Hemotopietic 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 hemotopietic stem cell.
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Key words: stem cell; life; gene; DNA; protein; hemotopietic stem cell

Literatures

Aiuti, A., B. Cassani, et al. (2007). "Multilineage hematopoietic reconstitution without clonal selection in ADA-SCID patients treated with stem cell gene therapy." J Clin Invest **117**(8): 2233-40.

Gene transfer into HSCs is an effective treatment for SCID, although potentially limited by the risk of insertional mutagenesis. We performed a genome-wide analysis of retroviral vector integrations in genetically corrected HSCs and their multilineage progeny before and up to 47 months after transplantation into 5 patients with adenosine deaminase-deficient SCID. Gene-dense regions, promoters, and transcriptionally active genes were preferred retroviral integrations sites (RISs) both in preinfusion transduced CD34(+) cells and in vivo after gene therapy. The occurrence of insertion sites proximal to protooncogenes or genes controlling cell growth and self renewal, including LMO2, was not associated with clonal selection or expansion in vivo. Clonal analysis of long-term repopulating cell progeny in vivo revealed highly polyclonal T cell populations and shared RISs among multiple lineages, demonstrating the engraftment of multipotent HSCs. These data have important implications for the biology of retroviral vectors, the dynamics of genetically modified HSCs, and the safety of gene therapy.

Akiyama, M., Y. Hoshi, et al. (1998). "Changes of telomere length in children after hematopoietic stem cell transplantation." Bone Marrow Transplant **21**(2): 167-71.

Telomeres are responsible for keeping the stability not only of chromosomes but also of genes. To investigate the effect of hematopoietic stem cell transplantation (HSCT) on telomeres, we studied telomere length in the peripheral blood mononuclear cells of 31 children who received HSCT. In the auto-HSCT groups telomere length ranged from 8.6 to 12.0 kb and in the allo-HSCT groups from 8.4 to 12.0 kb. Comparison of the telomere length between before and after auto-HSCT showed shorting up to 1.0 kb. Moreover, comparison between donors and recipients in allo-HSCT revealed that telomeres of recipients were up to 1.0 kb shorter than those of the donors. Patients who received allo-HSCT from donors older than 18 years had significantly shorter telomeres than those transplanted from donors under 18 years old ($P < 0.05$), indicating that donor age is an important factor for recipient's telomere length. These findings suggest that the effects which might be induced by shortening of telomeres in recipients are within the biologically tolerable range. However, if hematopoietic stem cells from elderly donors are transplanted into younger patients, the telomere length may become too short for acceptable lifetime risks of genetic instability in the recipient.

An, D. S., S. K. Kung, et al. (2001). "Lentivirus vector-mediated hematopoietic stem cell gene transfer of common gamma-chain cytokine receptor in rhesus macaques." J Virol **75**(8): 3547-55.

Nonhuman primate model systems of autologous CD34+ cell transplant are the most effective means to assess the safety and capabilities of lentivirus vectors. Toward this end, we tested the efficiency of marking, gene expression, and transplant of bone marrow and peripheral blood CD34+ cells using a self-inactivating lentivirus vector (CS-Rh-MLV-E) bearing an internal murine leukemia virus long terminal repeat derived from a murine retrovirus adapted to replicate in rhesus macaques. In vitro cytokine stimulation was not required to achieve

efficient transduction of CD34+ cells resulting in marking and gene expression of the reporter gene encoding enhanced green fluorescent protein (EGFP) following transplant of the CD34+ cells. Monkeys transplanted with mobilized peripheral blood CD34+ cells resulted in EGFP expression in 1 to 10% of multilineage peripheral blood cells, including red blood cells and platelets, stable for 15 months to date. The relative level of gene expression utilizing this vector is 2- to 10-fold greater than that utilizing a non-self-inactivating lentivirus vector bearing the cytomegalovirus immediate-early promoter. In contrast, in animals transplanted with autologous bone marrow CD34+ cells, multilineage EGFP expression was evident initially but diminished over time. We further tested our lentivirus vector system by demonstrating gene transfer of the human common gamma-chain cytokine receptor gene (gamma(c)), deficient in X-linked SCID patients and recently successfully used to treat disease. Marking was 0.42 and.001 HIV-1 vector DNA copy per 100 cells in two animals. To date, all EGFP- and gamma(c)-transplanted animals are healthy. This system may prove useful for expression of therapeutic genes in human hematopoietic cells.

Anzai, H., M. Nagayoshi, et al. (1999). "Self-renewal and differentiation of a basic fibroblast growth factor-dependent multipotent hematopoietic cell line derived from embryonic stem cells." Dev Growth Differ **41**(1): 51-8.

Despite the accumulation of informat on on the origin of hematopoietic stem cells, it is still unclear how these cells are generated in ontogeny. Isolation of cell lines equivalent to early embryonic hematopoietic progenitor cells can be helpful. A multipotent hematopoietic progenitor cell line, A-6, was isolated from H-1 embryonic stem (ES) cells. The self-renewal of A-6 cells was supported by basic-fibroblast growth factor (b-FGF) and their differentiation into definitive erythroid cells, granulocytes and macrophages was induced after co-culture with ST-2 stromal cells. A-6 cells were positive for the surface markers of hematopoietic stem cell, c-kit, CD31, CD34, Flt3/Flk2, PgP-1, and HSA, but were negative for that of the differentiated cells. Reverse transcription-polymerase chain reaction analysis showed that A-6 cells produced mRNA from SCL/tal-1 and GATA-2 genes. Among various cytokines examined, on y stem cell factor (SCF) and Flt3/Flk2 ligand (FL) supported the proliferation of A-6 cells instead of b-FGF. The FL, as well as b-FGF, supported the self-renewal of A-6 cells, whereas SCF induced differentiation into myeloid cells. A-6 cells will be useful for the characterization of hematopoietic progenitor cells derived from ES cells and provide a model system to realize the control mechanisms between self-renewal and different ation of hematopoietic stem cells.

Appel, S. H., J. I. Engelhardt, et al. (2008). "Hematopoietic stem cell transplantation in patients with sporadic amyotrophic lateral sclerosis." Neurology **71**(17): 1326-34.

BACKGROUND: Amyotrophic lateral sclerosis (ALS), an inexorably progressive motoneuron disease, is accompanied by significantly increased markers of inflammation. These inflammatory constituents could protect, harm, do neither, or do both. OBJECTIVE: Allogeneic hematopoietic stem cell transplantation (HSCT) was performed in patients with sporadic ALS to suppress neuroinflammation and improve clinical outcomes after CNS engraftment. METHODS: Six patients with definite ALS received total body irradiation followed by peripheral blood HSCT infusion from human leukocyte antigen identically matched sibling donors. Disease progression and survival were assessed monthly and compared with matched historic database patients. Autopsy samples from brain and spinal cord were examined immunohistochemically and by quantitative reverse-transcriptase polymerase chain reaction. Donor-derived DNA in brain and spinal cord tissue was evaluated for the extent of chimerism. RESULTS: No clinical benefits were evident. Four patients were 100% engrafted; postmortem tissue examination in two of the 100% engrafted patients demonstrated 16% to 38% donor-derived DNA at sites with motoneuron pathology, which may correspond to the observed increased CD68 or CD1a-positive cells. Neither donor DNA nor increased cell numbers were found in several unaffected brain regions. A third minimally engrafted patient had neither donor DNA nor increased infiltrating cells in the CNS. CONCLUSIONS: This study demonstrates that peripheral cells derived from donor hematopoietic stem cells can enter the human CNS primarily at sites of motoneuron pathology and engraft as immunomodulatory cells. Although unmodified hematopoietic stem cells did not benefit these sporadic amyotrophic lateral sclerosis patients, such cells may provide a cellular vehicle for future CNS gene therapy.

Armistead, P. M., M. Mohseni, et al. (2008). "Erythroid-lineage-specific engraftment in patients with severe hemoglobinopathy following allogeneic hematopoietic stem cell transplantation." Exp Hematol **36**(9): 1205-15.

OBJECTIVE: We aimed to create a molecular assay to monitor erythroid (red blood cell [RBC]) engraftment in any patient following

allogeneic hematopoietic stem cell transplantation, independent of disease-specific mutations. MATERIALS AND METHODS: We identified 10 common single nucleotide polymorphisms (SNPs), expressed by genes encoding RBC antigens and structural proteins. These SNPs were polymerase chain reaction-amplified from total RNA extracted from peripheral blood, which contains nucleated erythroid progenitors. Mixing studies validated that each SNP can quantitatively measure donor/recipient DNA and RNA. RESULTS: We directly genotyped 23 patients who underwent hematopoietic stem cell transplantation and their human leukocyte antigen-matched donors and found a median of three informative SNPs (i.e., discordant between donor and recipient) per pair. By using the informative RBC SNPs to quantify donor-derived RBC transcripts, we compared rates of RBC engraftment in 13 patients with hemoglobinopathies vs donor mononuclear cell (white blood cell [WBC]) engraftment. Consistent with known ineffective erythropoiesis associated with hemoglobinopathies, we detected up to threefold greater RBC-specific compared to overall WBC engraftment in five of eight patients who were mixed chimeras by transplant day 30. The remaining three of eight who received ABH-incompatible grafts, demonstrated at least 0.5-fold lower RBC compared to WBC engraftment that was related to persistence of host-derived anti-isohemagglutinin antibodies. CONCLUSION: This RNA-based assay can be used to monitor RBC-specific engraftment regardless of a patient's specific hemoglobin mutation or even diagnosis. We propose that panels of expressed SNPs informative for other cell lineages can be created to comprehensively assess the impact of novel stem cell-based therapies on lineage-specific engraftment.

Attema, J. L., P. Papathanasiou, et al. (2007). "Epigenetic characterization of hematopoietic stem cell differentiation using miniChIP and bisulfite sequencing analysis." Proc Natl Acad Sci U S A **104**(30): 12371-6.

Hematopoietic stem cells (HSC) produce all blood cell lineages by virtue of their capacity to self-renew and differentiate into progenitors with decreasing cellular potential. Recent studies suggest that epigenetic mechanisms play an important role in controlling stem cell potency and cell fate decisions. To investigate this hypothesis in HSC, we have modified the conventional chromatin immunoprecipitation assay allowing for the analysis of 50,000 prospectively purified stem and progenitor cells. Together with bisulfite sequencing analysis, we found that methylated H3K4 and AcH3 and unmethylated CpG dinucleotides colocalize across defined regulatory regions of lineage-affiliated genes in HSC. These active epigenetic histone modifications either accumulated or were replaced by increased DNA methylation and H3K27 trimethylation in committed progenitors consistent with gene expression. We also observed bivalent histone modifications at a lymphoid-affiliated gene in HSC and downstream transit-amplifying progenitors. Together, these data support a model in which epigenetic modifications serve as an important mechanism to control HSC multipotency.

Attema, J. L., C. J. Pronk, et al. (2009). "Hematopoietic stem cell ageing is uncoupled from p16 INK4A-mediated senescence." Oncogene **28**(22): 2238-43.

Somatic stem cells are ultimately responsible for mediating appropriate organ homeostasis and have therefore been proposed to represent a cellular origin of the ageing process-a state often characterized by inappropriate homeostasis. Specifically, it has been suggested that ageing stem cells might succumb to replicative senescence by a mechanism involving the cyclin-dependent kinase inhibitor p16(INK4A). Here, we tested multiple functional and molecular parameters indicative of p16(INK4A) activity in primary aged murine hematopoietic stem cells (HSCs). We found no evidence that replicative senescence accompanies stem cell ageing in vivo, and in line with p16(INK4A) being a critical determinant of such processes, most aged HSCs (>99%) failed to express p16(INK4A) at the mRNA level. Moreover, whereas loss of epigenetically guided repression of the INK4A/ARF locus accompanied replicative senescent murine embryonic fibroblasts, such repression was maintained in aged stem cells. Taken together, these studies indicate that increased senescence as mediated by the p16(INK4A) tumor suppressor has only a minor function as an intrinsic regulator of steady-state HSC ageing in vivo.

Au, W. Y., A. Pang, et al. (2007). "G6PD deficiency from lyonization after hematopoietic stem cell transplantation from female heterozygous donors." Bone Marrow Transplant **40**(7): 677-81.

To determine whether during hematopoietic stem cell transplantation (HSCT), X-chromosome inactivation (lyonization) of donor HSC might change after engraftment in recipients, the glucose-6-phosphate dehydrogenase (G6PD) gene of 180 female donors was genotyped by PCR/allele-specific primer extension, and MALDI-TOF mass spectrometry/Sequenom MassARRAY analysis. X-inactivation was determined by semiquantitative PCR for the HUMARA gene before/after HpaII digestion. X-inactivation was preserved in most cases post-HSCT, although altered skewing of lyonization might

occur to either of the X-chromosomes. Among pre-HSCT clinicopathologic parameters analyzed, only recipient gender significantly affected skewing. Seven donors with normal G6PD biochemically but heterozygous for G6PD mutants were identified. Owing to lyonization changes, some donor-recipient pairs showed significantly different G6PD levels. In one donor-recipient pair, extreme lyonization affecting the wild-type G6PD allele occurred, causing biochemical G6PD deficiency in the recipient. In HSCT from asymptomatic female donors heterozygous for X-linked recessive disorders, altered lyonization might cause clinical diseases in the recipients.

Battista, S., F. Pentimalli, et al. (2003). "Loss of Hmga1 gene function affects embryonic stem cell lympho-hematopoietic differentiation." Faseb J **17**(11): 1496-8.

By interacting with transcription machinery, high-mobility group A 1 (HMGA1) proteins alter the chromatin structure and thereby regulate the transcriptional activity of several genes. To assess their role in development, we studied the in vitro differentiation of embryonic stem (ES) cells that bear one or both disrupted Hmga1 alleles. Here, we report that Hmga1 null ES cells generate fewer T-cell precursors than do wild-type ES cells. Indeed, they preferentially differentiate to B cells, probably consequent to decreased interleukin 2 expression and increased interleukin 6 expression. Moreover, a lack of HMGA1 expression induces changes in hemopoietic differentiation, i.e., a reduced monocyte/macrophage population and an increase in megakaryocyte precursor numbers, erythropoiesis, and globin gene expression. Re-expression of the Hmga1 gene in Hmga1 null ES cells restores the wild-type phenotype. The effect on megakaryocyte/erythrocyte lineages seems, at least in part, mediated by the GATA-1 transcription factor, a key regulator of red blood cell differentiation. In fact, we found that Hmga1-/- ES cells overexpress GATA-1 and that HMGA1 proteins directly control GATA-1 transcription. Taken together, these data indicate that HMGA1 proteins play a prime role in lymphohematopoietic differentiation.

Bauer, T. R., Jr., K. E. Creevy, et al. (2004). "Very low levels of donor CD18+ neutrophils following allogeneic hematopoietic stem cell transplantation reverse the disease phenotype in canine leukocyte adhesion deficiency." Blood **103**(9): 3582-9.

Children with the severe phenotype of the genetic immunodeficiency disease leukocyte adhesion deficiency or LAD experience life-threatening bacterial infections because of molecular defects in the leukocyte integrin CD18 molecule and the resultant failure to express the CD11/CD18 adhesion molecules on the leukocyte surface. Hematopoietic stem cell transplantation remains the only definitive therapy for LAD; however, the degree of donor chimerism and particularly the number of CD18(+) donor-derived neutrophils required to reverse the disease phenotype are not known. We performed nonmyeloablative hematopoietic stem cell transplantations from healthy matched littermates in 9 dogs with the canine form of LAD known as CLAD and demonstrate that in the 3 dogs with the lowest level of donor chimerism, less than 500 CD18(+) donor-derived neutrophils/microL in the peripheral blood of the CLAD recipients resulted in reversal of the CLAD disease phenotype. These results demonstrate the value of a disease-specific, large-animal model for identifying the lowest therapeutic level required for successful cellular and gene therapy.

Baum, C., H. G. Eckert, et al. (1996). "Improved retroviral vectors for hematopoietic stem cell protection and in vivo selection." J Hematother **5**(4): 323-9.

Therapeutic gene transfer into hematopoietic cells is critically dependent on the evolution of methods that allow ex vivo expansion, high-frequency transduction, and selection of gene-modified long-term repopulating cells. Progress in this area needs elaboration of defined culture and transduction conditions for long-term repopulating cells and improvement of gene transfer systems. We have optimized retroviral vector constructions based on murine leukemia viruses (MuLV) to overcome the transcriptional repression encountered with the use of conventional Moloney MuLV (MoMuLV) vectors in early hematopoietic progenitor cells (HPC). Novel retroviral vectors, termed FMEV (for Friend-MCF/MESV hybrid vectors), were cloned that mediate greatly improved gene expression in the myeloerythroid compartment. Transfer of the selectable marker multidrug resistance 1 (mdr1), FMEV, in contrast to conventional MoMuLV-related vectors currently in use for clinical protocols, mediated background-free selectability of transduced human HPC in the presence of myeloablative doses of the cytostatic agent paclitaxel in vitro. Furthermore, FMEV also greatly improved chemo-protection of hematopoietic progenitor cells in a murine model system in vivo. Finally, when a second gene was transferred along with mdr1 in an FMEV-backbone, close to 100% coexpression was observed in multidrug-resistant colonies. These observations have significant consequences for a number of ongoing and planned gene therapy trials, for example, stem cell protection to reduce the myelotoxic side effects of

anticancer chemotherapy, correction of inherited disorders involving hematopoietic cells, and antagonism of HIV infection.

