167. Hoshi, N., T. Kusakabe, et al. (2007). "Side population cells in the mouse thyroid exhibit stem/progenitor cell-like characteristics." Endocrinology **148**(9): 4251-8.
168. Huang, H. M., J. C. Li, et al. (1999). "Optimal proliferation of a hematopoietic progenitor cell line requires either costimulation with stem cell factor or increase of receptor expression that can be replaced by overexpression of Bcl-2." Blood **93**(8): 2569-77.
169. Huang, X. J., D. H. Liu, et al. (2006). "Prophylactic infusion of donor granulocyte colony stimulating factor mobilized peripheral blood progenitor cells after allogeneic hematological stem cell transplantation in patients with high-risk leukemia." Leukemia **20**(2): 365-8.
170. Humpe, A., J. Riggert, et al. (1997). "Comparison of CD34+ cell numbers and colony growth before and after cryopreservation of peripheral blood progenitor and stem cell harvests: influence of prior chemotherapy." Transfusion **37**(10): 1050-7.
171. Hwang-Verslues, W. W., W. H. Kuo, et al. (2009). "Multiple lineages of human breast cancer stem/progenitor cells identified by profiling with stem cell markers." PLoS One **4**(12): e8377.

172. Ichihara, M., T. Hotta, et al. (1994). "Effects of stem cell factor (SCF) on human marrow neutrophil, neutrophil/macrophage mixed, macrophage and eosinophil progenitor cell growth." Int J Hematol **59**(2): 81-9.
173. Ikegami, T., M. Nakamura, et al. (2005). "Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury." Eur J Neurosci **22**(12): 3036-46.
174. Imamura, M., X. Zhu, et al. (1996). "In vitro expansion of murine hematopoietic progenitor cells by leukemia inhibitory factor, stem cell factor, and interleukin-1 beta." Exp Hematol **24**(11): 1280-8.
175. Irons, R. D. and W. S. Stillman (1996). "The effects of benzene and other leukaemogenic agents on haematopoietic stem and progenitor cell differentiation." Eur J Haematol Suppl **60**: 119-24.
176. Ito, C. Y., C. Y. Li, et al. (2003). "Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A-null mice." Blood **101**(2): 517-23.
177. Ito, M., K. Kizawa, et al. (2004). "Hair follicle stem cells in the lower bulge form the secondary germ, a biochemically distinct but functionally equivalent progenitor cell population, at the termination of catagen." Differentiation **72**(9-10): 548-57.
178. Jacobsen, F. W., T. Stokke, et al. (1995). "Transforming growth factor-beta potently inhibits the viability-promoting activity of stem cell factor and other cytokines and induces apoptosis of primitive murine hematopoietic progenitor cells." Blood **86**(8): 2957-66.
179. Jadhav, A. P., S. H. Cho, et al. (2006). "Notch activity permits retinal cells to progress through multiple progenitor states and acquire a stem cell property." Proc Natl Acad Sci U S A **103**(50): 18998-9003.
180. Ju, X. D., S. Q. Lou, et al. (2004). "Effect of hydroxyurea and etoposide on transduction of human bone marrow mesenchymal stem and progenitor cell by adeno-associated virus vectors." Acta Pharmacol Sin **25**(2): 196-202.
181. Jung, C. G., H. Hida, et al. (2004). "Pleiotrophin mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derived nestin-positive cells." Faseb J **18**(11): 1237-9.
182. Juttner, C. A., L. B. To, et al. (1987). "Successful peripheral blood stem-cell autograft with a near-critical dose of myeloid progenitor cells in acute non-lymphoblastic leukaemia in relapse." Med J Aust **147**(6): 292-3.
183. Kakinuma, S., H. Nakauchi, et al. (2009). "Hepatic stem/progenitor cells and stem-cell transplantation for the treatment of liver disease." J Gastroenterol **44**(3): 167-72.
184. Kakinuma, S., H. Ohta, et al. (2009). "Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver." J Hepatol **51**(1): 127-38.
185. Kanemura, Y., H. Mori, et al. (2005). "In vitro screening of exogenous factors for human neural stem/progenitor cell proliferation using measurement of total ATP content in viable cells." Cell Transplant **14**(9): 673-82.
186. Kasbia, G., F. Al-Gahtani, et al. (2008). "Reduced hemoglobin on day of peripheral blood progenitor cell collection is associated with low graft content of vascular progenitors and increased toxicity after autologous hematopoietic stem cell transplantation." Transfusion **48**(11): 2421-8.
187. Kasenda, B., S. H. Kassmer, et al. (2008). "The stromal cell-derived factor-1alpha dependent migration of human cord blood CD34 haematopoietic stem and progenitor cells switches from protein kinase C (PKC)-alpha dependence to PKC-alpha independence upon prolonged culture in the presence of Flt3-ligand and interleukin-6." Br J Haematol **142**(5): 831-5.
188. Kashiwakura, I., O. Inanami, et al. (2003). "Protective effects of thrombopoietin and stem cell factor on X-irradiated CD34+ megakaryocytic progenitor cells from human placental and umbilical cord blood." Radiat Res **160**(2): 210-6.
189. Kashofer, K., E. K. Siapati, et al. (2006). "In vivo formation of unstable heterokaryons after liver damage and hematopoietic stem cell/progenitor transplantation." Stem Cells **24**(4): 1104-12.
190. Kattman, S. J., E. D. Adler, et al. (2007). "Specification of multipotential cardiovascular progenitor cells during embryonic stem cell differentiation and embryonic development." Trends Cardiovasc Med **17**(7): 240-6.
191. Kaushansky, K., N. Fox, et al. (2002). "Lineage-specific growth factors can compensate for stem and progenitor cell deficiencies at the postprogenitor cell level: an analysis of doubly TPO- and G-CSF receptor-deficient mice." Blood **99**(10): 3573-8.
192. Kawano, Y., Y. Takaue, et al. (1993). "Effects of progenitor cell dose and preleukapheresis use of human recombinant granulocyte colony-stimulating factor on the recovery of hematopoiesis after blood stem cell autografting in children." Exp Hematol **21**(1): 103-8.
193. Ke, X., L. Jia, et al. (2002). "Effects of novel human chemokine-like factor 1 (CKLF1) on bone marrow hematopoietic stem cell/progenitor cell in vitro." Zhonghua Xue Ye Xue Za Zhi **23**(6): 301-3.
194. Keirstead, H. S., G. Nistor, et al. (2005). "Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury." J Neurosci **25**(19): 4694-705.
195. Keller, J. R., M. Ortiz, et al. (1995). "Steel factor (c-kit ligand) promotes the survival of hematopoietic stem/progenitor cells in the absence of cell division." Blood **86**(5): 1757-64.
196. Keller, J. R., S. H. Bartelmez, et al. (1994). "Distinct and overlapping direct effects of macrophage inflammatory protein-1 alpha and transforming growth factor beta on hematopoietic progenitor/stem cell growth." Blood **84**(7): 2175-81.
197. Kessinger, A. and J. G. Sharp (2003). "The whys and hows of hematopoietic progenitor and stem cell mobilization." Bone Marrow Transplant **31**(5): 319-29.