Beck-Engeser, G., C. Stocking, et al. (1991). "Retroviral vectors related to the myeloproliferative sarcoma virus allow efficient expression in hematopoietic stem and precursor cell lines, but retroviral infection is reduced in more primitive cells." Hum Gene Ther **2**(1): 61-70.

Retroviral vectors are considered to be the most suited vehicles for somatic gene therapy with hematopoietic stem cells as targets. Retrovirus-mediated gene transfer into differentiation-restricted hematopoietic precursor (FDC-P1, FDC-P2) and multipotent progenitor (stem) cell lines (FDC-Pmix) is inefficient. Two cellular restrictions are involved. One is specific for stem but not precursor cells and is at the level of transcription. Due to a unique property of the transcriptional control region of the myeloproliferative sarcoma virus (MPSV), vectors derived from MPSV are not affected by this block. The second restriction occurs before proviral DNA synthesis and integration. This inhibition of effective viral infection depends on the state of differentiation, being more pronounced in multipotent clonogenic blast cells. This block to retroviral infection affects all retroviral vectors tested.

Bloor, A. J., M. J. Sanchez, et al. (2002). "The role of the stem cell leukemia (SCL) gene in hematopoietic and endothelial lineage specification." J Hematother Stem Cell Res **11**(2): 195-206.

Anatomical observations made at the beginning of the twentieth century revealed an intimate association between the ontogeny of blood and endothelium and led to the hypothesis of a common cell of origin termed the hemangioblast. However, the precise nature of the cellular intermediates involved in the development of both lineages from uncommitted precursors to mature cell types is still the subject of ongoing studies, as are the molecular mechanisms driving this process. There is clear evidence that lineage-restricted transcription factors play a central role in the genesis of mature lineage committed cells from multipotent progenitors. Amongst these, the basic helix-loop-helix (bHLH) family is of key importance for cell fate determination in the development of the hematopoietic system and beyond. This article will review the current evidence for the common origin of blood and endothelium, focusing on the function of the bHLH protein encoded by the stem cell leukemia (SCL) gene, and its role as a pivotal regulator of hematopoiesis and vasculogenesis.

Bodine, D. M., K. T. McDonagh, et al. (1990). "Development of a high-titer retrovirus producer cell line capable of gene transfer into rhesus monkey hematopoietic stem cells." Proc Natl Acad Sci U S A **87**(10): 3738-42.

Retroviral-mediated gene transfer into primitive hematopoietic cells has been difficult to achieve in large-animal models. We have developed an amphotropic producer clone that generates greater than 10(10) recombinant retroviral particles (colony-forming units) per ml of culture medium. Autologous rhesus monkey bone-marrow cells were cocultured with either high (2 x 10(10) colony-forming units/ml) or low (5 x 10(6) colony-forming units/ml) titer producer clones for 4-6 days and reinfused into sublethally irradiated animals. The proviral genome was detected in blood and bone-marrow cells from all three animals reconstituted with cells cocultured with the high-titer producer cells. In contrast, three animals reconstituted with bone marrow cocultured with the low-titer producer clone exhibited no evidence of gene transfer.

Bodine, D. M., N. E. Seidel, et al. (1994). "Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte colony-stimulating factor and stem cell factor." Blood **84**(5): 1482-91.

Cytokine-mobilized peripheral blood cells have been shown to participate in hematopoietic recovery after bone marrow (BM) transplantation, and are proposed to be useful targets for retrovirus-mediated gene transfer protocols. We treated mice with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) to mobilize hematopoietic progenitor cells into the peripheral blood. These cells were analyzed for the number and frequency of pluripotent hematopoietic stem cells (PHSC). We found that splenectomized animals treated for 5 days with G-CSF and SCF showed a threefold increase in the absolute number of PHSC over normal mice. The number of peripheral-blood PHSC increased 250-fold from 29 per untreated mouse to 7,200 in peripheral-blood PHSC in splenectomized animals treated for 5 days with G-CSF and SCF. Peripheral blood PHSC mobilized by treatment with G-CSF and SCF were analyzed for their ability to be transduced by retroviral vectors. Peripheral-blood PHSC from splenectomized animals G-CSF and SCF were transduced with a recombinant retrovirus containing the human MDR-1 gene. The frequency of gene transfer into peripheral blood PHSC from animals treated for 5 and 7 days was two-fold and threefold higher than gene transfer into PHSC from the BM of 5-fluorouracil-treated mice ($P < .01$). We conclude that peripheral blood

stem cells mobilized by treatment with G-CSF and SCF are excellent targets for retrovirus-mediated gene transfer.

Bodine, D. M., N. E. Seidel, et al. (1993). "In vivo administration of stem cell factor to mice increases the absolute number of pluripotent hematopoietic stem cells." Blood **82**(2): 445-55.

We have examined the effects of administration of stem cell-factor (SCF) on the number and distribution of pluripotent hematopoietic stem cells (PHSC) in normal mice. Using the competitive repopulation assay we found that in vivo administration of SCF increases the absolute number of PHSC per mouse threefold. The increased numbers of PHSC are found in the peripheral blood and spleen of the SCF-treated animals. The spleen and peripheral blood stem cells completely repopulated the erythroid, myeloid, and lymphoid lineages of irradiated or W/Wv hosts, similar to bone marrow PHSC. PHSC from the peripheral blood of SCF-treated mice have a lineage marker-negative, c-kit-positive phenotype that is indistinguishable from that of bone marrow PHSC. The increase in the absolute number of spleen PHSC is associated with efficient gene transfer to these cells without prior treatment with 5-fluorouracil. This is a US government work. There are no restrictions on its use.

Bradley, H. L., T. S. Hawley, et al. (2002). "Cell intrinsic defects in cytokine responsiveness of STAT5-deficient hematopoietic stem cells." Blood **100**(12): 3983-9.

Secreted growth factors are integral components of the bone marrow (BM) niche and can regulate survival, proliferation, and differentiation of committed hematopoietic stem cells (HSCs). However, downstream genes activated in HSCs by early-acting cytokines are not well characterized. To better define intracellular cytokine signaling in HSC function, we have analyzed mice lacking expression of both signal transducer and activator of transcription 5a (STAT5a) and STAT5b (STAT5ab(-/-)). These studies specifically avoided possible autoimmune and/or splenomegaly disease-mediated indirect effects on HSC function by using 2 independent approaches: (1) by crossing onto the C57Bl/6 RAG2(-/-) background, and (2) by generation of wild-type chimeric mice reconstituted with transplanted STAT5ab(-/-) BM cells. These experiments demonstrated that STAT5-deficient HSCs have cell autonomous defects in competitive long-term repopulating activity. Furthermore, in the chimeric mice, injected wild-type BM cells showed a progressive multilineage competitive repopulating advantage in vivo, demonstrating that steady-state hematopoiesis was also highly STAT5-dependent. Consistent with the in vivo repopulating deficiency, when Sca-1(+)c-kit(+)lin(-) (KLS) cells were isolated and stimulated with growth factors in vitro, up to a 13-fold reduced expansion of total nucleated cells was observed in response to cocktails containing interleukin 3 (IL-3), IL-6, stem cell factor (SCF), Flt3 ligand, and thrombopoietin. Notably, a 10-fold reduction in expansion was observed with IL-3 and SCF. However, STAT5 activation was not required for regeneration of the KLS pool in vivo following transplant or for secondary repopulating ability. These studies support a major role for STAT5 activation as a cellular determinant of cytokine-mediated HSC repopulating potential but not self-renewal capacity.

Broske, A. M., L. Vockentanz, et al. (2009). "DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction." Nat Genet **41**(11): 1207-15.

DNA methylation is a dynamic epigenetic mark that undergoes extensive changes during differentiation of self-renewing stem cells. However, whether these changes are the cause or consequence of stem cell fate remains unknown. Here, we show that alternative functional programs of hematopoietic stem cells (HSCs) are governed by gradual differences in methylation levels. Constitutive methylation is essential for HSC self-renewal but dispensable for homing, cell cycle control and suppression of apoptosis. Notably, HSCs from mice with reduced DNA methyltransferase 1 activity cannot suppress key myeloerythroid regulators and thus can differentiate into myeloerythroid, but not lymphoid, progeny. A similar methylation dosage effect controls stem cell function in leukemia. These data identify DNA methylation as an essential epigenetic mechanism to protect stem cells from premature activation of predominant differentiation programs and suggest that methylation dynamics determine stem cell functions in tissue homeostasis and cancer.

Burn, T. C., A. B. Satterthwaite, et al. (1992). "The human CD34 hematopoietic stem cell antigen promoter and a 3' enhancer direct hematopoietic expression in tissue culture." Blood **80**(12): 3051-9.

The human CD34 hematopoietic stem cell antigen is a highly glycosylated type 1 membrane protein of unknown function. CD34 is expressed on 1% to 4% of bone marrow cells, including pluripotent stem cells and committed progenitors of each hematopoietic lineage. CD34 has also been shown to be expressed on the small vessel endothelium of a variety of tissues and on a subset of bone marrow stromal cells. We have chosen to use the human CD34 gene as model to examine the transcription factors and

cis-elements required for stem cell/progenitor cell-specific gene regulation. We show here that the CD34 gene is transcriptionally regulated in tissue culture cells. Using a luciferase reporter gene, we have isolated and characterized an active CD34 promoter. A CD34-luciferase construct, containing 4.5 kb of 5' flanking DNA from a CD34 genomic clone, was 30-fold more active in CD34+ tissue culture cells than in HeLa cells. Sequences from the 3' end of the CD34 gene were shown to have enhancing activity in CD34+ T-lymphoblastic RPMI-8402 cells and not in CD34- U937 cells or in nonhematopoietic HeLa cells. We also show that a cytidine-guanosine island in the 5' end of the CD34 gene is heavily methylated in two CD34- hematopoietic cell lines and demethylated in two CD34+ cell lines. Analysis of the CD34 promoter should result in the identification of stem cell/progenitor cell-specific transcription factors and should provide a means to direct the expression of heterologous genes in hematopoietic stem cells and progenitors.

Capel, B., R. G. Hawley, et al. (1990). "Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers." Blood **75**(12): 2267-70.

The proliferative longevity of totipotent hematopoietic stem cells (THSC) is a limiting factor in normal hematopoiesis and in therapy by cell- or gene-replacement, but has not yet been ascertained. We have followed the long-term fate of individual clones of mouse THSC from fetal liver or adult bone marrow, after labeling in culture, followed by engraftment and serial transplantation in unirradiated W/Wv-C57BL/6 hosts. The ancestor cell of each clone and its mitotic progeny were uniquely identifiable retrospectively by the DNA integration pattern experimentally produced by replication-incompetent recombinant murine retroviruses. These viruses provided physiologically neutral markers. The marked clones proved to be derived from THSC, based on their contributions to a wide array of myeloid and lymphoid blood lineages in the hosts. The label also identified the target cells as the population displaying clonal succession. The various labeled stem cell clones proliferated for substantially different periods of time. The longest observed clone endured, after the original cell was marked, for at least 2 1/2 years--the equivalent of a mouse's lifetime. However, the results suggest that THSC clones are not all long-lived and that even the longest-lived ones may not be potentially immortal. Thus, the unpredictable lifespan of any given THSC clone indicates the desirability of introducing multiple clones in therapeutic transplants.

Carr, M. J., G. P. McCormack, et al. (2006). "Unique BK virus non-coding control region (NCCR) variants in hematopoietic stem cell transplant recipients with and without hemorrhagic cystitis." J Med Virol **78**(4): 485-93.

Hematopoietic stem cell transplant recipients frequently develop BK virus (BKV)-associated hemorrhagic cystitis, which coincides with BK viruria. However, the precise role of BKV in the etiology of hemorrhagic cystitis in hematopoietic stem cell transplant recipients remains unclear, since approximately 50% of all such adult transplant recipients excrete BKV, yet do not develop this clinical condition. In the present study, BKV were analyzed to determine if mutations in the non-coding control region (NCCR), and specific BKV sub-types defined by sequence analysis of major capsid protein VP1, were associated with development of hemorrhagic cystitis in hematopoietic stem cell transplant recipients. The regions encoding VP1 and NCCRs of BKV in urine samples collected from 15 hematopoietic stem cell transplant recipients with hemorrhagic cystitis and 20 without this illness were amplified and sequenced. Sequence variations in the NCCRs of BKV were identified in urine samples from those with and without hemorrhagic cystitis. Furthermore, five unique sequence variations within transcription factor binding sites in the canonical NCCR, O-P-Q-R-S, were identified, representing new BKV variants from a population of cloned quasi-species obtained from patients with and without hemorrhagic cystitis. Thirty-five BKV VP1 sequences were analyzed by phylogenetic analysis but no specific BKV sub-type was associated with hemorrhagic cystitis. Five previously unrecognized naturally occurring variants of the BKV are described which involve amplifications, deletions, and rearrangements of the archetypal BKV NCCRs in individuals with and without hemorrhagic cystitis. Architectural rearrangements in the NCCRs of BKV did not appear to be a prerequisite for development of hemorrhagic cystitis in hematopoietic stem cell transplant recipients.

Chen, J., A. Larochelle, et al. (2006). "Mobilization as a preparative regimen for hematopoietic stem cell transplantation." Blood **107**(9): 3764-71.

Current myeloablative conditioning regimens for hematopoietic stem cell (HSC) transplantation are associated with significant morbidity and mortality. Thus, alternative strategies to promote engraftment of infused HSCs with increased safety warrant investigation. Using parabiotic mice, we determined that, after mobilization with AMD3100 (a CXCR4 antagonist), HSCs exited from marrow, transited blood, and engrafted in open niches in partner

marrow. We then hypothesized that mobilization before transplantation might vacate niches and improve HSC engraftment. When PeP3(b) mice were treated with AMD3100 at 2 hours before the transplantation of 4 x 10(7) marrow cells, donor cell engraftment was higher (4.6% +/- 1.1%) than in control animals (no AMD3100; 1.0% +/- 0.24%, P < .001). When mice received weekly injections of AMD3100 on 3 consecutive weeks and marrow cells were transplanted 2 hours after each mobilization, donor cell engraftment further increased (9.1% +/- 1.7%, P = .001). In contrast, in similar experiments with Balb/cByJ mice that mobilize poorly, there was no difference between the donor cell engraftment of AMD3100-treated and control recipients. These results indicate that the number of available niches regulates the number of HSCs. In addition, mobilization with AMD3100 may provide a safer preparative approach for HSC transplantation in genetic and other nonmalignant disorders.

Cowan, K. H., J. A. Moscow, et al. (1999). "Paclitaxel chemotherapy after autologous stem-cell transplantation and engraftment of hematopoietic cells transduced with a retrovirus containing the multidrug resistance complementary DNA (MDR1) in metastatic breast cancer patients." Clin Cancer Res **5**(7): 1619-28.

The MDR1 multidrug resistance gene confers resistance to natural-product anticancer drugs including paclitaxel. We conducted a clinical gene therapy study to determine whether retroviral-mediated transfer of MDR1 in human hematopoietic cells would result in stable engraftment, and possibly expansion, of cells containing this gene after treatment with myelosuppressive doses of paclitaxel. Patients with metastatic breast cancer who achieved a complete or partial remission after standard chemotherapy were eligible for the study. Hematopoietic stem cells (HSCs) were collected by both peripheral blood apheresis and bone marrow harvest after mobilization with a single dose of cyclophosphamide (4 g/m2) and daily filgrastim therapy (10 microg/kg/day). After enrichment for CD34+ cells, one-third of each collection was incubated ex vivo for 72 h with a replication-incompetent retrovirus containing the MDR1 gene (G1MD) in the presence of stem-cell factor, interleukin 3, and interleukin 6. The remaining CD34+ cells were stored without further manipulation. All of the CD34+ cells were reinfused for hematopoietic rescue after conditioning chemotherapy with ifosfamide, carboplatin, and etoposide regimen. After hematopoietic recovery, patients received six cycles of paclitaxel (175 mg/m2 every 3 weeks). Bone marrow and serial peripheral blood samples were obtained and tested for the presence of the MDR1 transgene using a PCR assay. Six patients were enrolled in the study and four patients received infusion of genetically altered cells. The ex vivo transduction efficiency, estimated by the PCR assay, ranged from 0.1 to 0.5%. Three of the four patients demonstrated engraftment of cells containing the MDR1 transgene. The estimated percentage of granulocytes containing the MDR1 transgene ranged from a maximum of 9% of circulating nucleated cells down to the limit of detection of 0.01%. One patient remained positive for the MDR1 transgene throughout all six cycles of paclitaxel therapy, whereas the other 2 patients showed a decrease in the number of cells containing the transgene to undetectable levels. Despite the low level of engraftment of MDR1-marked cells, a correlation was observed between the relative number of granulocytes containing the MDR1 transgene and the granulocyte nadir after paclitaxel therapy. No adverse reactions to the genetic manipulation procedures were detected. Therefore, engraftment of human HSCs transduced with the MDR1 gene can be achieved. However, the overall transduction efficiency and stable engraftment of gene-modified HSCs must be improved before MDR1 gene therapy and in vivo selection with anticancer drugs can be reliably used to protect cancer patients from drug-related myelosuppression.

Dou, L. P., H. Zheng de, et al. (2008). "The diversity of KIR gene in Chinese Northern Han population and the impact of donor KIR and patient HLA genotypes on outcome following HLA-identical sibling allogeneic hematopoietic stem cell transplantation for hematological malignancy in Chinese people." Int J Hematol **87**(4): 422-33.

Killer cell immunoglobulin-like receptors (KIRs) are members of a group of molecules that specifically recognize HLA class I ligands and are found on subsets of human lymphopoetic cells. The number of KIR loci can vary between individuals, resulting in a heterogeneous array of possible KIR genes. The range of observed profiles has been explained by the occurrence of two haplotype families termed A and B, which can be distinguished on the basis of certain KIR sequences. Immunogenetic analysis of different ethnic populations shows significant differences in terms of the distribution for group A and group B haplotypes. Recently, attention has been focused on the role of killer cell immunoglobulin-like receptor (KIR)-ligand incompatibility in the graft-versus-host direction between donor and recipient in allogeneic hematopoietic stem-cell transplantation (ASCT). The goal of this study was to study the frequency of specific KIR genes in Chinese Northern Han

population and evaluate the role of KIR-ligand mismatch in Chinese HLA-identical sibling hematopoietic stem cell transplantation patients with hematological malignancy. Here genomic DNA from 150 Northern Chinese Han individuals was typed for the presence or absence of KIR genes. Seventy-four allogeneic stem cell transplantation donor/recipient pairs were typed for HLA-A, B, C and KIR. Sixteen KIR genes were observed in the population, and framework genes 3DL3, 3DP1, 2DL4, and 3DL2 were present in all individuals. Twenty-two different genotypes were found. Group A haplotypes outnumbered group B haplotypes in frequency by approximately 3:1, with individuals having two group A haplotypes accounting for 51.9% (78/150). We observed that 57 out of 74 (77.3%) donor-recipient pairs could be characterized by lack of recipient HLA ligand for donor KIR. We observed that 36 out of 45 (80%) donor-recipient HLA-identical sibling transplant pairs could be characterized by lack of recipient HLA ligand for donor KIR. Cumulative incidence analysis of aGVHD in patients undergoing HLA-identical sibling hematopoietic stem cell transplantation in this study demonstrated a decreased incidence of severe aGVHD in patients lacking HLA ligand for donor-inhibitory KIR2DL1 (31.4 vs. 70%, P = 0.029). And also in AML (acute myeloid leukemia) patients lacking HLA ligand for donor-inhibitory KIR and KIR2DL1 (17.6 vs. 75%, P = 0.03). Our data demonstrated that the Chinese Han population is distinct in KIR gene frequencies and putative KIR haplotypes in comparison to some other populations. Almost all allogeneic donors could be characterized as having an inhibitory KIR for each of the three known class I ligands. KIR and KIR2DL1 mismatch is associated with lower aGVHD in Chinese after HLA-identical sibling hematopoietic stem cell transplantation.

Downing, J., M. G. Guttridge, et al. (2004). "Five-locus HLA typing of hematopoietic stem cell donor volunteers using PCR sequence specific primers." Genet Test **8**(3): 301-12.

We have developed a strategy for five-locus human leukocyte antigen (HLA) typing of hematopoeitic stem cell (HSC) donors using the polymerase chain reaction with sequence-specific primers (PCR-SSP). The PCR-SSP method is robust, reproducible, and accurate. New PCR-SSP mixtures can be added as required and all reactions are carried out under the same conditions, which can easily be applied to the typing of other loci, e.g., ABO blood groups. Initially, 127 PCR-SSP reactions were used to detect simultaneously HLA-A, -B, -C, -DRB1/3/4/5, and DQB1 alleles, differentiated generally to the level of the first two digits of the allele name, essentially equivalent to the serological split specificity. Approximately 40% of subjects were tested against a further 29 HLA-A, -B SSP mixtures to exclude rare alleles and unambiguously assign a two-digit HLA allele family. This gave an overall typing resolution equivalent to or greater than the split specificity level and covered all HLA-A, -B, -C, -DRBland DQB1 alleles listed in the WHO's Nomenclature for Factors of the HLA System, 2000. The Welsh Bone Marrow Donor Registry has used this strategy to HLA type over 35,000 HSC donors over 9 years. Comprehensive and accurate five-locus HLA typing allows confident and rapid identification of potential matched HSC donors for patients requiring stem cell transplantation generally without the need for typing additional loci. This allows resources to be focused directly on allele level typing of DRB1 and other loci. This strategy decreases overall donor work-up time, which is a major benefit to patients.

Du, J. W., J. Y. Gu, et al. (2007). "TCR spectratyping revealed T lymphocytes associated with graft-versus-host disease after allogeneic hematopoietic stem cell transplantation." Leuk Lymphoma **48**(8): 1618-27.

Clonal expansion of T cells after allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been observed, but their characteristics remain to be fully elucidated. We report here that CD8(+) T cells were the dominant T lymphocytes seen and T-cell repertoire diversity decreased dramatically during the first 3 months after allo-HSCT. Patients with GVHD grade II - IV had significantly lower T-cell repertoire diversity compared with non-GVHD patients. TCR beta variable gene (TCRBV) subfamily 8, 5.1, 5.2, 4, and 13 were the five most frequently expanded subfamilies among these patients. Among the 49 over-expanded clones identified, clonotype "TCR3-5" and "TCR18-5" were isolated from four patients with HLA-A2 allele and skin GVHD. Their frequencies correlated well with skin symptoms (i.e. rash). Moreover, they were detected in donors but not detected in recipients before transplantation. Lastly, three common TCRBV CDR3 motifs shared by T cells related with GVHD were discovered: TGDS, GLAG, and GGG. These findings suggest that TCR spectratyping is helpful for revealing GVHD-related T cells and may have utility in early diagnosis.

Dykstra, B. and G. de Haan (2008). "Hematopoietic stem cell aging and self-renewal." Cell Tissue Res **331**(1): 91-101.

A functional decline of the immune system occurs during organismal aging that is attributable, in large part, to changes in the hematopoietic stem cell (HSC) compartment. In the mouse, several hallmark age-dependent changes in the HSC compartment have

been identified, including an increase in HSC numbers, a decrease in homing efficiency, and a myeloid skewing of differentiation potential. Whether these changes are caused by gradual intrinsic changes within individual HSCs or by changes in the cellular composition of the HSC compartment remains unclear. However, of note, many of the aging properties of HSCs are highly dependent on their genetic background. In particular, the widely used C57Bl/6 strain appears to have unique HSC aging characteristics compared with those of other mouse strains. These differences can be exploited by using recombinant inbred strains to further our understanding of the genetic basis for HSC aging. The mechanism(s) responsible for HSC aging have only begun to be elucidated. Recent studies have reported co-ordinated variation in gene expression of HSCs with age, possibly as a result of epigenetic changes. In addition, an accumulation of DNA damage, in concert with an increase in intracellular reactive oxygen species, has been associated with aged HSCs. Nevertheless, whether age-related changes in HSCs are programmed to occur in a certain predictable fashion, or whether they are simply an accumulation of random changes over time remains unclear. Further, whether the genetic dysregulation observed in old HSCs is a cause or an effect of cellular aging is unknown.

Enczmann, J., M. Rinker, et al. (1999). "Donor selection process for allogeneic hematopoietic stem cell transplantation at the university hospital of Dusseldorf (1997-1998)." Klin Padiatr **211**(4): 218-23.

BACKGROUND: Transplantation of hematopoietic stem cells (HSC) is an effective treatment for a number of patients with life-threatening hematologic diseases. HSC donors can be found in the family of the patient or in registries of unrelated donors. In the present study, the search procedure within the last two years for an allogeneic HSC donor at the University of Dusseldorf is analyzed. PATIENTS AND METHODS: During 1997 and 1998, an early search for a related HSC donor in the family was performed for 70 high risk pediatric patients. During the same period, the search for an unrelated HSC donor for 116 adult and pediatric patients was performed. Low resolution HLA-A and -B typing was performed by serology in combination with DNA-typing. High resolution typing of HLA-A, -B and -C was carried out by DNA-sequencing. Low resolution HLA-DRB- und HLA-DQB1-typing was done solely by DNA-typing and high resolution typing of these genes was performed by DNA-sequencing. MAIN RESULTS: For 51 of 70 high risk pediatric patients (73%), no family donor could be defined, 16 of 70 patients (23%) had a genotypically identical sibling and for three of 70 patients (4%) an HLA-acceptable donor in the extended family could be identified. The search for an unrelated HSC donor was successful in 74% of the adult and pediatric patients lacking such a family donor. Most noteworthy, nearly all of the HLA-acceptable donors were identified from that group of donors in the registries, which were not only HLA-A and HLA-B, but also HLA-DR pretyped. CONCLUSION: These data show, that a growing number of pediatric patients with high risk leukemia need an unrelated HSC donor and that HLA-ABDR-pretyped registries present the optimal prerequisite to identify an HSC donor for most of the patients. In addition, 25% of the patients with no family or unrelated HSC donor require HSC transplants from alternative donors like unrelated Cord Blood (CB) from high quality cord blood banks.

Filippi, M. D., F. Porteu, et al. (2000). "Embryonic stem cell differentiation to hematopoietic cells: A model to study the function of various regions of the intracytoplasmic domain of cytokine receptors in vitro." Exp Hematol **28**(12): 1363-72.

To examine whether the in vitro model of embryonic stem (ES) cell hematopoietic differentiation is suitable to study the function of intracytoplasmic regions of cytokine receptors, we used the thrombopoietin receptor Mpl as a typical cytokine receptor.ES cells deficient in c-mpl (mpl(-/)-) were transfected with genes encoding the full-length or two mutated forms of the intracytoplasmic domain of Mpl using the pEF-BOS expression vector. The mutated forms lack box1 or box2.pEF-BOS was able to maintain protein production during ES cell differentiation. Reintroduction of full-length-c-mpl into mpl(-/)- ES cells restored the response of megakaryocyte progenitors to a truncated form of human Mpl-ligand conjugated to polyethylene glycol (PEG-rhuMGDF) and the formation of platelets, for which mpl(-/)- ES cells are defective. In addition, enforced expression of Mpl resulted in the development of all myeloid progenitors and mature cells in the presence of PEG-rhuMGDF. Blast colony-forming cells, the in vitro equivalent of the hemangioblast, also generated blast cell colonies with a hematopoietic potential equivalent to that of the wild type in the presence of PEG-rhuMGDF, although its growth is normally dependent on vascular endothelial cell growth factor (VEGF). Thus, Mpl acts as a substitute for other cytokine receptors and for a tyrosine kinase receptor, Flk-1, indicating that Mpl has no instructive role in hematopoietic cell commitment and differentiation. The Mpl mutant forms lacking box1 or box2 prevented response of ES cell-derived blast colony-forming cells or progenitors

to PEG-rhuMGDF. Therefore, these two regions, essential for signaling by cytokine receptors, are required for the responses of ES cell-derived hematopoietic cells to PEG-rhuMGDF.These results show that the in vitro hematopoietic differentiation of ES cells is suitable for studying the role of various intracytoplasmic regions of cytokine receptors.

Fischbach, N. A., S. Rozenfeld, et al. (2005). "HOXB6 overexpression in murine bone marrow immortalizes a myelomonocytic precursor in vitro and causes hematopoietic stem cell expansion and acute myeloid leukemia in vivo." Blood **105**(4): 1456-66.

The HOX family of homeobox genes plays an important role in normal and malignant hematopoiesis. Dysregulated HOX gene expression profoundly effects the proliferation and differentiation of hematopoietic stem cells (HSCs) and committed progenitors, and aberrant activation of HOX genes is a common event in human myeloid leukemia. HOXB6 is frequently overexpressed in human acute myeloid leukemia (AML). To gain further insight into the role of HOXB6 in hematopoiesis, we overexpressed HOXB6 in murine bone marrow using retrovirus-mediated gene transfer. We also explored structure-function relationships using mutant HOXB6 proteins unable to bind to DNA or a key HOX-binding partner, pre-B-cell leukemia transcription factor-1 (PBX1). Additionally, we investigated the potential cooperative interaction with myeloid ecotropic viral integration site 1 homolog (MEIS1). In vivo, HOXB6 expanded HSCs and myeloid precursors while inhibiting erythropoiesis and lymphopoiesis. Overexpression of HOXB6 resulted in AML with a median latency of 223 days. Coexpression of MEIS1 dramatically shortened the onset of AML. Cytogenetic analysis of a subset of HOXB6-induced AMLs revealed recurrent deletions of chromosome bands 2D-E4, a region frequently deleted in HOXA9-induced AMLs. In vitro, HOXB6 immortalized a factor-dependent myelomonocytic precursor capable of granulocytic and monocytic differentiation. These biologic effects of HOXB6 were largely dependent on DNA binding but independent of direct interaction with PBX1.

Fredriksson, M., G. Barbany, et al. (2004). "Assessing hematopoietic chimerism after allogeneic stem cell transplantation by multiplexed SNP genotyping using microarrays and quantitative analysis of SNP alleles." Leukemia **18**(2): 255-66.

Single-nucleotide polymorphisms (SNPs) have the potential to be particularly useful as markers for monitoring of chimerism after stem cell transplantation (SCT) because they can be analyzed by accurate and robust methods. We used a two-phased minisequencing strategy for monitoring chimerism after SCT. First, informative SNPs with alleles differing between donor and recipient were identified using a multiplex microarray-based minisequencing system screening 51 SNPs to ensure that multiple informative SNPs were detected in each donor-recipient pair. Secondly, the development of chimerism was followed up after SCT by sensitive, quantitative analysis of individual informative SNPs by applying the minisequencing method in a microtiter plate format. Using this panel of SNPs, we identified multiple informative SNPs in nine unrelated and in 16 related donor-recipient pairs. Samples from nine of the donor-recipient pairs taken at time points ranging from 1 month to 8 years after transplantation were available for analysis. In these samples, we monitored the allelic ratios of two or three informative SNPs in individual minisequencing reactions. The results agreed well with the data obtained by microsatellite analysis. Thus, we conclude that the two-phased minisequencing strategy is a useful approach in the following up of patients after SCT.

Gotoh, K., Y. Ito, et al. (2008). "Clinical and virological characteristics of 15 patients with chronic active Epstein-Barr virus infection treated with hematopoietic stem cell transplantation." Clin Infect Dis **46**(10): 1525-34.

BACKGROUND: Chronic active Epstein-Barr virus (EBV) infection is characterized by recurrent infectious mononucleosis-like symptoms, and infected patients have high viral loads in their peripheral blood. Standard therapy for the disease has not yet been established. Recently, hematopoietic stem cell transplantation (HSCT) has been introduced and has the potential to become a standard treatment, although guidelines for HSCT to treat chronic active EBV infection have not yet been proposed. METHODS: Fifteen patients were retrospectively analyzed, both clinically and virologically, to investigate the factors associated with prognosis of chronic active EBV infection treated with HSCT. RESULTS: After HSCT, 7 patients died after survival periods that ranged from 1 to 16 months (mean duration of survival after HSCT, 5 months). Three patients were considered to have died of transplantation-related complications. The duration between infection onset and diagnosis was significantly longer in patients who died than in those who survived. Five of the 7 patients who died experienced > or =3 life-threatening complications. The plasma concentrations of interferon-gamma, interleukin-10, thrombomodulin, and soluble E-selectin did not differ significantly between the groups of patients. With regard to sequence variations in the EBV latent membrane protein 1 gene, no specific

patterns were found in the patients who died. Importantly, the plasma EBV load at diagnosis was significantly higher in patients who died than in living patients. Moreover, plasma viral load was shown to be an important factor to monitor during follow-up for patients after HSCT. CONCLUSIONS: The number of life-threatening complications and plasma viral load are indicative of the stage of disease progression and may be useful factors for predicting the outcome of HSCT.

Gottgens, B., C. Broccardo, et al. (2004). "The scl +18/19 stem cell enhancer is not required for hematopoiesis: identification of a 5' bifunctional hematopoietic-endothelial enhancer bound by Fli-1 and Elf-1." Mol Cell Biol **24**(5): 1870-83.

Analysis of cis-regulatory elements is central to understanding the genomic program for development. The scl/tal-1 transcription factor is essential for lineage commitment to blood cell formation and previous studies identified an scl enhancer (the +18/19 element) which was sufficient to target the vast majority of hematopoietic stem cells, together with hematopoietic progenitors and endothelium. Moreover, expression of scl under control of the +18/19 enhancer rescued blood progenitor formation in scl(-/-) embryos. However, here we demonstrate by using a knockout approach that, within the endogenous scl locus, the +18/19 enhancer is not necessary for the initiation of scl transcription or for the formation of hematopoietic cells. These results led to the identification of a bifunctional 5' enhancer (-3.8 element), which targets expression to hematopoietic progenitors and endothelium, contains conserved critical Ets sites, and is bound by Ets family transcription factors, including Fli-1 and Elf-1. These data demonstrate that two geographically distinct but functionally related enhancers regulate scl transcription in hematopoietic progenitors and endothelial cells and suggest that enhancers with dual hematopoietic-endothelial activity may represent a general strategy for regulating blood and endothelial development.