198. Kikushige, Y., G. Yoshimoto, et al. (2008). "Human Flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival." J Immunol **180**(11): 7358-67.
199. Kim, A. C. and G. D. Hammer (2007). "Adrenocortical cells with stem/progenitor cell properties: recent advances." Mol Cell Endocrinol **265-266**: 10-6.
200. Kim, H. J., J. F. Tisdale, et al. (2000). "Many multipotential gene-marked progenitor or stem cell clones contribute to hematopoiesis in nonhuman primates." Blood **96**(1): 1-8.
201. Kim, M. K., S. Kim, et al. (2007). "A randomized comparison of peripheral blood hematopoietic progenitor cell level of 5/mm3 versus 50/mm3 as a surrogate marker to initiate efficient autologous blood stem cell collection." J Clin Apher **22**(5): 277-82.
202. Kim, T. S., S. Misumi, et al. (2008). "Increase in dopaminergic neurons from mouse embryonic stem cell-derived neural progenitor/stem cells is mediated by hypoxia inducible factor-1alpha." J Neurosci Res **86**(11): 2353-62.
203. King, A. G., D. Horowitz, et al. (2001). "Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta." Blood **97**(6): 1534-42.

204. Kirouac, D. C., G. J. Madlambayan, et al. (2009). "Cell-cell interaction networks regulate blood stem and progenitor cell fate." Mol Syst Biol **5**: 293.
205. Kirshenbaum, A. S., C. Akin, et al. (2005). "Thrombopoietin alone or in the presence of stem cell factor supports the growth of KIT(CD117)low/ MPL(CD110)+ human mast cells from hematopoietic progenitor cells." Exp Hematol **33**(4): 413-21.
206. Kirshenbaum, A. S., J. P. Goff, et al. (1992). "Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells." J Immunol **148**(3): 772-7.
207. Kleinman, M. E., M. R. Greives, et al. (2007). "Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma." Arterioscler Thromb Vasc Biol **27**(12): 2664-70.
208. Klimchenko, O., M. Mori, et al. (2009). "A common bipotent progenitor generates the erythroid and megakaryocyte lineages in embryonic stem cell-derived primitive hematopoiesis." Blood **114**(8): 1506-17.
209. Knudsen, L. M., T. Rasmussen, et al. (1999). "Reduced bone marrow stem cell pool and progenitor mobilisation in multiple myeloma after melphalan treatment." Med Oncol **16**(4): 245-54.
210. Koch, K. S., K. H. Son, et al. (2006). "Immune-privileged embryonic Swiss mouse STO and STO cell-derived progenitor cells: major histocompatibility complex and cell differentiation antigen expression patterns resemble those of human embryonic stem cell lines." Immunology **119**(1): 98-115.
211. Komitova, M., B. Mattsson, et al. (2005). "Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats." Stroke **36**(6): 1278-82.
212. Korbling, M., P. Anderlini, et al. (1996). "Delayed effects of rhG-CSF mobilization treatment and apheresis on circulating CD34+ and CD34+ Thy-1dim CD38- progenitor cells, and lymphoid subsets in normal stem cell donors for allogeneic transplantation." Bone Marrow Transplant **18**(6): 1073-9.
213. Korbling, M., Y. O. Huh, et al. (1995). "Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease." Blood **86**(7): 2842-8.
214. Krivtsov, A. V., D. Twomey, et al. (2006). "Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9." Nature **442**(7104): 818-22.
215. Kroger, N., W. Zeller, et al. (1999). "Stem cell mobilization with G-CSF alone in breast cancer patients: higher progenitor cell yield by delivering divided doses (2 x 5 microg/kg) compared to a single dose (1 x 10 microg/kg)." Bone Marrow Transplant **23**(2): 125-9.
216. Kruse, C., J. Kajahn, et al. (2006). "Adult pancreatic stem/progenitor cells spontaneously differentiate in vitro into multiple cell lineages and form teratoma-like structures." Ann Anat **188**(6): 503-17.
217. Kubo, F. and S. Nakagawa (2009). "Hairy1 acts as a node downstream of Wnt signaling to maintain retinal stem cell-like progenitor cells in the chick ciliary marginal zone." Development **136**(11): 1823-33.
218. Kulbatski, I., A. J. Mothe, et al. (2005). "Endogenous and exogenous CNS derived stem/progenitor cell approaches for neurotrauma." Curr Drug Targets **6**(1): 111-26.
219. Kumagai, G., Y. Okada, et al. (2009). "Roles of ES cell-derived gliogenic neural stem/progenitor cells in functional recovery after spinal cord injury." PLoS One **4**(11): e7706.
220. Kurata, H., G. C. Mancini, et al. (1998). "Stem cell factor induces proliferation and differentiation of fetal progenitor cells in the mouse." Br J Haematol **101**(4): 676-87.
221. Lardon, F., H. W. Snoeck, et al. (1994). "Transforming growth factor-beta regulates the cell cycle status of interleukin-3 (IL-3) plus IL-1, stem cell factor, or IL-6 stimulated CD34+ human hematopoietic progenitor cells through different cell kinetic mechanisms depending on the applied stimulus." Exp Hematol **22**(9): 903-9.
222. Lassmann, H. (2005). "Stem cell and progenitor cell transplantation in multiple sclerosis: the discrepancy between neurobiological attraction and clinical feasibility." J Neurol Sci **233**(1-2): 83-6.
223. Laterveer, L., J. M. Zijlmans, et al. (1996). "Improved survival of lethally irradiated recipient mice transplanted with circulating progenitor cells mobilized by IL-8 after pretreatment with stem cell factor." Exp Hematol **24**(12): 1387-93.
224. Lee, C., C. A. Evans, et al. (2003). "Generation of a conditionally immortalized myeloid progenitor cell line requiring the presence of both interleukin-3 and stem cell factor to survive and proliferate." Br J Haematol **122**(6): 985-95.
225. Lee, J. L., S. B. Kim, et al. (2003). "Clinical usefulness of the hematopoietic progenitor cell counts in predicting the optimal timing of peripheral blood stem cell harvest." J Korean Med Sci **18**(1): 27-35.
226. Lefrere, F., F. Audat, et al. (1999). "The timing of granulocyte-colony-stimulating factor administration after chemotherapy does not affect stem and progenitor cell apheresis yield: a retrospective study of 65 cases." Transfusion **39**(6): 561-4.
227. Li, J., D. P. Sejas, et al. (2006). "Nucleophosmin regulates cell cycle progression and stress response in hematopoietic stem/progenitor cells." J Biol Chem **281**(24): 16536-45.
228. Li, J., X. Meng, et al. (2008). "Prolongation of cardiac allograft survival by syngeneic hematopoietic stem/progenitor cell transplantation in mice." Adv Ther **25**(9): 935-42.
229. Li, L., O. Piloto, et al. (2007). "FLT3/ITD expression increases expansion, survival and entry into cell cycle of human haematopoietic stem/progenitor cells." Br J Haematol **137**(1): 64-75.
230. Li, L., Y. H. El-Hayek, et al. (2008). "Direct-current electrical field guides neuronal stem/progenitor cell migration." Stem Cells **26**(8): 2193-200.