Hacke, K., R. Falahati, et al. (2009). "Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation." Immunol Res **44**(1-3): 112-26.

Current approaches for hematopoietic stem cell (HSC) and organ transplantation are limited by donor and host-mediated immune responses to allo-antigens. Application of these therapies is limited by the toxicity of preparative and post-transplant immunosuppressive regimens and a shortage of appropriate HLA-matched donors. We have been exploring two complementary approaches for genetically modifying donor cells that achieve long-term suppression of cellular proteins that elicit host immune responses to mismatched donor antigens, and provide a selective advantage to genetically engineered donor cells after transplantation. The first approach is based on recent advances that make feasible targeted down-regulation of HLA expression. Suppression of HLA expression could help to overcome limitations imposed by extensive HLA polymorphisms that restrict the availability of suitable donors. Accordingly, we have recently investigated whether knockdown of HLA by RNA interference (RNAi) enables allogeneic cells to evade immune recognition. For efficient and stable delivery of short hairpin-type RNAi constructs (shRNA), we employed lentivirus-based gene transfer vectors that integrate into genomic DNA, thereby permanently modifying transduced donor cells. Lentivirus-mediated delivery of shRNA targeting pan-Class I and allele-specific HLA achieved efficient and dose-dependent reduction in surface expression of HLA in human cells, and enhanced resistance to allo-reactive T lymphocyte-mediated cytotoxicity, while avoiding non-MHC restricted killing. Complementary strategies for genetic engineering of HSC that would provide a selective advantage for transplanted donor cells and enable successful engraftment with less toxic preparative and immunosuppressive regimens would increase the numbers of individuals to whom HLA suppression therapy could be offered. Our second strategy is to provide a mechanism for in vivo selection of genetically modified HSC and other donor cells. We have uniquely combined transplantation during the neonatal period, when tolerance may be more readily achieved, with a positive selection strategy for in vivo amplification of drug-resistant donor HSC. This model system enables the evaluation of mechanisms of tolerance induction to neo-antigens, and allogeneic stem cells during immune ontogeny. HSC are transduced ex vivo by lentivirus-mediated gene transfer of P140K-O(6)-methylguanine-methyltransferase (MGMT(P140K)). The MGMT(P140K) DNA repair enzyme confers resistance to benzylguanine, an inhibitor of endogenous MGMT, and to chloroethylating agents such as BCNU. In vivo chemoselection enables enrichment of donor cells at the stem cell level. Using complementary approaches of in vivo chemoselection and RNAi-induced silencing of HLA expression may enable the generation of histocompatibility-enhanced, and eventually, perhaps "universally" compatible cellular grafts.

Hansen, J. E., G. J. Gram, et al. (1997). "Transduction potential of human retroviruses in highly proliferating

small-cell lung cancer cells as well as non-proliferating hematopoietic stem cells." Apmis **105**(9): 723-9.

Direct gene transfer to solid tissues or metastatic cancer cells requires vectors capable of in vivo transduction to specific cells. The predominant retroviral vectors of murine origin are inactivated by human complement, which precludes their use in vivo. Such inactivation does not take place with vectors based on human retroviruses. Murine retroviral vectors are also limited to proliferating cells, which human retroviruses are not. In this study we examined whether or not a vector using components from the human retroviruses HIV-1 and HTLV-1 could infect small-cell lung cancer cells and resting CD34+ hematopoietic stem cells. While HIV-1 itself was unable to infect cells lacking the CD4-membrane molecule, chimeric viral particles (pseudotype virus) with HIV-1 genome and HTLV-1 envelope components were able to infect both CD4-containing lymphocytic cells, CD4-negative tumour cells and hematopoietic stem cells. After infection with the pseudotype vector, the RNA genome was reverse transcribed and integrated. Transduction efficiency and gene expression under the HIV-1 LTR promoter in both tumour and stem cells were found to be of a similar or greater magnitude than in lymphocytic cells. These results suggest that gene transfer targeting proliferating as well as resting cells in vivo may be realized using components from human retroviruses.

Hauser, H., O. Zach, et al. (2008). "A single nucleotide polymorphism at chromosome 2q21.3 (LCT -13910C>T) associates with clinical outcome after allogeneic hematopoietic stem cell transplantation." Blood **112**(5): 2156-9.

A single nucleotide polymorphism (SNP) responsible for lactase persistence (LCT -13910C>T) changes intestinal microflora. Considering the influence of bacterial microflora on various immune effects, we tested DNA from 111 recipients/donors and analyzed whether this SNP interferes with survival and the incidence of acute graft-versus-host disease (aGVHD) after allogeneic hematopoetic stem cell transplantations (HSCT). Median overall survival (OS) was significantly longer when donors had a CC genotype (not reached after 133 vs 11.1 months, P = .004). Multivariate analysis identified a donor T allele (hazard ratio 2.63, 95% confidence interval 1.29-5.33, P = .008) as independent risk factor for death. Surprisingly, recipient genotypes did not influence outcome and there were no differences regarding aGVHD. Transplantation-related mortality (TRM), relapse and pneumonia were significantly less frequent in patients with CC donors. These findings add to the growing list of non-HLA polymorphisms with impact on outcome after allogeneic HSCT.

Hochberg, E. P., D. B. Miklos, et al. (2003). "A novel rapid single nucleotide polymorphism (SNP)-based method for assessment of hematopoietic chimerism after allogeneic stem cell transplantation." Blood **101**(1): 363-9.

A major end point of nonmyeloablative hematopoietic stem cell transplantation is the attainment of either mixed chimerism or full donor hematopoiesis. Because the majority of human genetic disparity is generated by single nucleotide polymorphisms (SNPs), direct measurement of SNPs should provide a robust tool for the detection and quantitation of chimerism. Using pyrosequencing, a rapid quantitative sequencing technology, we developed a SNP-based assay for hematopoietic chimerism. Based on 14 SNPs with high allele frequencies, we were able to identify at least 1 informative SNP locus in 55 patients with HLA-identical donors. The median number of informative SNPs in related pairs was 5 and in unrelated pairs was 8 (P <.0001). Assessment of hematopoietic chimerism in posttransplantation DNA was shown to be quantitative, accurate, and highly reproducible. The presence of 5% donor cells was reliably detected in replicate assays. Compared with current measures of engraftment based on identification of short tandem repeats (STRs), variable number of tandem repeats (VNTRs), or microsatellite polymorphisms, this SNP-based method provides a more rapid and quantitative assessment of chimerism. A large panel of SNPs enhances the ability to identify an informative marker in almost all patient/donor pairs and also facilitates the simultaneous use of multiple markers to improve the statistical validity of chimerism measurements. The inclusion of SNPs that encode minor histocompatibility antigens or other genetic polymorphisms that may influence graft-versus-host disease or other transplantation outcomes can provide additional clinically relevant data. SNP-based assessment of chimerism is a promising technique that will assist in the analysis of outcomes following transplantation.

Hock, H., E. Meade, et al. (2004). "Tel/Etv6 is an essential and selective regulator of adult hematopoietic stem cell survival." Genes Dev **18**(19): 2336-41.

Hematopoietic stem cells (HSCs) sustain blood formation throughout life. Pathways regulating maintenance of adult HSCs are largely unknown. Here we report that the Ets-related transcription factor Tel/Etv6, the product of a locus frequently involved in translocations in leukemia, is a selective regulator of

HSC survival. Following inactivation of Tel/Etv6, HSCs are lost in the adult bone marrow but their progeny are unaffected and transiently sustain blood formation. Accordingly, absence of Tel/Etv6 after lineage commitment is ostensibly without consequence except for unexpected impairment of maturation of megakaryocytes. Thus, we establish Tel/Etv6 as a selective and essential regulator of postembryonic HSCs.

Hole, N., G. J. Graham, et al. (1996). "A limited temporal window for the derivation of multilineage repopulating hematopoietic progenitors during embryonal stem cell differentiation in vitro." Blood **88**(4): 1266-76.

Embryonal stem cells have been shown to differentiate in vitro into all hematopoietic lineages. This has been used successfully as one approach to the study of genetic events occurring during haematopoiesis. However, studies on the commitment of mesodermal precursors to the hematopoietic lineage have been limited due to the inability to define a system in which embryonal stem (ES) cells will give rise to primitive hematopoietic stem cells in vitro. Using a colony forming assay (CFU-A), we determined that the earliest time point at which primitive multilineage hematopoietic precursors can be detected during ES cell differentiation in vitro in the absence of exogenous conditioned medium or stromal cell culture is 4 days. Lethally irradiated adult recipient mice that received differentiated ES cells from this time point survived for more than 3 weeks; and in two out three experiments, peripheral blood from these animals contained ES-derived progeny. Fluorescence activated cell sorting (FACS) found ES-derived CD45+ hematopoietic cells in both lymphoid and myeloid compartments at 12 weeks posttransplantation, suggesting that the population of day 4 differentiated ES cells contains primitive hematopoietic precursors. A preliminary RT-PCR analysis of gene expression around this time point suggests that there are very few hematopoietic cells present. This approach should prove useful in studies of genetic control of commitment to and maintenance of hematopoietic lineages in vitro and in vivo.

Husain, S. M., Y. Shou, et al. (2006). "Isolation, molecular cloning and in vitro expression of rhesus monkey (Macaca mulatta) prominin-1.s1 complementary DNA encoding a potential hematopoietic stem cell antigen." Tissue Antigens **68**(4): 317-24.

Human prominin-1 (CD133 or AC133) is an important cell surface marker used to isolate primitive hematopoietic stem cells. The commercially available antibody to human prominin-1 does not recognize rhesus prominin-1. Therefore, we isolated, cloned and characterized the complementary DNA (cDNA) of rhesus prominin-1 gene and determined its coding potential. Following the nomenclature of prominin family of genes, we named this cDNA as rhesus prominin-1.s1. The amino acid sequence data of the putative rhesus prominin-1.s1 could be used in designing antigenic peptides to raise antibodies for use in isolation of pure populations of rhesus prominin-1(+) hematopoietic cells. To the best of our knowledge, there has been no previously published report about the isolation of a prominin-1 cDNA from rhesus monkey (Macaca mulatta).

Jaskula, E., D. Dlubek, et al. (2009). "Interferon gamma 13-CA-repeat homozygous genotype and a low proportion of CD4(+) lymphocytes are independent risk factors for cytomegalovirus reactivation with a high number of copies in hematopoietic stem cell transplantation recipients." Biol Blood Marrow Transplant **15**(10): 1296-305.

Cytomegalovirus (CMV) reactivation was analyzed in 92 recipients of allogeneic hematopoietic stem cell transplantation (HSCT) in relation to the proportion of CD4(+) lymphocytes in blood and a microsatellite polymorphism within the first intron of the interferon-gamma (IFNG) gene. CMV reactivation was found in 50% of the HSCT recipients; in 30% of these individuals, the level of CMV copies exceeded 100 per 10(5) peripheral blood (PB) cells on at least one occasion during the 100-day post-HSCT observation period. This high CMV copy level was most frequently found between 31 and 60 days post-HSCT (P = .021). Patients with > or = 100 CMV copies/10(5) cells were characterized by poorer overall survival (OS) compared with those lacking CMV copies or having < 100 CMV copies/10(5) cells (P = .04), and they suffered from severe post-HSCT complications, including acute graft-versus-host disease (aGVHD) and relapse. Thus, patients with > or = 100 CMV copies/10(5) cells were designated as having clinically significant CMV reactivation. Patients with < 10% CD4(+) lymphocytes had a higher number of CMV DNA copies than those with higher proportions of CD4(+) lymphocytes (0.62 vs 0.21, P = .001; mean +/- SEM, 4422 +/- 1667 vs 937 +/- 662 CMV copies/10(5) cells, P < .001, for the proportion of cases with reactivation and numbers of copies, respectively). Similarly, patients carrying 2 IFNG 13-CA-repeat alleles (homozygotes) had more frequent CMV reactivation (0.50 vs 0.26; P = .039) and a higher CMV load (4111 +/- 1699 vs 950+/-591 CMV copies/10(5) cells; P = .041) compared with those with other IFNG microsatellite allele constellations. Multivariate analysis demonstrated that the IFNG 13-CA-repeat homozygous genotype (odds

ratio [OR] = 0.221; P = .044), a low proportion of CD4(+) lymphocytes (OR = 0.276; P = .050), and a lack of optimal (10/10 alleles) donor-recipient HLA match (OR = 15.19; P = .006) were independent risk factors for CMV reactivation with a high number of copies.

Kajiume, T., Y. Ninomiya, et al. (2004). "Polycomb group gene mel-18 modulates the self-renewal activity and cell cycle status of hematopoietic stem cells." Exp Hematol **32**(6): 571-8.

OBJECTIVE: Mel-18 is a member of the mammalian Polycomb group (PcG) genes. This family of genes regulates global gene expression in many biologic processes, including hematopoiesis and anterior-posterior axis formation by manipulating specific target genes, including members of the Hox family. Here, we demonstrate that mel-18 negatively regulates the self-renewal activity of hematopoietic stem cells (HSCs). MATERIALS AND METHODS: Long-term reconstitution activity was evaluated by competitive repopulating unit (CRU) and mean activity of the stem cells (MAS) assays in vivo in bone marrow cells (BMCs) derived from mel-18(-/-) and mel-18 tg mice. The expression levels of mel-18 and Hoxb4 were measured by quantitative real-time reverse transcription polymerase chain reaction. RESULTS: The Hoxb4 gene was highly expressed in HSCs derived from mel-18(-/-) mice. The observed CRUs were 3.21, 4.77, 3.32, and 1.64 CRU per 10(5) BMCs in mel-18(+/+), mel-18(-/-), C57BL/6, and mel-18 tg, respectively. MAS was 0.58, 0.18, 0.41, and 5.89 in mel-18(+/+), mel-18(-/-), C57BL/6, and mel-18 tg, respectively. The percentage in G0 phase HSCs (lin(-)flk2(-)c-Kit(+)Sca1+ cells) was increased in mel-18(-/-) mice and decreased in mel-18 tg mice. CONCLUSION: Loss or knockdown of mel-18 leads to the expression of Hoxb4, an increase in the proportion of HSCs in G0 phase, and the subsequent promotion of HSC self-renewal. These findings will enable us to develop new approaches for controlling HSC activity for hematopoietic transplantations based on ex vivo expansion of HSCs.

Kamble, R. T., D. A. Clark, et al. (2007). "Transmission of integrated human herpesvirus-6 in allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **40**(6): 563-6.

Human herpesvirus 6 (HHV-6) viremia, as detected by polymerase chain amplification, occurs in approximately half of allogeneic hematopoietic stem cell transplant recipients. The significance of such viremia is incompletely understood, but HHV-6 encephalitis and bone marrow suppression are increasingly being recognized in patients with high viral DNA. We report two patients in whom donor-to-recipient transmission occurred through hematopoietic transplant by means of chromosomally integrated (CI) HHV-6. Iatrogenic transmission manifested at engraftment as asymptomatic elevation of HHV-6 viral DNA of 3600 and 15 400 DNA copies/ml in plasma and 6.1 x 10(6) and 9.7 x 10(5) DNA copies/ml in the whole blood. Both donors had elevated plasma HHV-6 PCR at 5.6 x 10(4) and 1.3 x 10(5) DNA copies/ml and strikingly elevated whole blood HHV-6 levels at 4.1 x 10(6) and 4.7 x 10(6) DNA copies/ml, respectively. CI of the virus was traced to the mother of one patient and his donor. CI of HHV-6 may confound the interpretation of HHV-6 viremia after stem cell transplantation; consideration of the possibility of CI HHV-6 will avoid unnecessary antiviral therapy.

Kamminga, L. M. and G. de Haan (2006). "Cellular memory and hematopoietic stem cell aging." Stem Cells **24**(5): 1143-9.

Hematopoietic stem cells (HSCs) balance self-renewal and differentiation in order to sustain lifelong blood production and simultaneously maintain the HSC pool. However, there is clear evidence that HSCs are subject to quantitative and qualitative exhaustion. In this review, we briefly discuss several known aspects of the stem cell aging process, including DNA damage, telomere shortening, and oxidative stress. Besides these known players, there is increasing evidence that higher order chromatin structure, largely defined by the histone code and affecting transcriptional activity, is important. A model is suggested which describes how epigenetic regulation of gene transcription by modulation of the chromatin structure in stem cells can account for regulation of the aging program.

Kaneko, S., M. Onodera, et al. (2001). "Simplified retroviral vector gcsap with murine stem cell virus long terminal repeat allows high and continued expression of enhanced green fluorescent protein by human hematopoietic progenitors engrafted in nonobese diabetic/severe combined immunodeficient mice." Hum Gene Ther **12**(1): 35-44.

Despite efforts toward improvements in retrovirus-mediated gene transfer, stable high-level expression of a therapeutic gene in human hematopoietic stem cells remains a great challenge. We have evaluated the efficiency of different viral long terminal repeats (LTRs) in long-term expression of a transgene in vivo, using severe combined immunodeficiency (SCID)-repopulating cell assays. Vectors used were variants of the simplified retroviral vector GCsap with the different LTRs of Moloney murine leukemia virus (MLV), myeloproliferative sarcoma virus (MPSV), and murine stem cell virus

(MSCV). The enhanced green fluorescent protein (EGFP) gene was used as a marker to assess levels of transduction efficiency. CD34+ cells isolated from human cord blood were transduced by exposure to virus-containing supernatants on fibronectin fragments and in the presence of stem cell factor, interleukin 6, Flt-3 ligand, and thrombopoietin, and then transplanted into nonobese diabetic/SCID mice. Engraftment of human cells highly expressing EGFP, with differentiation along multiple cell lineages, was demonstrated for up to 18 weeks posttransplant, although the three different vectors showed different transduction frequencies (MLV, <0.1-33.2%; MPSV, <0.1-22.8%; MSCV, 0.3-51.7%). Of importance is that high-level transduction frequencies in human progenitor cells were also confirmed by colony-forming cell assays using bone marrow from transplanted mice, in which EGFP-expressing, highly proliferative potential colonies were observed by fluorescence microscopy. In these mice the vector carrying the MSCV LTR generated more EGFP-expressing human cells than did either of the other two constructs, indicating that GCsap carrying the MSCV LTR may be an efficient tool for stem cell gene therapy.

Kang, J., J. Wither, et al. (1990). "Long-term expression of a T-cell receptor beta-chain gene in mice reconstituted with retrovirus-infected hematopoietic stem cells." Proc Natl Acad Sci U S A **87**(24): 9803-7.

To determine the feasibility of retrovirus-mediated gene transfer into stem cells for studying T-cell development, we constructed a high-titer retrovirus vector containing the neomycin phosphotransferase (neo) gene and a murine T-cell receptor (TCR) beta-chain gene with the V beta 6 variable segment. The TCR gene was placed under the control of the human beta-actin promoter and enhancer. Bone marrow cells pretreated with 5-fluorouracil were infected by coculturing with psi-2 virus-producing cells in the presence of recombinant interleukins 1, 2, 4, and 6 as well as interleukin 3 from WEHI-3 conditioned medium. The infected cells were transplanted into irradiated mice, and expression of the exogenous V beta 6 gene was examined with a V beta 6-specific monoclonal antibody, RNase protection, and polymerase chain reaction amplification. Three of seven mice expressed the retroviral TCR gene on the surface of a significant proportion of mature T cells 5-6 months after transplantation. In mice analyzed less than 1 month after transplantation, up to 30% of mature T cells expressed V beta 6 TCRs, an increase of at least 20% above the level of endogenous V beta 6 expression. DNA analysis revealed that pluripotent hematopoietic stem cells were infected by the retroviral vector in a long-term reconstituted mouse that showed increased V beta 6 expression.

Kimura, H., E. Morii, et al. (2006). "Role of DNA methylation for expression of novel stem cell marker CDCP1 in hematopoietic cells." Leukemia **20**(9): 1551-6.

CDCP1, a novel stem cell marker, is expressed in hematopoietic cell line K562 but not in Jurkat. When CDCP1 promoter was transfected exogenously, Jurkat showed comparable promoter activity with K562, suggesting that the factor to enhance transcription was present but interfered to function in Jurkat. The reporter assay and si-RNA-mediated knockdown experiment revealed that zfp67, a zinc-finger protein, enhanced CDCP1 transcription. Amount of zfp67 in Jurkat was comparable with K562, but chromatin immunoprecipitation showed that zfp67 bound to CDCP1 promoter in K562 but not in Jurkat. There are CpG sequences around the promoter of CDCP1, which were heavily methylated in Jurkat but not in K562. Addition of demethylating reagent to Jurkat induced CDCP1 expression, and increased the zfp67 binding to CDCP1 promoter. Among normal hematopoietic cells such as CD34+CD38- cells, lymphocytes and granulocytes, inverse correlation between proportion of methylated CpG sequences and CDCP1 expression level was found. Demethylation of CpG sequences in lymphocytes, in which CpG sequences were heavily methylated, induced CDCP1 expression and its expression level further increased through zfp67 overexpression. The methylation of DNA appeared to regulate the cell-type-specific expression of CDCP1 through the control of interaction between chromatin DNA and transcription factors.

Kitazawa, J., C. Tono, et al. (2005). "Successful outcome of mismatched hematopoietic stem cell transplantation from a related donor in an infant with acute lymphoblastic leukemia and 9;11 translocation: case report and review of the literature." Int J Hematol **81**(5): 428-32.

Although infants with acute lymphoblastic leukemia (ALL) and MLL gene rearrangements have a poor prognosis, those with acute myeloid leukemia (AML) have been shown to have a superior outcome with intensive chemotherapy alone despite the presence of MLL gene rearrangements. We report the case of an ALL infant with t(9;11), a common cytogenetic abnormality in infant AML, who after relapse underwent successful hematopoietic stem cell transplantation (HSCT) from her HLA 2-loci-mismatched mother. Analysis of the outcome among ALL infants with MLL gene rearrangements

registered in the Japan Infant Leukemia Study between 1996 and 1999 showed the event-free survival of patients with t(9;11) was not different from that of those with other 11q23 translocations. Most of the patients with t(9;11) described in the reviewed literature also experienced either induction failure or early relapse after achievement of complete remission, but some of them were rescued with subsequent HSCT. These findings suggest that infant ALL with t(9;11) has features distinct from those of infant AML with the same karyotype and that the prognosis among these patients can be improved only with the combination of intensive chemotherapy and HSCT An appropriate strategy for the treatment of ALL infants with different 11q23 translocations must be clarified.

Kosaka, Y., K. Koh, et al. (2004). "Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation." Blood **104**(12): 3527-34.

Forty-four infants with acute lymphoblastic leukemia (ALL) characterized by MLL gene rearrangements were treated on a protocol of intensive chemotherapy followed by hematopoietic stem cell transplantation (HSCT) between November 1998 and June 2002. The remission induction rate was 91.0%, and the 3-year overall survival and event-free survival (EFS) rates, with 95% confidence intervals, were 58.2% (43.5%-72.9%) and 43.6% (28.5%-58.7%), respectively. Univariate analysis of EFS by presenting features indicated a poorer outcome in patients younger than 6 months of age with high white blood cell counts (>/= 100 x 10(9)/L; EFS rate, 9.4% versus 55.1% for all others, P = .0036) and in those with central nervous system invasion (EFS rate, 10.0% versus 56.9% for all others, P = .0073). The 3-year posttransplantation EFS rate for the 29 patients who underwent HSCT in first remission was 64.4% (46.4%-82.4%). In this subgroup, only the timing of HSCT (first remission versus others) was a significant risk factor by multivariate analysis (P < .0001). These results suggest that early introduction of HSCT, possibly with a less toxic conditioning regimen, may improve the prognosis for infants with MLL(+) ALL. Identification of subgroups or patients who respond well to intensified chemotherapy alone should have a high priority in future investigations.

Kunisato, A., S. Chiba, et al. (2004). "Stem cell leukemia protein directs hematopoietic stem cell fate." Blood **103**(9): 3336-41.

Stem cell leukemia (SCL) protein has been shown to be an essential transcription factor during hematopoietic development in the embryo. In adult hematopoiesis, however, the role for SCL has remained largely unknown, whereas it is expressed in bone marrow hematopoietic stem cells (HSCs). In this study, we performed HSC transplantation and an in vitro HSC differentiation assay using retrovirally transduced HSCs with wild-type (WT) and dominant-negative (DN) SCL. The transplantation experiments showed that SCL does not affect the long-term repopulating capacity of HSCs but that WT SCL and DN SCL increase the short-term contribution of the transduced HSCs in myeloid and lymphoid lineages, respectively. An in vitro single-cell assay using a fetal thymus organ culture system further demonstrated that WT SCL facilitates HSCs to differentiate into the myeloid lineage but that DN SCL facilitates HSCs to differentiate into the lymphoid lineage. We conclude that the up-regulation or down-regulation of SCL directs HSCs toward myeloid or lymphoid lineage, respectively, although SCL does not affect their long-term repopulating capacity.

Lacey, S. F., J. Martinez, et al. (2005). "Simultaneous reconstitution of multiple cytomegalovirus-specific CD8+ cell populations with divergent functionality in hematopoietic stem-cell transplant recipients." J Infect Dis **191**(6): 977-84.

A panel of 7 human cytomegalovirus (CMV) epitope peptides and corresponding major histocompatibility class 1 tetramers was used to evaluate cellular immunity in healthy seropositive donors and in hematopoietic stem-cell transplant recipients. Broad CMV-specific T cell responses to epitopes were found within several CMV polypeptides and were restricted by multiple human leukocyte antigen alleles. Their cytotoxic functionality was evaluated by use of an assay that measures transient surface levels of lysosomal membrane proteins LAMP-1 (CD107a) and LAMP-2 (CD107b) after peptide stimulation. This assay can be combined with tetramer staining of antigen-specific CD8(+) T lymphocytes and has potential as a surrogate marker for cytotoxic function. CD8(+) T lymphocytes specific for epitopes within the pp65 or pp50 gene products exhibited significantly higher functionality, compared with populations recognizing CMV major immediate early-1 epitopes. These functional differences between T lymphocyte populations within the same individual may have implications for protection against CMV.

Lee, D. G., S. T. Park, et al. (2003). "Prevalence of human herpesvirus-6B in Korean hematopoietic stem cell transplantation recipients." Mol Cells **16**(3): 307-15.

Human herpesvirus-6 (HHV-6) is a major pathogen associated with diseases of recipients of hematopoietic stem cell transplants (HSCT). We have

isolated HHV-6 in Korean HSCT recipients and carried out a prospective investigation of its prevalence. We obtained peripheral blood from HSCT recipients who had signs of HHV-6 infection. Cord blood mononuclear cells (CBMC) and Sup-T1 cells were used to culture the HHV-6. Indirect immunofluorescence assays (IFA), and the polymerase chain reaction (PCR) were employed to detect HHV-6. The prevalence of HHV-6 infection in HSCT recipients was calculated on the basis of the PCR results. HHV-6 was isolated from four clinical samples. After culturing the HHV-6 in CBMC, the standard strain and the four clinical isolates were propagated in Sup-T1 cells. The infected cells became grossly enlarged and multinucleate after 7-21 days. The virus was identified primarily on the basis of the morphological changes of the cultured cells, and confirmed by specific IFA with monoclonal antibody to HHV-6. HHV-6 was detected in each sample by PCR with primers specific for the major immediate early gene. Sequencing of the standard strain and PCR products confirmed identification of the HHV-6B variant. By PCR we detected 415 instances of HHV-6 in 3966 samples (14.6% of peripheral blood mononuclear cells and 6.3% of sera), and HHV-6 DNAemia was most frequent from the second to the fourth week after HSCT.

Lee, K. H., S. S. Park, et al. (2007). "P2X7 receptor polymorphism and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation." Haematologica **92**(5): 651-7.

BACKGROUND AND OBJECTIVES: The P2X7 receptor (P2X7 R) is a key player in the processing and release of interleukin (IL)-1. To evaluate whether the A1513C polymorphism of the P2X7 R gene is related to allogeneic stem cell transplantation outcome, we performed an association analysis between this polymorphism and clinical outcomes in patients treated with an HLA-matched sibling stem cell transplant. DESIGN AND METHODS: Patients (n=152) with a malignancy or aplastic anemia underwent allogeneic stem cell transplantation at a single institute. Peripheral blood DNA of these 152 patients and their 152 donors was genotyped. Genotypes of 145 recipients and 150 donors were obtained and analyzed for the polymorphism. RESULTS: The frequencies of the A and C alleles in all 295 study subjects were 72% and 28%, respectively. The genotypes in patients were AA in 75, AC in 58, and CC in 12; the genotypes in donors were AA in 74, AC in 70, and CC in 6. Overall survival was significantly shorter for recipients with the CC genotype than for those with the AA or AC genotype (92 days for 1513CC vs. 821 days for 1513AA or 1513AC, p=0.012), and for recipients from donors with the CC genotype than for recipients from donors with the AA or AC genotype (63 days for 1513CC vs. 702 days for 1513AA or 1513AC, p=0.024). Multivariate analyses, which included sex, age, transplant method (reduced intensity conditioning vs. conventional conditioning), stem cell source, risk group, and P2X7R recipient and donor genotypes, as parameters, identified high-risk group (hazard ratio 3.25, 95% confidence interval 1.83~5.77) and a donor 1513CC genotype (hazard ratio 2.66, 95% confidence interval 1.02~6.91) as risk factors for a shorter survival. Microbiologically documented bacteremia occurred in 66.7% of recipients with the CC donor genotype and in 17.6% of recipients of transplants of AA or AC genotype (p=0.014). INTERPRETATION AND CONCLUSIONS: We conclude that the A1513C polymorphism in the P2X7R gene is related to the occurrence of infections and survival after allogeneic stem cell transplantation. Thus, the determination of this polymorphism may be helpful for the optimal selection of patients and donors.

Leen, A. M., C. M. Bollard, et al. (2006). "Adenoviral infections in hematopoietic stem cell transplantation." Biol Blood Marrow Transplant **12**(3): 243-51.

Adenoviruses are lytic DNA viruses that are ubiquitous in human communities. In total, 51 different serotypes with varying tissue tropisms have been identified. Adenovirus infections, although frequent, are rarely fatal in immunocompetent individuals who have potent innate and adaptive immunity. But in immunosuppressed individuals, adenoviruses are a significant cause of morbidity and mortality, with limited treatment options. In particular, pediatric recipients of allogeneic hematopoietic stem cell transplantation frequently develop infections early in the posttransplantation period. Because the endogenous recovery of adenovirus-specific T cells has proven important in controlling infection, we explore the potential of adoptive T-cell immunotherapy as a therapeutic strategy. We discuss the advantages and limitations of T-cell therapy for the prophylaxis and treatment of adenovirus infection posttransplantation.

Li, M. X., D. Banerjee, et al. (1994). "Development of a retroviral construct containing a human mutated dihydrofolate reductase cDNA for hematopoietic stem cell transduction." Blood **83**(11): 3403-8.

A double-copy Moloney leukemia virus-based retroviral construct containing both the NeoR gene and a mutant human dihydrofolate reductase (DHFR) cDNA (Ser31 mutant) was used to transduce NIH 3T3 and mouse bone marrow (BM) progenitor cells. This resulted in increased resistance of these cells to methotrexate (MTX). The transduced BM

progenitor cells were returned to lethally irradiated mice. The recipients transplanted with marrow cells infected with the recombinant virus showed protection from lethal MTX toxicity as compared with mock-infected animals. Evidence for integration of the proviral DNA was obtained by amplification of proviral DNA by polymerase chain reaction (PCR) and Southern analysis. Sequencing a portion of the PCR-amplified human DHFR cDNA showed the presence of the mutation. These studies with the human Ser31 mutant DHFR cDNA gave results comparable with those obtained with the mutant murine DHFR cDNA (Leu to Arg22) in developing MTX-resistant BM. The Ser31 mutant human DHFR cDNA is currently being tested for infection of human CD34+ human BM and peripheral blood stem cells in vitro.

Little, A. M. (2007). "An overview of HLA typing for hematopoietic stem cell transplantation." Methods Mol Med **134**: 35-49.

Selection of a related or unrelated haematopoietic stem cell donor for a patient requires accurate matching of human leukocyte antigen (HLA) genes in order to maximise the beneficial effects of the transplant. There are a number of different approaches that can be made in order to achieve HLA type depending on the number of samples being processed, the level of resolution to be achieved, and the cost of providing the various tests. Each method has its advantages and disadvantages and in most laboratories, a combination of methods may be used.

Liu, Y., S. E. Elf, et al. (2009). "The p53 tumor suppressor protein is a critical regulator of hematopoietic stem cell behavior." Cell Cycle **8**(19): 3120-4.

In response to diverse stresses, the tumor suppressor p53 differentially regulates its target genes, variably inducing cell-cycle arrest, apoptosis or senescence. Emerging evidence indicates that p53 plays an important role in regulating hematopoietic stem cell (HSC) quiescence, self-renewal, apoptosis and aging. The p53 pathway is activated by DNA damage, defects in ribosome biogenesis, oxidative stress and oncogene induced p19 ARF upregulation. We present an overview of the current state of knowledge about p53 (and its target genes) in regulating HSC behavior, with the hope that understanding the molecular mechanisms that control p53 activity in HSCs and how p53 mutations affect its role in these events may facilitate the development of therapeutic strategies for eliminating leukemia (and cancer) propagating cells.

Liu, Y., S. E. Elf, et al. (2009). "p53 regulates hematopoietic stem cell quiescence." Cell Stem Cell **4**(1): 37-48.

The importance of the p53 protein in the cellular response to DNA damage is well known, but its function during steady-state hematopoiesis has not been established. We have defined a critical role of p53 in regulating hematopoietic stem cell quiescence, especially in promoting the enhanced quiescence seen in HSCs that lack the MEF/ELF4 transcription factor. Transcription profiling of HSCs isolated from wild-type and p53 null mice identified Gfi-1 and Necdin as p53 target genes, and using lentiviral vectors to upregulate or knockdown the expression of these genes, we show their importance in regulating HSC quiescence. Establishing the role of p53 (and its target genes) in controlling the cell-cycle entry of HSCs may lead to therapeutic strategies capable of eliminating quiescent cancer (stem) cells.

Lubovy, M., S. McCune, et al. (1996). "Stable transduction of recombinant adeno-associated virus into hematopoietic stem cells from normal and sickle cell patients." Biol Blood Marrow Transplant **2**(1): 24-30.