231. Li, X., B. Z. Barkho, et al. (2008). "Epigenetic regulation of the stem cell mitogen Fgf-2 by Mbd1 in adult neural stem/progenitor cells." J Biol Chem **283**(41): 27644-52.
232. Li, X., P. A. Plett, et al. (2003). "Fanconi anemia type C-deficient hematopoietic stem/progenitor cells exhibit aberrant cell cycle control." Blood **102**(6): 2081-4.
233. Liao, H., W. Huang, et al. (2008). "Beta 1 integrin-mediated effects of tenascin-R domains EGFL and FN6-8 on neural stem/progenitor cell proliferation and differentiation in vitro." J Biol Chem **283**(41): 27927-36.
234. Liao, H., W. Huang, et al. (2008). "Cross-talk between the epidermal growth factor-like repeats/fibronectin 6-8 repeats domains of Tenascin-R and microglia modulates neural stem/progenitor cell proliferation and differentiation." J Neurosci Res **86**(1): 27-34.
235. Lie, A. K., T. P. Rawling, et al. (1996). "Progenitor cell yield in sequential blood stem cell mobilization in the same patients: insights into chemotherapy dose escalation and combination of haemopoietic growth factor and chemotherapy." Br J Haematol **95**(1): 39-44.
236. Link, H. and L. Arseniev (1997). "CD34 positive blood cells for allogeneic progenitor and stem cell transplantation." Leuk Lymphoma **26**(5-6): 451-65.
237. Linnekin, D., C. S. DeBerry, et al. (1997). "Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells." J Biol Chem **272**(43): 27450-5.

238. Liu, S., C. Ginestier, et al. (2008). "BRCA1 regulates human mammary stem/progenitor cell fate." Proc Natl Acad Sci U S A **105**(5): 1680-5.
239. Liu, X. and J. F. Engelhardt (2008). "The glandular stem/progenitor cell niche in airway development and repair." Proc Am Thorac Soc **5**(6): 682-8.
240. Liu, Z. and L. J. Martin (2006). "The adult neural stem and progenitor cell niche is altered in amyotrophic lateral sclerosis mouse brain." J Comp Neurol **497**(3): 468-88.
241. Lock, L. T. and E. S. Tzanakakis (2007). "Stem/Progenitor cell sources of insulin-producing cells for the treatment of diabetes." Tissue Eng **13**(7): 1399-412.
242. Loizou, J. I., G. Oser, et al. (2009). "Histone acetyltransferase cofactor Trrap is essential for maintaining the hematopoietic stem/progenitor cell pool." J Immunol **183**(10): 6422-31.
243. Lord-Grignon, J., M. Abdouh, et al. (2006). "Identification of genes expressed in retinal progenitor/stem cell colonies isolated from the ocular ciliary body of adult mice." Gene Expr Patterns **6**(8): 992-9.
244. Luis, T. C., F. Weerkamp, et al. (2009). "Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation." Blood **113**(3): 546-54.
245. Luo, Y., J. Cai, et al. (2003). "Designing, testing, and validating a focused stem cell microarray for characterization of neural stem cells and progenitor cells." Stem Cells **21**(5): 575-87.
246. Ma, W., W. Fitzgerald, et al. (2004). "CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels." Exp Neurol **190**(2): 276-88.
247. Machold, R., S. Hayashi, et al. (2003). "Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches." Neuron **39**(6): 937-50.
248. Maciejewski, J. P., C. O'Keefe, et al. (2007). "Immune-mediated bone marrow failure syndromes of progenitor and stem cells: molecular analysis of cytotoxic T cell clones." Folia Histochem Cytobiol **45**(1): 5-14.
249. Madlambayan, G. J., J. M. Butler, et al. (2009). "Bone marrow stem and progenitor cell contribution to neovasculogenesis is dependent on model system with SDF-1 as a permissive trigger." Blood **114**(19): 4310-9.
250. Madras, N., A. L. Gibbs, et al. (2002). "Modeling stem cell development by retrospective analysis of gene expression profiles in single progenitor-derived colonies." Stem Cells **20**(3): 230-40.
251. Maeda, K., M. Takemura, et al. (2008). "E-cadherin prolongs the moment for interaction between intestinal stem cell and its progenitor cell to ensure Notch signaling in adult Drosophila midgut." Genes Cells **13**(12): 1219-27.
252. Mahmud, D. L., G. A. M, et al. (2002). "Phosphorylation of forkhead transcription factors by erythropoietin and stem cell factor prevents acetylation and their interaction with coactivator p300 in erythroid progenitor cells." Oncogene **21**(10): 1556-62.
253. Mahmud, N., N. Katayama, et al. (1996). "A possible change in doubling time of haemopoietic progenitor cells with stem cell development." Br J Haematol **94**(2): 242-9.
254. Mahmut, N., Y. Katayama, et al. (1999). "Analysis of circulating hematopoietic progenitor cells after peripheral blood stem cell transplantation." Int J Hematol **69**(1): 36-42.
255. Malaterre, J., T. Mantamadiotis, et al. (2008). "c-Myb is required for neural progenitor cell proliferation and maintenance of the neural stem cell niche in adult brain." Stem Cells **26**(1): 173-81.
256. Marley, S. B. and M. Y. Gordon (2005). "Chronic myeloid leukaemia: stem cell derived but progenitor cell driven." Clin Sci (Lond) **109**(1): 13-25.
257. Marquez-Curtis, L., A. Jalili, et al. (2008). "Carboxypeptidase M expressed by human bone marrow cells cleaves the C-terminal lysine of stromal cell-derived factor-1alpha: another player in hematopoietic stem/progenitor cell mobilization?" Stem Cells **26**(5): 1211-20.
258. Martin, C. H., P. S. Woll, et al. (2008). "Differences in lymphocyte developmental potential between human embryonic stem cell and umbilical cord blood-derived hematopoietic progenitor cells." Blood **112**(7): 2730-7.
259. Martinez-Serrano, A., F. J. Rubio, et al. (2001). "Human neural stem and progenitor cells: in vitro and in vivo properties, and potential for gene therapy and cell replacement in the CNS." Curr Gene Ther **1**(3): 279-99.
260. Masaka, T., M. Miyazaki, et al. (2008). "Derivation of hepato-pancreatic intermediate progenitor cells from a clonal mesenchymal stem cell line of rat bone marrow origin." Int J Mol Med **22**(4): 447-52.
261. Maslov, A. Y., K. J. Bailey, et al. (2007). "Stem/progenitor cell-specific enhanced green fluorescent protein expression driven by the endogenous Mcm2 promoter." Stem Cells **25**(1): 132-8.
262. Mathieu, C., K. Sii-Felice, et al. (2008). "Endothelial cell-derived bone morphogenetic proteins control proliferation of neural stem/progenitor cells." Mol Cell Neurosci **38**(4): 569-77.
263. McNiece, I. K., K. E. Langley, et al. (1991). "Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and epo to stimulate human progenitor cells of the myeloid and erythroid lineages." Exp Hematol **19**(3): 226-31.
264. McNiece, I. K., R. A. Briddell, et al. (1994). "The role of stem cell factor in mobilization of peripheral blood progenitor cells." Leuk Lymphoma **15**(5-6): 405-9.
265. McQuaker, I. G., A. P. Haynes, et al. (1997). "Stem cell mobilization in resistant or relapsed lymphoma: superior yield of progenitor cells following a salvage regimen comprising ifosphamide, etoposide and epirubicin compared to intermediate-dose cyclophosphamide." Br J Haematol **98**(1): 228-33.
266. Mebius, R. E., S. van Tuijl, et al. (1998). "Transfer of primitive stem/progenitor bone marrow cells from LT alpha-/- donors to wild-type hosts: implications for the generation of architectural events in lymphoid B cell domains." J Immunol **161**(8): 3836-43.
267. Meehan, K. R., U. N. Verma, et al. (1997). "Suppression of progenitor cell growth by vancomycin following autologous stem cell transplantation." Bone Marrow Transplant **19**(10): 1029-32.
268. Meisenberg, B. R., M. Callaghan, et al. (1997). "Complications associated with central venous catheters used for the collection of peripheral blood progenitor cells to support high-dose chemotherapy and autologous stem cell rescue." Support Care Cancer **5**(3): 223-7.
269. Metcalf, D., S. Mifsud, et al. (2002). "Stem cell factor can stimulate the formation of eosinophils by two types of murine eosinophil progenitor cells." Stem Cells **20**(5): 460-9.
270. Mielcarek, M., E. Bryant, et al. (1999). "Haemopoietic reconstitution by donor-derived myelodysplastic progenitor cells after haemopoietic stem cell transplantation." Br J Haematol **105**(2): 361-5.
271. Migliaccio, A. R., M. Baiocchi, et al. (1994). "Stem cell factor and the amplification of progenitor cells from CD34+ cord blood cells." Blood Cells **20**(1): 129-38; discussion 138-9.
272. Migliaccio, G., A. R. Migliaccio, et al. (1991). "Effects of recombinant human stem cell factor (SCF) on the growth of human progenitor cells in vitro." J Cell Physiol **148**(3): 503-9.

273. Mimeault, M. and S. K. Batra (2008). "Recent progress on normal and malignant pancreatic stem/progenitor cell research: therapeutic implications for the treatment of type 1 or 2 diabetes mellitus and aggressive pancreatic cancer." Gut **57**(10): 1456-68.
274. Mimeault, M. and S. K. Batra (2008). "Targeting of cancer stem/progenitor cells plus stem cell-based therapies: the ultimate hope for treating and curing aggressive and recurrent cancers." Panminerva Med **50**(1): 3-18.
275. Mimeault, M., R. Hauke, et al. (2007). "Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers." J Cell Mol Med **11**(5): 981-1011.
276. Miura, Y., Z. Gao, et al. (2006). "Mesenchymal stem cell-organized bone marrow elements: an alternative hematopoietic progenitor resource." Stem Cells **24**(11): 2428-36.
277. Miyazaki, T., I. Matsuda, et al. (2001). "Flow cytometric analysis of hemetopoietic progenitor cells in peripheral blood stem cell harvest from patients with CD34 positive acute leukemia." J Immunol Methods **247**(1-2): 9-15.
278. Molofsky, A. V., R. Pardal, et al. (2003). "Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation." Nature **425**(6961): 962-7.
279. Moskowitz, C. H., P. Stiff, et al. (1997). "Recombinant methionyl human stem cell factor and filgrastim for peripheral blood progenitor cell mobilization and transplantation in non-Hodgkin's lymphoma patients--results of a phase I/II trial." Blood **89**(9): 3136-47.
280. Muschler, G. F., R. J. Midura, et al. (2003). "Practical Modeling Concepts for Connective Tissue Stem Cell and Progenitor Compartment Kinetics." J Biomed Biotechnol **2003**(3): 170-193.
281. Musso, M., F. Porretto, et al. (1998). "Autologous peripheral blood stem and progenitor (CD34+) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome." Lupus **7**(7): 492-4.
282. Musso, M., F. Porretto, et al. (1999). "Successful treatment of resistant thrombotic thrombocytopenic purpura/hemolytic uremic syndrome with autologous peripheral blood stem and progenitor (CD34+) cell transplantation." Bone Marrow Transplant **24**(2): 207-9.
283. Muta, K., S. B. Krantz, et al. (1994). "Distinct roles of erythropoietin, insulin-like growth factor I, and stem cell factor in the development of erythroid progenitor cells." J Clin Invest **94**(1): 34-43.
284. Muta, K., S. B. Krantz, et al. (1995). "Stem cell factor retards differentiation of normal human erythroid progenitor cells while stimulating proliferation." Blood **86**(2): 572-80.
285. Myers, S. E., R. Mick, et al. (1994). "High-dose chemotherapy with autologous stem cell rescue in women with metastatic breast cancer with involved bone marrow: a role for peripheral blood progenitor transplant." Bone Marrow Transplant **13**(4): 449-54.
286. Nagamatsu, G., M. Ohmura, et al. (2006). "A CTX family cell adhesion molecule, JAM4, is expressed in stem cell and progenitor cell populations of both male germ cell and hematopoietic cell lineages." Mol Cell Biol **26**(22): 8498-506.
287. Naito, H., H. Kidoya, et al. (2009). "Induction and expression of anti-angiogenic vasohibins in the hematopoietic stem/progenitor cell population." J Biochem **145**(5): 653-9.
288. Nakano, I., J. D. Dougherty, et al. (2007). "Phosphoserine phosphatase is expressed in the neural stem cell niche and regulates neural stem and progenitor cell proliferation." Stem Cells **25**(8): 1975-84.
289. Nakayama, N., D. Duryea, et al. (2003). "Macroscopic cartilage formation with embryonic stem-cell-derived mesodermal progenitor cells." J Cell Sci **116**(Pt 10): 2015-28.
290. Nalapareddy, K., H. Jiang, et al. (2008). "Determining the influence of telomere dysfunction and DNA damage on stem and progenitor cell aging: what markers can we use?" Exp Gerontol **43**(11): 998-1004.
291. Neben, S., K. Marcus, et al. (1993). "Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor." Blood **81**(7): 1960-7.
292. Nikolova, T., J. Czyz, et al. (2005). "Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells." Faseb J **19**(12): 1686-8.
293. Nilsson, L., I. Astrand-Grundstrom, et al. (2000). "Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level." Blood **96**(6): 2012-21.
294. Nishihara, M., Y. Wada, et al. (1998). "A combination of stem cell factor and granulocyte colony-stimulating factor enhances the growth of human progenitor B cells supported by murine stromal cell line MS-5." Eur J Immunol **28**(3): 855-64.
295. Noble, M., D. Wren, et al. (1992). "The O-2A(adult) progenitor cell: a glial stem cell of the adult central nervous system." Semin Cell Biol **3**(6): 413-22.
296. Oertel, M., A. Menthena, et al. (2006). "Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells." Gastroenterology **130**(2): 507-20; quiz 590.
297. Ogawa, D., Y. Okada, et al. (2009). "Evaluation of human fetal neural stem/progenitor cells as a source for cell replacement therapy for neurological disorders: properties and tumorigenicity after long-term in vitro maintenance." J Neurosci Res **87**(2): 307-17.