Stable introduction of genes into human hematopoietic stem cells with self-renewing potential is a necessary requirement for gene therapy strategies. We have developed an adeno-associated virus (AAV) vector and a partial packaging cell line that produces recombinant AAV at a titer of 10(8) transducing particles per milliliter. A high-titer viral stock containing the CMV/lacZ gene was used to transfer lacZ sequences into CD34+ Lin-Thy+ hematopoietic stem cells purified from normal and homozygous sickle cell patients. After infection, the cells were cultured in two ways. In the first set of experiments, the cell were expanded 300-fold in liquid culture for 21 days and plated in methylcellulose. Burst-forming units-erythroid (BFU-E) and colony-forming units-granulocyte/macrophage (CFU-GM) were then analyzed for lacZ sequences. In the second set of experiments, infected cells were cultured for 6 weeks under conditions that maintain long-term culture-initiating cells (LTC-IC). Progenitors were plated in methylcellulose, and BFU-E were analyzed for lacZ DNA. Stable transduction of lacZ sequences was observed in 25% of the colonies in both sets of experiments. These results demonstrate for the first time that LTC-IC can be transduced stably with a recombinant AAV vector. The results suggest that AAV may be a useful vector for genetic therapy of sickle cell disease and other hematopoietic disorders.

Luskey, B. D., M. Rosenblatt, et al. (1992). "Stem cell factor, interleukin-3, and interleukin-6 promote

retroviral-mediated gene transfer into murine hematopoietic stem cells." Blood **80**(2): 396-402.

The efficiency of retroviral-mediated gene transfer into hematopoietic stem cells (HSC) is dependent on the survival and self-renewal of HSC in vitro during retroviral infection. We have examined the effect of prestimulation of bone marrow with various cytokines, including the product of the Steel gene, Steel factor or stem cell factor (SCF) (the ligand for the c-kit receptor) on the efficiency of retroviral transduction of the human adenosine deaminase (hADA) cDNA into murine HSC. Bone marrow cells were prestimulated for 48 hours with hematopoietic growth factors, then cocultivated with the packaging cell line producing the ZipPGK-ADA simplified retrovirus for an additional 48 hours with continued growth factor exposure. Nonadherant cells from these cocultures were injected into lethally irradiated recipients. The content of day 12 colony-forming unit-spleen (CFU-S12) in SCF/interleukin 6 (IL-6)-prestimulated and cocultured bone marrow was more than threefold greater than that of IL-3/IL-6-prestimulated bone marrow cells. All mice receiving bone marrow cells infected with the PGK-ADA virus after prestimulation with IL-3/IL-6 or SCF/IL-6 demonstrated hADA expression in the peripheral blood after full hematopoietic reconstitution. While all recipients of IL-3/IL-6-prestimulated bone marrow expressed hADA at 4 months posttransplant, in three independent experiments examining a total of 33 mice, in most recipients of SCF/IL-6-prestimulated and infected bone marrow cells, the expression of human enzyme was higher than IL-3/IL-6 mice. Southern blot analysis of DNA from hematopoietic tissues from these same mice prepared at least 4 months posttransplantation also demonstrated a higher infection efficiency of HSC as measured by proviral integration patterns and genome copy number analysis. These results suggest that the higher level of hADA expression seen in mice receiving marrow prestimulated with SCF/IL-6 before retroviral infection is due to more efficient infection of reconstituting HSC. Other growth factor combinations were also studied; however, prestimulation with SCF/IL-6 or IL-3/IL-6 appeared optimal. Using retroviral-mediated gene transfer and viral integration patterns, Steel factor (SCF) in combination with IL-6 appears to increase the survival and self-renewal of reconstituting hematopoietic stem cells and proves useful in effecting expression of foreign genes in transplant recipients. Such pretreatment may also be useful in the application of retroviral transfer methods to human cells.

Madlambayan, G. J., I. Rogers, et al. (2005). "Dynamic changes in cellular and microenvironmental composition can be controlled to elicit in vitro human hematopoietic stem cell expansion." Exp Hematol **33**(10): 1229-39.

OBJECTIVE: The absence of effective strategies for the ex vivo expansion of human hematopoietic stem cells (HSCs) limits the development of many cell-based therapies. Prior attempts to stimulate HSC expansion have focused on media supplementation using cytokines and growth factors. In these cultures, cellular and microenvironmental compositions change with time. In this study, the impact of controlling these dynamic changes on HSC output is determined. MATERIALS AND METHODS: Cord blood-derived lin(-) cells were cultured for 8 days in serum-free medium supplemented with stem cell factor, Flt3 ligand, and thrombopoietin. Functional, phenotypic, and molecular (gene and protein) analyses were used to characterize dynamic changes in cellular and microenvironmental composition. The effects of these changes and the mechanism behind their effects on HSC expansion were assessed using a selection/media exchange-based global culture manipulation (GCM) technique. RESULTS: We show that the direct secretion of negative regulators by culture-generated lin(+) cells, and the indirect stimulation of cells to secrete negative regulators by culture-conditioned media, limits in vitro HSC generation. The GCM strategy was able to abrogate these effects to produce elevated numbers of LTC-ICs (14.6-fold relative to input), migrating rapid NOD/SCID repopulating cells (12.1-fold), and long-term NOD/SCID repopulating cells (5.2-fold). CONCLUSIONS: Cellular and microenvironmental changes that occur during all in vitro HSC cultures can significantly affect HSC output through the direct or indirect secretion of negative regulators. This study provides insight into the mechanisms regulating HSC fate in vitro and describes a novel methodology to regulate overall in vitro microenvironmental dynamics to enable the generation of clinically relevant numbers of HSCs.

Maina, N., Z. Han, et al. (2008). "Recombinant self-complementary adeno-associated virus serotype vector-mediated hematopoietic stem cell transduction and lineage-restricted, long-term transgene expression in a murine serial bone marrow transplantation model." Hum Gene Ther **19**(4): 376-83.

Although conventional recombinant single-stranded adeno-associated virus serotype 2 (ssAAV2) vectors have been shown to efficiently transduce numerous cells and tissues such as brain and muscle, their ability to transduce primary hematopoietic stem cells (HSCs) has been reported to be controversial. We have previously documented that among the ssAAV serotype 1 through 5 vectors, ssAAV1 vectors

are more efficient in transducing primary murine HSCs, but that viral second-strand DNA synthesis continues to be a rate-limiting step. In the present studies, we evaluated the transduction efficiency of several novel serotype vectors (AAV1, AAV7, AAV8, and AAV10) and documented efficient transduction of HSCs in a murine serial bone marrow transplantation model. Self-complementary AAV (scAAV) vectors were found to be more efficient than ssAAV vectors, and the use of hematopoietic cell-specific enhancers/promoters, such as the human beta-globin gene DNase I-hypersensitive site 2 enhancer and promoter (HS2-betap) from the beta-globin locus control region (LCR), and the human parvovirus B19 promoter at map unit 6 (B19p6), allowed sustained transgene expression in an erythroid lineage-restricted manner in both primary and secondary transplant recipient mice. The proviral AAV genomes were stably integrated into progenitor cell chromosomal DNA, and did not lead to any overt hematological abnormalities in mice. These studies demonstrate the feasibility of the use of novel scAAV vectors for achieving high-efficiency transduction of HSCs as well as erythroid lineage-restricted expression of a therapeutic gene for the potential gene therapy of beta-thalassemia and sickle cell disease.

Manfredini, R., R. Zini, et al. (2005). "The kinetic status of hematopoietic stem cell subpopulations underlies a differential expression of genes involved in self-renewal, commitment, and engraftment." Stem Cells **23**(4): 496-506.

The gene expression profile of CD34(-) hematopoietic stem cells (HSCs) and the correlations with their biological properties are still poorly understood. To address this issue, we used the DNA microarray technology to compare the expression profiles of different peripheral blood hemopoietic stem/progenitor cell subsets, lineage-negative (Lin(-)) CD34(-), Lin(-)CD34(+), and Lin(+)CD34(+) cells. The analysis of gene categories differentially expressed shows that the expression of CD34 is associated with cell cycle entry and metabolic activation, such as DNA, RNA, and protein synthesis. Moreover, the significant upregulation in CD34(-) cells of pathways inhibiting HSC proliferation induces a strong differential expression of cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors, and growth-arrest genes. According to the expression of their receptors and transducers, interleukin (IL)-10 and IL-17 showed an inhibitory effect on the clonogenic activity of CD34(-) cells. Conversely, CD34(+) cells were sensitive to the mitogenic stimulus of thrombopoietin. Furthermore, CD34(-) cells express preferentially genes related to neural, epithelial, and muscle differentiation. The analysis of transcription factor expression shows that the CD34 induction results in the upregulation of genes related to self-renewal and lineage commitment. The preferential expression in CD34(+) cells of genes supporting the HSC mobilization and homing to the bone marrow, such as chemokine receptors and integrins, gives the molecular basis for the higher engraftment capacity of CD34(+) cells. Thus, the different kinetic status of CD34(-) and CD34(+) cells, detailed by molecular and functional analysis, significantly influences their biological behavior.

Matthews, W., C. T. Jordan, et al. (1991). "A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations." Cell **65**(7): 1143-52.

To elucidate the molecular biology of the hematopoietic stem cell, we have begun to isolate genes from murine cell populations enriched in stem cell activity. One such cDNA encodes a novel receptor tyrosine kinase, designated fetal liver kinase-2 or flk-2, which is related to the W locus gene product c-kit. Expression analyses suggest an extremely restricted distribution of flk-2. It is expressed in populations enriched for stem cells and primitive uncommitted progenitors, and is absent in populations containing more mature cells. Therefore, this receptor may be a key signal transducing component in the totipotent hematopoietic stem cell and its immediate self-renewing progeny.

Mayack, S. R. and A. J. Wagers (2008). "Osteolineage niche cells initiate hematopoietic stem cell mobilization." Blood **112**(3): 519-31.

Recent studies have implicated bone-lining osteoblasts as important regulators of hematopoietic stem cell (HSC) self-renewal and differentiation; however, because much of the evidence supporting this notion derives from indirect in vivo experiments, which are unavoidably complicated by the presence of other cell types within the complex bone marrow milieu, the sufficiency of osteoblasts in modulating HSC activity has remained controversial. To address this, we prospectively isolated mouse osteoblasts, using a novel flow cytometry-based approach, and directly tested their activity as HSC niche cells and their role in cyclophosphamide/granulocyte colony-stimulating factor (G-CSF)-induced HSC proliferation and mobilization. We found that osteoblasts expand rapidly after cyclophosphamide/G-CSF treatment and exhibit phenotypic and functional changes that directly influence HSC proliferation and maintenance of reconstituting potential. Effects of mobilization on osteoblast number and function depend on the function of ataxia telangiectasia mutated (ATM), the product of the Atm gene, demonstrating a new role for

ATM in stem cell niche activity. These studies demonstrate that signals from osteoblasts can directly initiate and modulate HSC proliferation in the context of mobilization. This work also establishes that direct interaction with osteolineage niche cells, in the absence of additional environmental inputs, is sufficient to modulate stem cell activity.

Mazurier, F., A. Fontanellas, et al. (1999). "A novel immunodeficient mouse model--RAG2 x common cytokine receptor gamma chain double mutants--requiring exogenous cytokine administration for human hematopoietic stem cell engraftment." J Interferon Cytokine Res **19**(5): 533-41.

Gene transduction into immature human hematopoietic cells collected from umbilical cord blood, bone marrow, or mobilized peripheral blood cells could be useful for the treatment of genetic and acquired disorders of the hematopoietic system. Immunodeficient mouse models have been used frequently as recipients to assay the growth and differentiation of human hematopoietic stem/progenitor cells. Indeed, high levels of human cell engraftment were first reported in human/murine chimeras using NOD/SCID mice, which now are considered as the standard for these types of experiments. However, NOD/SCID mice have some clear disadvantages (including spontaneous tumor formation) that limit their general use. We have developed a new immunodeficient mouse model by combining recombinase activating gene-2 (RAG2) and common cytokine receptor gamma chain (gamma c) mutations. The RAG2-/-/gamma c- double mutant mice are completely alymphoid (T-, B-, NK-), show no spontaneous tumor formation, and exhibit normal hematopoietic parameters. Interestingly, human cord blood cell engraftment in RAG2-/-/gamma c- mice was greatly enhanced by the exogenous administration of human cytokines interleukin-(IL-3) granulocyte-macrophage colony-stimulating factor, (GM-CSF), and erythropoietin in contrast to the NOD/SCID model. This unique feature of the RAG2-/-/gamma c- mouse model should be particularly well suited for assessing the role of different cytokines in human lymphopoiesis and stem/progenitor cell function in vivo.

Melotti, P. and B. Calabretta (1994). "Ets-2 and c-Myb act independently in regulating expression of the hematopoietic stem cell antigen CD34." J Biol Chem **269**(41): 25303-9.

CD34 is currently the only well defined human hematopoietic stem cell marker and is expressed on 1-4% of normal bone marrow cells. Putative binding sites for Ets proteins, a family of transcription factors involved in the regulation of cell differentiation and proliferation in many cell systems, are present in the 5'-flanking region of the CD34 gene. Some of these sites are in close proximity to binding sequences of the encoded product of the proto-oncogene c-myb, which regulates CD34 expression by interacting with the Myb binding sites. Here we demonstrate that Ets-2 (i) transactivates the CD34 promoter in rodent fibroblasts upon interaction with Ets binding sites and (ii) induces expression of CD34 mRNA and protein in the CD34- human glioblastoma T98G cells. Ets-2 and c-Myb transactivate the CD34 promoter independently because specific transactivation is abrogated by site-specific mutations of the binding sites or by competition with oligomers that include wild type but not mutated Myb or Ets binding sites. Ets-2 and c-Myb appear to have addictive effects on transactivation of the CD34 promoter and on induction of CD34 mRNA. Instead, CD34 surface protein levels might be induced synergistically, raising the possibility of a posttranslational mechanism of CD34 expression in cells constitutively expressing c-Myb and Ets-2.

Milhem, M., N. Mahmud, et al. (2004). "Modification of hematopoietic stem cell fate by 5aza 2'deoxycytidine and trichostatin A." Blood **103**(11): 4102-10.

Efforts to change the fate of human hematopoietic stem cells (HSCs) and progenitor cells (HPCs) in vitro have met with limited success. We hypothesized that previously utilized in vitro conditions might result in silencing of genes required for the maintenance of primitive HSCs/HPCs. DNA methylation and histone deacetylation are components of an epigenetic program that regulates gene expression. Using pharmacologic agents in vitro that might possibly interfere with DNA methylation and histone deacetylation, we attempted to maintain and expand cells with phenotypic and functional characteristics of primitive HSCs/HPCs. Human marrow CD34(+) cells were exposed to a cytokine cocktail favoring differentiation in combination with 5aza 2'deoxycytidine (5azaD) and trichostatin A (TSA), resulting in a significant expansion of a subset of CD34(+) cells that possessed phenotypic properties as well as the proliferative potential characteristic of primitive HSCs/HPCs. In addition, 5azaD- and TSA-pretreated cells but not the CD34(+) cells exposed to cytokines alone retained the ability to repopulate immunodeficient mice. Our findings demonstrate that 5azaD and TSA can be used to alter the fate of primitive HSCs/HPCs during in vitro culture.

Miyazato, A., S. Ueno, et al. (2001). "Identification of myelodysplastic syndrome-specific genes by DNA

microarray analysis with purified hematopoietic stem cell fraction." Blood **98**(2): 422-7.

Myelodysplastic syndrome (MDS) is a slowly progressing hematologic malignancy associated with a poor outcome. Despite the relatively high incidence of MDS in the elderly, differentiation of MDS from de novo acute myeloid leukemia (AML) still remains problematic. Identification of genes expressed in an MDS-specific manner would allow the molecular diagnosis of MDS. Toward this goal, AC133 surface marker-positive hematopoietic stem cell (HSC)-like fractions have been collected from a variety of leukemias in a large-scale and long-term genomics project, referred to as "Blast Bank," and transcriptome of these purified blasts from the patients with MDS were then compared with those from AML through the use of oligonucleotide microarrays. A number of genes were shown to be expressed in a disease-specific manner either to MDS or AML. Among the former found was the gene encoding the protein Delta-like (Dlk) that is distantly related to the Delta-Notch family of signaling proteins. Because overexpression of Dlk may play a role in the pathogenesis of MDS, the disease specificity of Dlk expression was tested by a quantitative "real-time" polymerase chain reaction analysis. Examination of the Blast Bank samples from 22 patients with MDS, 31 with AML, and 8 with chronic myeloid leukemia confirmed the highly selective expression of the Dlk gene in the individuals with MDS. Dlk could be the first candidate molecule to differentiate MDS from AML. The proposal is made that microarray analysis with the Blast Bank samples is an efficient approach to extract transcriptome data of clinical relevance for a wide range of hematologic disorders.

Mochizuki, K., C. Sugimori, et al. (2008). "Expansion of donor-derived hematopoietic stem cells with PIGA mutation associated with late graft failure after allogeneic stem cell transplantation." Blood **112**(5): 2160-2.