298. Ohta, K., T. Yamane, et al. (1999). "An effective method for recovering CD34 positive progenitor cells from peripheral blood stem cell apheresis products cryopreserved with simplified method." Osaka City Med J **45**(2): 139-48.
299. Oikawa, T., A. Kamiya, et al. (2009). "Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells." Gastroenterology **136**(3): 1000-11.
300. Okada, Y., A. Matsumoto, et al. (2008). "Spatiotemporal recapitulation of central nervous system development by murine embryonic stem cell-derived neural stem/progenitor cells." Stem Cells **26**(12): 3086-98.
301. Okumura, N., K. Tsuji, et al. (1996). "Chemotactic and chemokinetic activities of stem cell factor on murine hematopoietic progenitor cells." Blood **87**(10): 4100-8.
302. Olweus, J., L. W. Terstappen, et al. (1996). "Expression and function of receptors for stem cell factor and erythropoietin during lineage commitment of human hematopoietic progenitor cells." Blood **88**(5): 1594-607.
303. Oritani, K., K. Aoyama, et al. (2000). "Stromal cell CD9 and the differentiation of hematopoietic stem/progenitor cells." Leuk Lymphoma **38**(1-2): 147-52.
304. Paczesny, S., Y. P. Li, et al. (2007). "Efficient generation of CD34+ progenitor-derived dendritic cells from G-CSF-mobilized peripheral mononuclear cells does not require hematopoietic stem cell enrichment." J Leukoc Biol **81**(4): 957-67.
305. Padmanabhan, A., R. Reich-Slotky, et al. (2009). "Use of the haematopoietic progenitor cell parameter in optimizing timing of peripheral blood stem cell harvest." Vox Sang **97**(2): 153-9.
306. Paguirigan, A., D. J. Beebe, et al. (2007). "Simulating mouse mammary gland development: cell ageing and its relation to stem and progenitor activity." Cell Prolif **40**(1): 106-24.

307. Panzenbock, B., P. Bartunek, et al. (1998). "Growth and differentiation of human stem cell factor/erythropoietin-dependent erythroid progenitor cells in vitro." Blood **92**(10): 3658-68.
308. Papayannopoulou, T. (1999). "Hematopoietic stem/progenitor cell mobilization. A continuing quest for etiologic mechanisms." Ann N Y Acad Sci **872**: 187-97; discussion 197-9.
309. Papayannopoulou, T. (2000). "Mechanisms of stem-/progenitor-cell mobilization: the anti-VLA-4 paradigm." Semin Hematol **37**(1 Suppl 2): 11-8.
310. Papayannopoulou, T. (2004). "Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization." Blood **103**(5): 1580-5.
311. Papayannopoulou, T., G. V. Priestley, et al. (2003). "The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization." Blood **101**(12): 4739-47.
312. Partlow, K. C., J. Chen, et al. (2007). "19F magnetic resonance imaging for stem/progenitor cell tracking with multiple unique perfluorocarbon nanobeacons." Faseb J **21**(8): 1647-54.
313. Passegue, E., A. J. Wagers, et al. (2005). "Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates." J Exp Med **202**(11): 1599-611.
314. Pearson, T., L. D. Shultz, et al. (2008). "A new immunodeficient hyperglycaemic mouse model based on the Ins2Akita mutation for analyses of human islet and beta stem and progenitor cell function." Diabetologia **51**(8): 1449-56.
315. Pellegatta, S., P. Tunici, et al. (2006). "The therapeutic potential of neural stem/progenitor cells in murine globoid cell leukodystrophy is conditioned by macrophage/microglia activation." Neurobiol Dis **21**(2): 314-23.
316. Perino, M. G., S. Yamanaka, et al. (2008). "Cardiomyogenic stem and progenitor cell plasticity and the dissection of cardiopoiesis." J Mol Cell Cardiol **45**(4): 475-94.
317. Pierelli, L., M. Marone, et al. (2002). "Transforming growth factor-beta1 causes transcriptional activation of CD34 and preserves haematopoietic stem/progenitor cell activity." Br J Haematol **118**(2): 627-37.
318. Pierre-Louis, O., D. Clay, et al. (2009). "Dual SP/ALDH functionalities refine the human hematopoietic Lin-CD34+CD38- stem/progenitor cell compartment." Stem Cells **27**(10): 2552-62.
319. Pituch-Noworolska, A., M. Majka, et al. (2003). "Circulating CXCR4-positive stem/progenitor cells compete for SDF-1-positive niches in bone marrow, muscle and neural tissues: an alternative hypothesis to stem cell plasticity." Folia Histochem Cytobiol **41**(1): 13-21.
320. Ploemacher, R. E., P. L. van Soest, et al. (1993). "Autocrine transforming growth factor beta 1 blocks colony formation and progenitor cell generation by hemopoietic stem cells stimulated with steel factor." Stem Cells **11**(4): 336-47.
321. Pode-Shakked, N., S. Metsuyanim, et al. (2009). "Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population." J Cell Mol Med **13**(8B): 1792-808.
322. Pollard, Y., M. J. Watts, et al. (1999). "Use of the haemopoietic progenitor cell count of the Sysmex SE-9500 to refine apheresis timing of peripheral blood stem cells." Br J Haematol **106**(2): 538-44.
323. Ponti, D., A. Costa, et al. (2005). "Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties." Cancer Res **65**(13): 5506-11.
324. Porta, C., R. Caporali, et al. (2004). "Impaired bone marrow hematopoietic progenitor cell function in rheumatoid arthritis patients candidated to autologous hematopoietic stem cell transplantation." Bone Marrow Transplant **33**(7): 721-8.
325. Preijers, F. W., P. B. van Hennik, et al. (1999). "Counterflow centrifugation allows addition of appropriate numbers of T cells to allogeneic marrow and blood stem cell grafts to prevent severe GVHD without substantial loss of mature and immature progenitor cells." Bone Marrow Transplant **23**(10): 1061-70.
326. Preti, R. A., T. Ahmed, et al. (1992). "Hemopoietic stem cell processing: comparison of progenitor cell recovery using the Cobe 2991 cell washer and the Haemonetics V50 apheresis system." Bone Marrow Transplant **9**(5): 377-81.
327. Priestley, G. V., T. Ulyanova, et al. (2007). "Sustained alterations in biodistribution of stem/progenitor cells in Tie2Cre+ alpha4(f/f) mice are hematopoietic cell autonomous." Blood **109**(1): 109-11.
328. Pruitt, S. C., K. J. Bailey, et al. (2007). "Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer." Stem Cells **25**(12): 3121-32.
329. Puglisi, M. A., L. Giuliani, et al. (2008). "Identification and characterization of a novel expandable adult stem/progenitor cell population in the human exocrine pancreas." J Endocrinol Invest **31**(6): 563-72.
330. Pyatt, D. W., W. S. Stillman, et al. (1996). "Reactive oxygen species mediate stem cell factor synergy with granulocyte/macrophage colony-stimulating factor in a subpopulation of primitive murine hematopoietic progenitor cells." Mol Pharmacol **49**(6): 1097-1103.
331. Qian, H., K. Tryggvason, et al. (2006). "Contribution of alpha6 integrins to hematopoietic stem and progenitor cell homing to bone marrow and collaboration with alpha4 integrins." Blood **107**(9): 3503-10.
332. Qian, Z., L. Chen, et al. (2008). "A critical role for Apc in hematopoietic stem and progenitor cell survival." J Exp Med **205**(9): 2163-75.
333. Rafii, S. and D. Lyden (2003). "Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration." Nat Med **9**(6): 702-12.
334. Raghavachar, A., O. Prummer, et al. (1983). "Progenitor cell (CFUc) reconstitution after autologous stem cell transfusion in lethally irradiated dogs: decreased CFUc populations in blood and bone marrow correlate with the fraction mobilizable by dextran sulphate." Exp Hematol **11**(10): 996-1004.
335. Ramirez, M., G. A. Rottman, et al. (1998). "Mature human hematopoietic cells in donor bone marrow complicate interpretation of stem/progenitor cell assays in xenogeneic hematopoietic chimeras." Exp Hematol **26**(4): 332-44.
336. Reddy, G. P., C. Y. Tiarks, et al. (1997). "Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells." Blood **90**(6): 2293-9.
337. Reynolds, S. D., A. Giangreco, et al. (2004). "Airway injury in lung disease pathophysiology: selective depletion of airway stem and progenitor cell pools potentiates lung inflammation and alveolar dysfunction." Am J Physiol Lung Cell Mol Physiol **287**(6): L1256-65.
338. Robinson, S., R. van Os, et al. (2000). "Reduction of marrow hematopoietic progenitor and stem cell content is not sufficient for enhanced syngeneic engraftment." Stem Cells **18**(2): 93-101.
339. Roessger, A., L. Denk, et al. (2009). "Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules." Biomaterials **30**(22): 3723-32.
340. Roitbak, T., L. Li, et al. (2008). "Neural stem/progenitor cells promote endothelial cell morphogenesis and protect endothelial cells against ischemia via HIF-1alpha-regulated VEGF signaling." J Cereb Blood Flow Metab **28**(9): 1530-42.
341. Roszko, I., P. Faure, et al. (2007). "Stem cell growth becomes predominant while neural plate progenitor pool decreases during spinal cord elongation." Dev Biol **304**(1): 232-45.

342. Rusten, L. S., E. B. Smeland, et al. (1994). "Tumor necrosis factor-alpha inhibits stem cell factor-induced proliferation of human bone marrow progenitor cells in vitro. Role of p55 and p75 tumor necrosis factor receptors." J Clin Invest **94**(1): 165-72.
343. Sakaguchi, D. S., S. J. Van Hoffelen, et al. (2004). "Transplantation of neural progenitor cells into the developing retina of the Brazilian opossum: an in vivo system for studying stem/progenitor cell plasticity." Dev Neurosci **26**(5-6): 336-45.
344. Sales, V. L., B. A. Mettler, et al. (2007). "Endothelial progenitor and mesenchymal stem cell-derived cells persist in tissue-engineered patch in vivo: application of green and red fluorescent protein-expressing retroviral vector." Tissue Eng **13**(3): 525-35.
345. Salter, A. B., S. K. Meadows, et al. (2009). "Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo." Blood **113**(9): 2104-7.
346. Sandhu, J. S., P. M. Petkov, et al. (2001). "Stem cell properties and repopulation of the rat liver by fetal liver epithelial progenitor cells." Am J Pathol **159**(4): 1323-34.
347. Sartor, M. M., F. Garvin, et al. (2007). "Failure to achieve a threshold dose of CD34+ CD110+ progenitor cells in the graft predicts delayed platelet engraftment after autologous stem cell transplantation." Bone Marrow Transplant **40**(9): 851-7.
348. Satoh, M., H. Sugino, et al. (2000). "Activin promotes astrocytic differentiation of a multipotent neural stem cell line and an astrocyte progenitor cell line from murine central nervous system." Neurosci Lett **284**(3): 143-6.
349. Schaffer, P., J. A. Gleave, et al. (2008). "Isostructural fluorescent and radioactive probes for monitoring neural stem and progenitor cell transplants." Nucl Med Biol **35**(2): 159-69.
350. Schaumburg, C., B. A. O'Hara, et al. (2008). "Human embryonic stem cell-derived oligodendrocyte progenitor cells express the serotonin receptor and are susceptible to JC virus infection." J Virol **82**(17): 8896-9.
351. Schreiber, T. D., C. Steinl, et al. (2009). "The integrin alpha9beta1 on hematopoietic stem and progenitor cells: involvement in cell adhesion, proliferation and differentiation." Haematologica **94**(11): 1493-501.
352. Shapiro, F., T. J. Yao, et al. (1997). "Effects of prior therapy on the in vitro proliferative potential of stem cell factor plus filgrastim-mobilized CD34-positive progenitor cells." Clin Cancer Res **3**(9): 1571-8.
353. Sharma, A. D., T. Cantz, et al. (2008). "Murine embryonic stem cell-derived hepatic progenitor cells engraft in recipient livers with limited capacity of liver tissue formation." Cell Transplant **17**(3): 313-23.
354. Shen, H., M. Boyer, et al. (2008). "Flow cytometry-based cell cycle measurement of mouse hematopoietic stem and progenitor cells." Methods Mol Biol **430**: 77-86.
355. Shetty, A. K., B. Hattiangady, et al. (2005). "Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes." Glia **51**(3): 173-86.
356. Shitara, T., H. Ijima, et al. (1994). "Increased cytokine levels and abnormal response of myeloid progenitor cells to granulocyte colony-stimulating factor in a case of severe congenital neutropenia. In vitro effects of stem cell factor." Am J Pediatr Hematol Oncol **16**(2): 167-72.
357. Shlebak, A. A., I. A. Roberts, et al. (1998). "The impact of antenatal and perinatal variables on cord blood haemopoietic stem/progenitor cell yield available for transplantation." Br J Haematol **103**(4): 1167-71.
358. Sirko, S., A. von Holst, et al. (2007). "Chondroitin sulfate glycosaminoglycans control proliferation, radial glia cell differentiation and neurogenesis in neural stem/progenitor cells." Development **134**(15): 2727-38.
359. Sitnicka, E., D. Bryder, et al. (2002). "Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool." Immunity **17**(4): 463-72.
360. Slovak, M. L., S. T. Traweek, et al. (1995). "Trisomy 11: an association with stem/progenitor cell immunophenotype." Br J Haematol **90**(2): 266-73.
361. Smalley, M. J. and R. B. Clarke (2005). "The mammary gland "side population": a putative stem/progenitor cell marker?" J Mammary Gland Biol Neoplasia **10**(1): 37-47.
362. Smit, W. M., M. Rijnbeek, et al. (1998). "T cells recognizing leukemic CD34(+) progenitor cells mediate the antileukemic effect of donor lymphocyte infusions for relapsed chronic myeloid leukemia after allogeneic stem cell transplantation." Proc Natl Acad Sci U S A **95**(17): 10152-7.
363. Snowden, J. A., V. Nink, et al. (1998). "Composition and function of peripheral blood stem and progenitor cell harvests from patients with severe active rheumatoid arthritis." Br J Haematol **103**(3): 601-9.