A small population of CD55(-)CD59(-) blood cells was detected in a patient who developed donor-type late graft failure after allogeneic stem cell transplantation (SCT) for treatment of aplastic anemia (AA). Chimerism and PIGA gene analyses showed the paroxysmal nocturnal hemoglobinuria (PNH)-type granulocytes to be of a donor-derived stem cell with a thymine insertion in PIGA exon 2. A sensitive mutation-specific polymerase chain reaction (PCR)-based analysis detected the mutation exclusively in DNA derived from the donor bone marrow (BM) cells. The patient responded to immunosuppressive therapy and achieved transfusion independence. The small population of PNH-type cells was undetectable in any of the 50 SCT recipients showing stable engraftment. The de novo development of donor cell-derived AA with a small population of PNH-type cells in this patient supports the concept that glycosyl phosphatidylinositol-anchored protein-deficient stem cells have a survival advantage in the setting of immune-mediated BM injury.

Muller, A. M., A. Medvinsky, et al. (1994). "Development of hematopoietic stem cell activity in the mouse embryo." Immunity **1**(4): 291-301.

The precise time of appearance of the first hematopoietic stem cell activity in the developing mouse embryo is unknown. Recently the aorta-gonad-mesonephros region of the developing mouse embryo has been shown to possess hematopoietic colony-forming activity (CFU-S) in irradiated recipient mice. To determine whether the mouse embryo possesses definitive hematopoietic stem cell activity in the analogous AGM region and to determine the order of appearance of stem cells in the yolk sac, AGM region, and liver, we transferred these embryonic tissues into adult irradiated recipients. We report here the long-term, complete, and functional hematopoietic repopulation of primary and serial recipients with AGM-derived cells. We observe potent hematopoietic stem cell activity in the AGM region before the appearance of yolk sac and liver stem cell activity and discuss a model for the maturation of stem cell activity in mouse embryogenesis.

Murphy, N., M. Diviney, et al. (2006). "Donor methylenetetrahydrofolate reductase genotype is associated with graft-versus-host disease in hematopoietic stem cell transplant patients treated with methotrexate." Bone Marrow Transplant **37**(8): 773-9.

Methotrexate (MTX), used as a graft-versus-host disease (GvHD) prophylactic agent in hematopoietic stem cell transplantation (HSCT), exerts its effect via folate cycle inhibition. A critical enzyme involved in folate metabolism is 5,10-methylenetetrahydrofolate reductase (MTHFR). We examined the association of a single nucleotide polymorphism (SNP) at position 677 in the MTHFR gene on GvHD outcomes in allogeneic HSCT patients administered MTX. MTHFR genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) on 193 HSCT patients and donors. A total of 140 patients were transplanted with an HLA-matched related donor and 53 with an unrelated donor. GvHD outcomes were compared between genotypes by univariate and multivariate analysis. The combined donor 677CT and TT genotypes were associated with a decreased incidence of GvHD (acute and chronic combined) in HSCT recipients with an HLA-matched related donor

(75% at 1 year in the CT and TT group compared with 91% in the wild type CC group, P=0.01), increased time to onset of first GvHD (P=0.001) and time to first GvHD treated with systemic therapy (P=0.022). Unrelated donor MTHFR genotype was not associated with outcome parameters and no associations of recipient genotype in either related or unrelated donor cohorts were observed.

Myers, G. D., C. M. Bollard, et al. (2007). "Reconstitution of adenovirus-specific cell-mediated immunity in pediatric patients after hematopoietic stem cell transplantation." Bone Marrow Transplant **39**(11): 677-86.

Adenovirus (adv) is a significant cause of morbidity and mortality in pediatric hematopoietic stem cell transplant recipients, and control of infection seems to require antigen-specific T cells. We evaluated the recovery of adv-specific cellular immunity in this patient population related to degree of T-cell immunosuppressive therapy and compared this to adv cellular immunity of normal donors. Over 12 months, we monitored for adv DNA in stool and blood of patients and in the blood of a normal donor group. Twenty-two pediatric hematopoietic stem cell transplant (HSCT) patients (14 months-20 years) who received matched-related (MRD n=6), mismatched related (Haplo n=6) or matched unrelated donor (MUD n=10) grafts, were followed and results compared to healthy controls (n=8). Adv was detected by polymerase chain reaction in blood and/or stool from 81.8% of patients on at least one occasion post-HSCT, but only 68% of patients developed symptomatic adv infections. Recovery of adv-specific T cells was significantly delayed in the MUD and Haplo recipients, whereas recovery in the MRD group was similar to levels detected in healthy donors within 30 days post-transplant. In conclusion, recipients of alternative donor transplants at our institution have significantly delayed adv-specific cellular immune recovery, which correlates to an increased risk of adv-associated morbidity and mortality.

Ng, Y. Y., B. van Kessel, et al. (2004). "Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity." J Leukoc Biol **75**(2): 314-23.

The replacement of bone marrow (BM) as a conventional source of stem cell (SC) by umbilical cord blood (UCB) and granulocyte-colony stimulating factor-mobilized peripheral blood SC (PBSC) has brought about clinical advantages. However, several studies have demonstrated that UCB CD34(+) cells and PBSC significantly differ from BM CD34(+) cells qualitatively and quantitatively. Here, we quantified the number of SC in purified BM, UCB CD34(+) cells, and CD34(+) PBSC using in vitro and in vivo assays for human hematopoietic SC (HSC) activity. A cobblestone area-forming cell (CAFC) assay showed that UCB CD34(+) cells contained the highest frequency of CAFC(wk6) (3.6- to tenfold higher than BM CD34(+) cells and PBSC, respectively), and the engraftment capacity in vivo by nonobese diabetic/severe combined immunodeficiency repopulation assay was also significantly greater than BM CD34(+), with a higher proportion of CD45(+) cells detected in the recipients at a lower cell dose. To understand the molecular characteristics underlying these functional differences, we performed several DNA microarray experiments using Affymetrix gene chips, containing 12,600 genes. Comparative analysis of gene-expression profiles showed differential expression of 51 genes between BM and UCB CD34(+) SC and 64 genes between BM CD34(+) cells and PBSC. These genes are involved in proliferation, differentiation, apoptosis, and engraftment capacity of SC. Thus, the molecular expression profiles reported here confirmed functional differences observed among the SC sources. Moreover, this report provides new insights to describe the molecular phenotype of CD34(+) HSC and leads to a better understanding of the discrepancy among the SC sources.

Nottingham, W. T., A. Jarratt, et al. (2007). "Runx1-mediated hematopoietic stem-cell emergence is controlled by a Gata/Ets/SCL-regulated enhancer." Blood **110**(13): 4188-97.

The transcription factor Runx1/AML1 is an important regulator of hematopoiesis and is critically required for the generation of the first definitive hematopoietic stem cells (HSCs) in the major vasculature of the mouse embryo. As a pivotal factor in HSC ontogeny, its transcriptional regulation is of high interest but is largely undefined. In this study, we used a combination of comparative genomics and chromatin analysis to identify a highly conserved 531-bp enhancer located at position + 23.5 in the first intron of the 224-kb mouse Runx1 gene. We show that this enhancer contributes to the early hematopoietic expression of Runx1. Transcription factor binding in vivo and analysis of the mutated enhancer in transient transgenic mouse embryos implicate Gata2 and Ets proteins as critical factors for its function. We also show that the SCL/Lmo2/Ldb-1 complex is recruited to the enhancer in vivo. Importantly, transplantation experiments demonstrate that the intronic Runx1 enhancer targets all definitive HSCs in the mouse embryo, suggesting that it functions as a crucial cis-regulatory element that

integrates the Gata, Ets, and SCL transcriptional networks to initiate HSC generation.

Ohtsubo, M., S. Yasunaga, et al. (2008). "Polycomb-group complex 1 acts as an E3 ubiquitin ligase for Geminin to sustain hematopoietic stem cell activity." Proc Natl Acad Sci U S A **105**(30): 10396-401.

Polycomb-group (PcG) genes encode multimeric nuclear protein complexes, PcG complex 1 and 2. PcG complex 2 was proved to induce transcription repression and to further methylate histone H3 at lysine-27 (H3K27). Subsequently PcG complex 1 is recruited through recognition of methylated H3K27 and maintains the transcription silencing by mediating monoubiquitination of histone H2A at lysine-119. Genetic evidence demonstrated a crucial role for PcG complex 1 in stem cells, and Bmi1, a member of PcG complex 1, was shown to sustain adult stem cells through direct repression of the INK4a locus encoding cyclin-dependent kinase inhibitor, p16CKI, and p19ARF. The molecular functions of PcG complex 1, however, remain insufficiently understood. In our study, deficiency of Rae28, a member of PcG complex 1, was found to impair ubiquitin-proteasome-mediated degradation of Geminin, an inhibitor of DNA replication licensing factor Cdt1, and to increase protein stability. The resultant accumulation of Geminin, based on evidence from retroviral transduction experiments, presumably eliminated hematopoietic stem cell activity in Rae28-deficient mice. Rae28 mediates recruiting Scmh1, which provides PcG complex 1 an interaction domain for Geminin. Moreover, PcG complex 1 acts as the E3 ubiquitin ligase for Geminin, as we demonstrated in vivo as well as in vitro by using purified recombinant PcG complex 1 reconstituted in insect cells. Our findings suggest that PcG complex 1 supports the activity of hematopoietic stem cells, in which high-level Geminin expression induces quiescence securing genome stability, by enhancing cycling capability and hematopoietic activity through direct regulation of Geminin.

Omazic, B., I. Nasman-Bjork, et al. (2001). "Altered expression of receptors for thyroid hormone and insulin-like growth factor-I during reconstitution after allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **27**(11): 1163-71.

Treatment with neuroendocrine hormones has been suggested to promote reconstitution of the immune system after hematopoietic stem cell transplantation (HSCT). We investigated the expression of genes encoding receptors for growth hormone (GH), insulin-like growth factor-I (IGF-I) and triiodothyronine (T3), at various time points after HSCT in 16 patients and 15 healthy controls. Peripheral blood mononuclear cells were isolated and RNA for GH receptor (GHR), IGF-I receptor (IGF-IR) and thyroid hormone receptor (TRalpha1) was amplified by RT-PCR. The expression of the genes was compared with the expression of beta-actin. We demonstrate increased expression of TRalpha1 RNA in patients at 1.5 months post HSCT, compared to a group of healthy controls, and decreased expression of IGF-IR RNA at 2 and 3 months post HSCT, compared to the controls. Serum from three of the patients was also analyzed for levels of T3, T4, TSH and IGF-I at several time points after HSCT. Serum levels for T3, thyroxine (T4), thyroid stimulating hormone (TSH) and IGF-I were within the normal range in all samples. Our results on the molecular level indicate a role for thyroid hormones and IGF-I in immune reconstitution after HSCT, even though the serum levels of T3, T4, TSH and IGF-I are normal.

Orlic, D., S. L. Laprise, et al. (1999). "Isolation of stem cell-specific cDNAs from hematopoietic stem cell populations." Ann N Y Acad Sci **872**: 243-54; discussion 254-5.

We have begun to isolate gene sequences that are specifically expressed in hematopoietic stem cells (HSCs). There are at least three fundamental requirements for the isolation of HSC-specific transcripts. First, highly enriched populations of HSCs, and an HSC-depleted cell population for comparison must be isolated. Secondly, the gene isolation procedures must be adapted to accommodate the small amounts of RNA obtained from purified HSCs. Finally, a defined screening strategy must be developed to focus on sequences to be examined in more detail. In this report, we describe the characterization of populations of HSCs that are highly enriched (Lin- c-kitHI) or depleted (Lin- c-kitNEG) of HSCs. We compared two methods for gene isolation, differential display polymerase chain reaction (DD-PCR) and subtractive hybridization (SH), and found that the latter was more powerful and efficient in our hands. Lastly we describe the strategy that we have developed to screen clones for further study.

Orsal, A. S., M. Ozsan, et al. (2006). "Comparison of hybrid capture and reverse transcriptase polymerase chain reaction methods in terms of diagnosing human cytomegalovirus infection in patients following hematopoietic stem cell transplantation." Saudi Med J **27**(7): 967-74.

OBJECTIVE: Human cytomegalovirus (CMV) is a life threatening cause of infection among hematopoietic stem cell recipients. Developing reliable methods in detecting the CMV infection is important to identify the patients at risk of CMV

infection and disease. The aim of this study was to compare the 2 tests- hybrid capture test, which is routinely used in the diagnosis of CMV infection among hematopoietic stem cell recipients, and reverse transcriptase polymerase chain reaction (RT-PCR) detecting UL21.5 mRNA transcripts of the active virus. METHODS: In this prospective study, a total of 178 blood samples obtained from 35 patients following allogeneic hematopoietic stem cell transplantation at the Bone Marrow Transplantation Unit of the Hematology Department, Ibn-i Sina Hospital of Ankara University School of Medicine, Turkey between January 2003 and September 2003 were analyzed. Hybrid capture and RT-PCR using UL21.5 gene transcript method to investigate HCMV in blood samples were performed at the Department of Microbiology and Clinic Microbiology Ankara University School of Medicine, Turkey. RESULTS: When hybrid capture test was accepted as the golden standard, the sensitivity of RT-PCR was 33%, specificity 100%, false negativity 67%, false positivity 0%, positive predictive value 100%, negative predictive value 74%, and accuracy was 77%. CONCLUSION: Improving this test by quantification, and application of additional gene transcripts, primarily the late gene transcripts can help increase the sensitivity and feasibility.

Oshima, K., Y. Kanda, et al. (2008). "Case report: persistent cytomegalovirus (CMV) infection after haploidentical hematopoietic stem cell transplantation using in vivo alemtuzumab: emergence of resistant CMV due to mutations in the UL97 and UL54 genes." J Med Virol **80**(10): 1769-75.

Addition of in vivo alemtuzumab to the conditioning regimen enabled 2- or 3-locus-mismatched hematopoietic stem cell transplantation with an acceptable incidence of graft-versus-host-disease. However, the procedure was associated with a high incidence of cytomegalovirus (CMV) reactivation. Although preemptive therapy with ganciclovir prevented successfully severe CMV diseases and CMV-related mortality, a patient developed persistent positive CMV antigenemia for more than 1 year after transplantation and CMV disease, despite the use of ganciclovir and foscarnet. The in vitro susceptibility assay showed that the clinical isolate was resistant to foscarnet, moderately resistant to ganciclovir, but sensitive to cidofovir. Therefore, cidofovir was administered. CMV antigenemia became negative within 2 weeks and never developed again. Nucleotide sequence of the UL54 and UL97 of the clinical isolate showed 4 amino acid substitutions (V11L, Q578H, S655L, and G874R) in UL54 and 2 mutations (A140V and A594V) in UL97 compared with the Towne and AD169 strains. Ganciclovir resistance was suspected to be caused by both A594V of UL97 and Q578H of UL54, whereas foscarnet resistance was due mainly to Q578H of UL54. In conclusion, the in vitro susceptibility assay as well as nucleotide sequence of clinical isolate is important to choose appropriate antiviral agents for patients who have persistent CMV reactivation after stem cell transplantation.

Oshima, Y., M. Ueda, et al. (2003). "DNA microarray analysis of hematopoietic stem cell-like fractions from individuals with the M2 subtype of acute myeloid leukemia." Leukemia **17**(10): 1990-7.

Acute myeloid leukemia (AML) may develop de novo or secondarily to myelodysplastic syndrome (MDS). Although the clinical outcome of MDS-related AML is worse than that of de novo AML, it is not easy to differentiate between these two clinical courses without a record of prior MDS. Large-scale profiling of gene expression by DNA microarray analysis is a promising approach with which to identify molecular markers specific to de novo or MDS-related AML. This approach has now been adopted with AC133-positive hematopoietic stem cell-like fractions purified from 10 individuals, each with either de novo or MDS-related AML of the M2 subtype. Sets of genes whose activity was associated with either disease course were identified. Furthermore, on the basis of the expression profiles of these genes, it was possible to predict correctly the clinical diagnosis for 17 (85%) of the 20 cases in a cross-validation trial. Similarly, different sets of genes were identified whose expression level was associated with clinical outcome after induction chemotherapy. These data suggest that, at least in terms of gene expression profiles, de novo AML and MDS-related AML are distinct clinical entities.

Ott, D. E., J. Keller, et al. (1994). "10A1 MuLV induces a murine leukemia that expresses hematopoietic stem cell markers by a mechanism that includes fli-1 integration." Virology **205**(2): 563-8.

The 10A1 murine leukemia virus induces tumors that lack lineage-specific markers found on myeloid, T-cell, and B-cell lineages. Either erythroid or multipotent stem cells can have this phenotype; therefore we have used fluorescence-activated cell sorter analysis with either multipotent stem cell markers or markers found on lineage-restricted precursors to differentiate between these two possibilities. The results showed that tumors induced by 10A1 expressed multipotent stem cell markers as well as some lineage-restricted precursor markers. To further study the tumor phenotype, we analyzed total RNAs from 10A1-induced tumors by Northern blotting for c-kit, erythropoietin receptor, and T-cell

gamma receptor mRNAs. Most of the tumors contained these mRNAs, which are characteristic of early hematopoietic cells. These results are consistent with the hypothesis that 10A1-induced tumor cells are early multipotent hematopoietic stem cells. Southern blot analysis revealed that 14 of 14 10A1-induced tumor cell DNAs examined contained MuLV integrations into the fli-1 gene. The results strongly suggested that promoter insertion into fli-1 is required for tumor formation.