364. Strohsnitter, W. C., T. M. Savarese, et al. (2008). "Correlation of umbilical cord blood haematopoietic stem and progenitor cell levels with birth weight: implications for a prenatal influence on cancer risk." Br J Cancer **98**(3): 660-3.
365. Stuart, S. A., Y. Minami, et al. (2009). "The CML stem cell: evolution of the progenitor." Cell Cycle **8**(9): 1338-43.
366. Suetake, K., S. S. Liour, et al. (2003). "Expression of gangliosides in an immortalized neural progenitor/stem cell line." J Neurosci Res **74**(5): 769-76.
367. Sui, X., S. B. Krantz, et al. (2000). "Stem cell factor and erythropoietin inhibit apoptosis of human erythroid progenitor cells through different signalling pathways." Br J Haematol **110**(1): 63-70.
368. Summerfield, A., M. P. Horn, et al. (2003). "C-kit positive porcine bone marrow progenitor cells identified and enriched using recombinant stem cell factor." J Immunol Methods **280**(1-2): 113-23.
369. Sussman, M. (2007). ""AKT"ing lessons for stem cells: regulation of cardiac myocyte and progenitor cell proliferation." Trends Cardiovasc Med **17**(7): 235-40.
370. Sutton, R. E., M. J. Reitsma, et al. (1999). "Transduction of human progenitor hematopoietic stem cells by human immunodeficiency virus type 1-based vectors is cell cycle dependent." J Virol **73**(5): 3649-60.
371. Suzuki, A., Y. W. Zheng, et al. (2001). "Clonal expansion of hepatic stem/progenitor cells following flow cytometric cell sorting." Cell Transplant **10**(4-5): 393-6.
372. Suzuki, H., T. Watabe, et al. (2005). "Roles of vascular endothelial growth factor receptor 3 signaling in differentiation of mouse embryonic stem cell-derived vascular progenitor cells into endothelial cells." Blood **105**(6): 2372-9.
373. Szilvassy, S. J. (2006). "Haematopoietic stem and progenitor cell-targeted therapies for thrombocytopenia." Expert Opin Biol Ther **6**(10): 983-92.
374. Szotek, P. P., H. L. Chang, et al. (2008). "Normal ovarian surface epithelial label-retaining cells exhibit stem/progenitor cell characteristics." Proc Natl Acad Sci U S A **105**(34): 12469-73.
375. Takami, A., S. Nakao, et al. (1999). "Impaired response of granulocyte-committed progenitor cells to stem cell factor and granulocyte colony-stimulating factor in human cyclic neutropenia." Ann Hematol **78**(4): 197-9.
376. Tang, B., N. Yoo, et al. (2007). "Transforming growth factor-beta can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model." Cancer Res **67**(18): 8643-52.
377. Toda, H., M. Tsuji, et al. (2003). "Stem cell-derived neural stem/progenitor cell supporting factor is an

autocrine/paracrine survival factor for adult neural stem/progenitor cells." J Biol Chem **278**(37): 35491-500.
378. Tokar, E. J., B. B. Ancrile, et al. (2005). "Stem/progenitor and intermediate cell types and the origin of human prostate cancer." Differentiation **73**(9-10): 463-73.
379. Torella, D., G. M. Ellison, et al. (2005). "Cardiac stem and progenitor cell biology for regenerative medicine." Trends Cardiovasc Med **15**(6): 229-36.
380. Torres, A., J. Sanchez, et al. (2001). "Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation." Haematologica **86**(1): 24-9.
381. Tsatalas, C., P. Chalkia, et al. (1995). "Increased peripheral blood normal myeloid progenitor cells (CFU-GM) in chronic lymphocytic leukemia: a perspective for autologous peripheral blood stem cell transplantation." Eur J Haematol **54**(4): 235-40.
382. Tsuzuki, S., D. Hong, et al. (2007). "Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1." PLoS Med **4**(5): e172.
383. Tycko, B., J. Ritz, et al. (1992). "Changing antigen receptor gene rearrangements in a case of early pre-B cell leukemia: evidence for a tumor progenitor cell with stem cell features and implications for monitoring residual disease." Blood **79**(2): 481-8.
384. Ural, S. H., M. D. Sammel, et al. (2005). "Determination of engraftment potential of human cord blood stem-progenitor cells as a function of donor cell dosage and gestational age in the NOD/SCID mouse model." Am J Obstet Gynecol **193**(3 Pt 2): 990-4.
385. Ushio-Fukai, M. and N. Urao (2009). "Novel role of NADPH oxidase in angiogenesis and stem/progenitor cell function." Antioxid Redox Signal **11**(10): 2517-33.
386. van Beusechem, V. W., J. A. Bart-Baumeister, et al. (1995). "Influence of interleukin-3, interleukin-6, and stem cell factor on retroviral transduction of rhesus monkey CD34+ hematopoietic progenitor cells measured in vitro and in vivo." Gene Ther **2**(4): 245-55.
387. Van Hennik, P. B., D. A. Breems, et al. (2000). "Stroma-supported progenitor production as a prognostic tool for graft failure following autologous stem cell transplantation." Br J Haematol **111**(2): 674-84.
388. van Pel, M., H. Hagoort, et al. (2008). "Differential role of CD97 in interleukin-8-induced and granulocyte-colony stimulating factor-induced hematopoietic stem and progenitor cell mobilization." Haematologica **93**(4): 601-4.
389. Vascotto, S. G. and M. Griffith (2006). "Localization of candidate stem and progenitor cell markers within the human cornea, limbus, and bulbar conjunctiva in vivo and in cell culture." Anat Rec A Discov Mol Cell Evol Biol **288**(8): 921-31.
390. Ventura, J. J., S. Tenbaum, et al. (2007). "p38alpha MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation." Nat Genet **39**(6): 750-8.
391. Viallard, J. F., C. Grosset, et al. (1996). "Effect of stem cell factor on leukemic progenitor cell growth and sensitivity to cytosine-arabinoside." Leuk Res **20**(11-12): 915-23.
392. Wagemaker, G. and T. P. Visser (1986). "Enumeration of stem cells and progenitor cells in alpha-thalassemic mice reveals lack of specific regulation of stem cell differentiation." Exp Hematol **14**(4): 303-6.
393. Wagner, W., A. Ansorge, et al. (2004). "Molecular evidence for stem cell function of the slow-dividing fraction among human hematopoietic progenitor cells by genome-wide analysis." Blood **104**(3): 675-86.
394. Wagner, W., F. Wein, et al. (2007). "Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction." Exp Hematol **35**(2): 314-25.
395. Wallington-Beddoe, C. T., D. J. Gottlieb, et al. (2009). "Failure to achieve a threshold dose of CD34+CD110+ progenitor cells in the graft predicts delayed platelet engraftment after autologous stem cell transplantation for multiple myeloma." Biol Blood Marrow Transplant **15**(11): 1386-93.
396. Wan, H., M. Yuan, et al. (2007). "Stem/progenitor cell-like properties of desmoglein 3dim cells in primary and immortalized keratinocyte lines." Stem Cells **25**(5): 1286-97.
397. Wandzioch, E., C. E. Edling, et al. (2004). "Activation of the MAP kinase pathway by c-Kit is PI-3 kinase dependent in hematopoietic progenitor/stem cell lines." Blood **104**(1): 51-7.
398. Wang, L., L. Yang, et al. (2006). "Genetic deletion of Cdc42GAP reveals a role of Cdc42 in erythropoiesis and hematopoietic stem/progenitor cell survival, adhesion, and engraftment." Blood **107**(1): 98-105.
399. Wiesmann, A., M. Kim, et al. (2000). "Modulation of hematopoietic stem/progenitor cell engraftment by transforming growth factor beta." Exp Hematol **28**(2): 128-39.
400. Xie, Z. and L. S. Chin (2008). "Molecular and cell biology of brain tumor stem cells: lessons from neural progenitor/stem cells." Neurosurg Focus **24**(3-4): E25.
401. Xu, Q., S. Wang, et al. (2007). "Hypoxia-induced astrocytes promote the migration of neural progenitor cells via vascular endothelial factor, stem cell factor, stromal-derived factor-1alpha and monocyte chemoattractant protein-1 upregulation in vitro." Clin Exp Pharmacol Physiol **34**(7): 624-31.
402. Yamaguchi, H., E. Ishii, et al. (1996). "Umbilical vein endothelial cells are an important source of c-kit and stem cell factor which regulate the proliferation of haemopoietic progenitor cells." Br J Haematol **94**(4): 606-11.
403. Yamamoto, K., T. Kondo, et al. (2004). "Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: therapeutic effect by stem cell transplantation." Arterioscler Thromb Vasc Biol **24**(12): e192-6.
404. Yamashita, T., J. Ji, et al. (2009). "EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features." Gastroenterology **136**(3): 1012-24.
405. Yan, X. Q., C. Hartley, et al. (1995). "Peripheral blood progenitor cells mobilized by recombinant human granulocyte colony-stimulating factor plus recombinant rat stem cell factor contain long-term engrafting cells capable of cellular proliferation for more than two years as shown by serial transplantation in mice." Blood **85**(9): 2303-7.
406. Yang, L., M. H. Soonpaa, et al. (2008). "Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population." Nature **453**(7194): 524-8.
407. Yasutake, M., Y. Zheng, et al. (2005). "SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem and/or progenitor cells in a nonobese diabetic/Shi-SCID mice serial xenotransplantation model and immune cell activities in vitro: a comparative study of the filter method and the hydroxyethyl starch method." Transfusion **45**(12): 1899-908.
408. Yoder, M. C., L. E. Mead, et al. (2007). "Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals." Blood **109**(5): 1801-9.
409. Yokota, T., K. Oritani, et al. (1998). "Growth-supporting activities of fibronectin on hematopoietic stem/progenitor cells in vitro and in vivo: structural requirement for fibronectin activities of CS1 and cell-binding domains." Blood **91**(9): 3263-72.

410. Yuan, Q., M. F. Gurish, et al. (1998). "Generation of a novel stem cell factor-dependent mast cell progenitor." J Immunol **161**(10): 5143-6.
411. Zahir, T., H. Nomura, et al. (2008). "Bioengineering neural stem/progenitor cell-coated tubes for spinal cord injury repair." Cell Transplant **17**(3): 245-54.
412. Zangiacomi, V., N. Balon, et al. (2008). "Cord blood-derived neurons are originated from CD133+/CD34 stem/progenitor cells in a cell-to-cell contact dependent manner." Stem Cells Dev **17**(5): 1005-16.
413. Zardo, G., G. Cimino, et al. (2008). "Epigenetic plasticity of chromatin in embryonic and hematopoietic stem/progenitor cells: therapeutic potential of cell reprogramming." Leukemia **22**(8): 1503-18.
414. Zenzmaier, C., G. Untergasser, et al. (2008). "Aging of the prostate epithelial stem/progenitor cell." Exp Gerontol **43**(11): 981-5.
415. Zhang, J., X. C. He, et al. (2006). "Bone morphogenetic protein signaling inhibits hair follicle anagen induction by restricting epithelial stem/progenitor cell activation and expansion." Stem Cells **24**(12): 2826-39.
416. Zhang, Y., N. Mukaida, et al. (1997). "Induction of dendritic cell differentiation by granulocyte-macrophage colony-stimulating factor, stem cell factor, and tumor necrosis factor alpha in vitro from lineage phenotypes-negative c-kit+ murine hematopoietic progenitor cells." Blood **90**(12): 4842-53.
417. Zhao, B., S. L. Allinson, et al. (2008). "Targeted cornea limbal stem/progenitor cell transfection in an organ culture model." Invest Ophthalmol Vis Sci **49**(8): 3395-401.
418. Zhao, M., S. A. Amiel, et al. (2007). "Evidence for the presence of stem cell-like progenitor cells in human adult pancreas." J Endocrinol **195**(3): 407-14.
419. Zheng, H., H. Ying, et al. (2008). "p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation." Nature **455**(7216): 1129-33.
420. Zheng, Y., A. Sun, et al. (2005). "Stem cell factor improves SCID-repopulating activity of human umbilical cord blood-derived hematopoietic stem/progenitor cells in xenotransplanted NOD/SCID mouse model." Bone Marrow Transplant **35**(2): 137-42.
421. Zheng, Y., N. Watanabe, et al. (2003). "Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their transmigratory potential." Exp Hematol **31**(12): 1237-46.
422. Zhong, L., W. Li, et al. (2006). "Evaluation of primitive murine hematopoietic stem and progenitor cell transduction in vitro and in vivo by recombinant adeno-associated virus vector serotypes 1 through 5." Hum Gene Ther **17**(3): 321-33.
423. Zhou, Z., A. Flesken-Nikitin, et al. (2007). "Prostate cancer associated with p53 and Rb deficiency arises from the stem/progenitor cell-enriched proximal region of prostatic ducts." Cancer Res **67**(12): 5683-90.
424. Bavister BD, Wolf DP, Brenner CA. Challenges of primate embryonic stem cell research. Cloning Stem Cells 2005;7(2):82-94.
425. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VAC, George NJ, Clarke NW. Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A. 2003;54(2):89-99.
426. Condorelli G, Peschle C. Stem cells for cardiac repair: state of the art. Front Biosci 2005;10:3143-50.
427. Daar AS, Sheremeta L. The science of stem cells: ethical, legal and social issues. Exp Clin Transplant. 2003;1(2):139-46.
428. Kashofer K, Bonnet D. Gene Therapy Progress and Prospects: Stem cell plasticity. Gene Ther. 2005 (Epub ahead of print).
429. Ma H. Technique of Animal Clone. Nature and Science 2004;2(1):29-35.
430. Stedman's Medical Dictionary. The American Heritage®. Houghton Mifflin Company. http://dictionary.reference.com/search?q=stem%20cell. 2002.
431. Williams D. Stem cells in medical techonology. Med Device Technol 2005;16(3):9-11.
432. Ma H, Chen G (2005). Stem Cell. J Am Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
433. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
434. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
435. Yang Y, Ma H (2010). Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
436. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
437. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

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