Pan, X., N. Minegishi, et al. (2000). "Identification of human GATA-2 gene distal IS exon and its expression in hematopoietic stem cell fractions." J Biochem **127**(1): 105-12.

Transcription factor GATA-2 is essential for the proper function of hematopoietic stem cells and progenitors. Two first exons/promoters have been found in the mouse GATA-2 gene, and a distal IS promoter shows activity specific to hematopoietic progenitors and neural tissues. To ascertain whether the two-promoter system is also utilized in the human GATA-2 gene, we isolated and analyzed a P1 phage clone containing this gene. The nucleotide sequence of the human GATA-2 gene 5' flanking region was determined over 10 kbp, and a human IS exon was identified in the locus through sequence comparison analysis with that of the mouse GATA-2 IS exon. RNA blotting and reverse-transcribed PCR analyses identified a transcript that starts from the IS exon in human leukemia-derived cell lines. The IS-originated transcript was also identified in CD34-positive bone marrow and cord blood mononuclear cells, which are recognized as clinically important hematopoietic stem cell-enriched fractions. Phylogenic comparison of the human and mouse GATA-2 gene sequences revealed several regions in the locus that exhibit high sequence similarity. These results demonstrate that the GATA-2 gene regulatory machinery is conserved among vertebrates. The fact that the human IS promoter is active in the hematopoietic stem cell/progenitor fraction may be an important clue for the design of a vector system that can specifically express various genes in hematopoietic stem cells and progenitors.

Piekorz, R. P., A. Hoffmeyer, et al. (2002). "The centrosomal protein TACC3 is essential for hematopoietic stem cell function and genetically interfaces with p53-regulated apoptosis." Embo J **21**(4): 653-64.

TACC3 is a centrosomal/mitotic spindle-associated protein that is highly expressed in a cell cycle-dependent manner in hematopoietic lineage cells. During embryonic development, TACC3 is expressed in a variety of tissues in addition to the hematopoietic lineages. TACC3 deficiency causes an embryonic lethality at mid- to late gestation involving several lineages of cells. Hematopoietic stem cells, while capable of terminal differentiation, are unable to be expanded in vitro or in vivo in reconstitution approaches. Although gross alterations in centrosome numbers and chromosomal segregation are not observed, TACC3 deficiency is associated with a high rate of apoptosis and expression of the p53 target gene, p21(Waf1/Cip1). Hematopoietic stem cell functions, as well as deficiencies in other cell lineages, can be rescued by combining the TACC3 deficiency with p53 deficiency. The results support the concept that TACC3 is a critical component of the centrosome/mitotic spindle apparatus and its absence triggers p53-mediated apoptosis.

Porecha, N. K., K. English, et al. (2006). "Enhanced functional response to CXCL12/SDF-1 through retroviral overexpression of CXCR4 on M07e cells: implications for hematopoietic stem cell transplantation." Stem Cells Dev **15**(3): 325-33.

The chemokine CXCL12 (stromal cell derived factor-1/SDF-1) stimulates hematopoietic stem and progenitor cells (HSCs/HPCs) through the corresponding chemokine receptor CXCR4. CXCL12 is thought to be important for both proper HSC homing, retention, and engraftment into the bone marrow (BM) and mobilization out of the BM. Previous studies suggest that breaking the CXCL12-CXCR4 interaction mobilizes HPCs, blocking CXCR4 inhibits HSC homing, and overexpression increases HSC/HPC repopulation. The efficiency of mobilization and engraftment therefore appears to be dependent on the response of HSCs/HPCs to CXCL12, which is in turn dependent upon levels of CXCR4 expressed on HSCs/HPCs. However, expression of CXCR4 on the surface of HSCs/HPCs appears to be variable. To study the function of CXCR4 on HSCs/HPCs, we used the MSCV-based bicistronic (EGFP) retroviral vector MIEG3 to overexpress CXCR4 on M07e cells, an established model of human HPC. CXCR4 overexpression resulted in significant increases in CXCL12-induced chemotaxis and cell survival. Most importantly, cells overexpressing CXCR4 responded to CXCL12 at levels typically too low induce a response. These data suggest that an increased transplant efficiency resulting from CXCR4 overexpression is likely a function of increased HSC/HPC homing and increased HSC/HPC survival in the recipient's BM. These experiments also validate the ability of the MIEG3-CXCR4 retroviral construct to overexpress CXCR4 efficiently and the use of MIEG3-CXCR4 M07e cells for further study. Finally, this information may have future potential therapeutic implications for improvements in transplant efficiency.

Pumannova, M., K. Roubalova, et al. (2006). "Comparison of quantitative competitive polymerase chain reaction-enzyme-linked immunosorbent assay with LightCycler-based polymerase chain reaction for measuring cytomegalovirus DNA in patients after hematopoietic stem cell transplantation." Diagn Microbiol Infect Dis **54**(2): 115-20.

Development of highly sensitive quantitative assays for cytomegalovirus (CMV) DNA detection is crucial for identification of immunodeficient patients at high risk of CMV disease. We designed 2 internally controlled competitive quantitative assays, enzyme-linked immunosorbent assay (ELISA)-based and real-time polymerase chain reaction (PCR) tests, using amplification of the same segment of the CMV genome. The aim of this study was to compare sensitivity, specificity, and laboratory performance characteristics of these assays. In both assays, a 159-bp segment of UL83 gene was amplified. External and internal controls were constructed by cloning the amplification product and heterogenous DNA segment flanked by target sequences for CMV-derived primers into bacterial plasmids, respectively. Real-time PCR was performed on LightCycler (Roche Diagnostics, Mannheim, Germany), and amplicons were detected using fluorescence resonance energy transfer probes. Alternatively, PCR products were labeled by digoxigenin, hybridized to immobilized probes, and detected by ELISA. The assays were tested on genomic DNA isolated from laboratory strains of CMV, QCMD control panel, and CMV DNA-positive peripheral blood DNA samples from hematopoietic stem cell transplant recipients, previously characterized by pp65 antigenemia and qualitative nested PCR. Real-time and ELISA-based PCR assays showed a linear course of 1-10(8) and 10-10(5) copies of CMV DNA per reaction, respectively. When compared with ELISA-based PCR, real-time PCR showed superiority in inter- and intra-assay reproducibility. Both assays were highly specific in detecting CMV DNA. No difference in amplification efficiency of internal or external standards and wild-type CMV DNA was found. The assays exhibited 83% concordance in CMV DNA detection from clinical samples, all discrepant samples having low CMV DNA copy numbers. There was a good correlation between viral DNA loads measured by the 2 assays. Statistically significant correlation was observed between the numbers of CMV DNA copies and pp65-positive leukocytes in the samples tested. Both variants of competitive PCR are adequately sensitive to be used for CMV DNA quantitation in clinical samples. LightCycler PCR, having superior performance characteristics and being less time-consuming, seems to be more suitable for routine diagnosis.

Roddie, C., J. P. Paul, et al. (2009). "Allogeneic hematopoietic stem cell transplantation and norovirus gastroenteritis: a previously unrecognized cause of morbidity." Clin Infect Dis **49**(7): 1061-8.

BACKGROUND: A retrospective study of the clinical, epidemiologic, and virologic features of norovirus gastroenteritis in 12 adult allogeneic hematopoietic stem cell transplant (HSCT) recipients. METHODS: Norovirus infection was diagnosed by reverse-transcriptase polymerase chain reaction. Strains were genotyped by nucleic acid sequence of the most highly conserved region of the norovirus gene encoding the capsid S (shell) domain. RESULTS: Ten of 12 patients presented with vomiting of short duration, but diarrhea was present in all. The median time from onset to norovirus diagnosis was 1 month (range, 0.25-6.0 months). Eleven patients were receiving immunosuppression when norovirus infection was diagnosed: 8 for graft-versus-host disease (GVHD) in an organ other than gut, 1 for previous gut GVHD, and 2 for presumed gut GVHD that proved to be norovirus gastroenteritis. Six patients required enteral or parenteral nutrition for severe weight loss. In 10 patients, diarrhea lasted a median of 3 months (range, 0.5-14 months) and virus was shed at a high level throughout. The remaining 2 patients died after 4 months of diarrhea (one died of unrelated complications, and the other died of malnutrition). The noroviruses found were GII (untyped), GII-3, GII-4, and GII-7 in 1, 1, 9, and 1 patients, respectively. Eleven of the 12 patients had acquired their infection in the community. Phylogenetic analysis of the GII-4 strains demonstrated that all differed. CONCLUSIONS: Noroviruses are a hitherto unsuspected cause of prolonged morbidity and mortality in adults after allogeneic HSCT. The use of reverse-transcriptase polymerase chain reaction to detect high viral load levels in feces distinguishes norovirus gastroenteritis from gut GVHD.

Rodrigues, N. P., V. Janzen, et al. (2005). "Haploinsufficiency of GATA-2 perturbs adult hematopoietic stem-cell homeostasis." Blood **106**(2): 477-84.

The zinc finger transcription factor GATA-2 plays a fundamental role in generating hematopoietic stem-cells in mammalian development. Less well defined is whether GATA-2 participates in adult stem-cell regulation, an issue we addressed using GATA-2 heterozygote mice that express reduced levels of GATA-2 in hematopoietic cells. While GATA-2+/- mice demonstrated decreases in some colony-forming

progenitors, the most prominent changes were observed within the stem-cell compartment. Heterozygote bone marrow had a lower abundance of Lin(-)c-kit(+)Sca-1(+)CD34- cells and performed poorly in competitive transplantation and quantitative week-5 cobblestone area-forming cell (CAFC) assays. Furthermore, a stem-cell-enriched population from GATA1+/- marrow was more quiescent and exhibited a greater frequency of apoptotic cells associated with decreased expression of the anti-apoptotic gene Bcl-xL. Yet the self-renewal potential of the +/- stem-cell compartment, as judged by serial transplantations, was unchanged. These data indicate compromised primitive cell proliferation and survival in the setting of a lower GATA-2 gene dose without a change in the differentiation or self-renewal capacity of the stem-cells that remain. Thus, GATA-2 dose regulates adult stem-cell homeostasis by affecting select aspects of stem cell function.

Sakaguchi, T., M. Nishimoto, et al. (2006). "Putative "stemness" gene jam-B is not required for maintenance of stem cell state in embryonic, neural, or hematopoietic stem cells." Mol Cell Biol **26**(17): 6557-70.

Many genes have been identified that are specifically expressed in multiple types of stem cells in their undifferentiated state. It is generally assumed that at least some of these putative "stemness" genes are involved in maintaining properties that are common to all stem cells. We compared gene expression profiles between undifferentiated and differentiated embryonic stem cells (ESCs) using DNA microarrays. We identified several genes with much greater signal in undifferentiated ESCs than in their differentiated derivatives, among them the putative stemness gene encoding junctional adhesion molecule B (Jam-B gene). However, in spite of the specific expression in undifferentiated ESCs, Jam-B mutant ESCs had normal morphology and pluripotency. Furthermore, Jam-B homozygous mutant mice are fertile and have no overt developmental defects. Moreover, we found that neural and hematopoietic stem cells recovered from Jam-B mutant mice are not impaired in their ability to self-renew and differentiate. These results demonstrate that Jam-B is dispensable for normal mouse development and stem cell identity in embryonic, neural, and hematopoietic stem cells.

Salgar, S. K., D. Yang, et al. (2004). "Viral interleukin-10-engineered autologous hematopoietic stem cell therapy: a novel gene therapy approach to prevent graft rejection." Hum Gene Ther **15**(2): 131-44.

The Epstein-Barr virus-encoded protein BCRF1 (viral interleukin [vIL]-10) is a biologically active homologue of cellular interleukin (IL)-10. In this study, a novel gene therapy approach to prolong allograft survival was designed. Autologous (syngeneic) hematopoietic progenitor/stem cell-enriched (HSC; lineage(-ve)) population derived from CBA/J mouse bone marrow were transduced with retrovirus encoding vIL-10 gene (vIL-10-HSC), ex vivo; vIL-10-HSC were injected (4-6 x 10(6) cells intravenously) into lethally (9.5 Gy) or sublethally (4 Gy) irradiated CBA/J mice. Six weeks after vIL-10-HSC administration, vascular heterotopic heart (C57BL/6) transplantation was performed. Ex vivo, the vIL-10-HSC produced 5.4 +/- 0.5 ng of vIL-10 protein/2 x 10(5) cells per 24 hr. In vivo, serum vIL-10 production was 187 +/- 205 pg/ml during 3-10 weeks after vIL-10-HSC administration. Cardiac allograft survival was prolonged ($p < 0.004$) in lethally (71 +/- 40 days) and sublethally (114 +/- 15 days) irradiated mice that received vIL-10-HSC compared to controls that received unengineered (UE) HSC or vector DNA-engineered HSC (12-16 days). However, secondary skin graft (C57BL/6) survival was not prolonged in cardiac allograft-tolerant animals. In the vIL-10-HSC-administered group, graft histopathology demonstrated mild arteritis/venulitis (grade 0.7) and rejection (grade 1.0). Intragraft expression of costimulatory molecules (B7.1, B7.2), cytokines (IL-2, IL-4, mIL-10, interferon [IFN]-gamma), and inducible nitric oxide synthase (iNOS) molecules was markedly lower in vIL-10-HSC-treated tolerant grafts that survived more than 100 days compared to vector DNA-HSC- or UE-HSC-treated controls. Furthermore, T lymphocytes derived from vIL-10-HSC-treated tolerant recipients demonstrated hyporeactivity to donor antigens in mixed lymphocyte cultures. Administration of autologous vIL-10-engineered HSC prior to organ transplantation prolonged cardiac allograft survival significantly.

Sandberg, M. L., S. E. Sutton, et al. (2005). "c-Myb and p300 regulate hematopoietic stem cell proliferation and differentiation." Dev Cell **8**(2): 153-66.

Precise control of hematopoietic stem cell (HSC) proliferation and differentiation is needed to maintain a lifetime supply of blood cells. Using genome-wide ENU mutagenesis and phenotypic screening, we have identified a mouse line that harbors a point mutation in the transactivation (TA) domain of the transcription factor c-Myb (M303V), which reduces c-Myb-dependent TA by disrupting its interaction with the transcriptional coactivator p300. The biological consequences of the c-Myb(M303V/M303V) mutation include

thrombocytosis, megakaryocytosis, anemia, lymphopenia, and the absence of eosinophils. Detailed analysis of hematopoiesis in c-Myb(M303V/M303V) mice reveals distinct blocks in T cell, B cell, and red blood cell development, as well as a remarkable 10-fold increase in the number of HSCs. Cell cycle analyses show that twice as many HSCs from c-Myb(M303V/M303V) animals are actively cycling. Thus c-Myb, through interaction with p300, controls the proliferation and differentiation of hematopoietic stem and progenitor cells.

Satterthwaite, A. B., T. C. Burn, et al. (1992). "Structure of the gene encoding CD34, a human hematopoietic stem cell antigen." Genomics **12**(4): 788-94.

CD34 is a cell surface antigen of unknown function expressed in humans in hematopoietic stem cells, vascular endothelium, and blasts from 30% of patients with acute myeloid and lymphocytic leukemia. To begin to investigate the cis-acting elements required for this tissue-specific expression, the human CD34 locus was isolated and its genomic structure and transcriptional start site were characterized. The human CD34 gene spans 26 kb and has 8 exons, a structure quite similar to that of the murine gene. The start site of CD34 transcription was determined to be 258 bp upstream of the translational start site using RNase protection. These experiments also indicated that the 5' untranslated region has extensive secondary structure. In addition, fluorescence in situ hybridization was used to map the CD34 locus to band 1q32.

Schambach, A., B. Schiedlmeier, et al. (2006). "Towards hematopoietic stem cell-mediated protection against infection with human immunodeficiency virus." Gene Ther **13**(13): 1037-47.

The failure of pharmacological approaches to cure infection with the human immunodeficiency virus (HIV) has renewed the interest in gene-based therapies. Among the various strategies that are currently explored, the blockade of HIV entry into susceptible T cells and macrophages promises to be the most powerful intervention. For long-term protection of both of these lineages, genetic modification of hematopoietic stem cells (HSCs) would be required. Here, we tested whether HSCs and their progeny can be modified to express therapeutic levels of M87o, a gammaretroviral vector encoding an artificial transmembrane molecule that blocks fusion-mediated uptake of HIV. In serial murine bone marrow transplantations, efficient and multilineage expression of M87o was observed for more than 1 year (range 37-75% of mononuclear cells), without signs of toxicity related to the transmembrane molecule. To allow enrichment of M87o-modified HSCs after transplant, we constructed vectors coexpressing the P140K mutant of O(6)-methylguanine-DNA-methyltransferase (MGMT-P140K). This clinically relevant selection marker mediates a survival advantage in HSCs if exposed to combinations of methylguanine-methyltransferase (MGMT) inhibitors and alkylating agents. A bicistronic vector mediated sufficient expression of both M87o and MGMT to confer a selective survival advantage in the presence of HIV and alkylating agents, respectively. These data encourage further investigations in large animal models and clinical trials.

Scherr, M., K. Battmer, et al. (2003). "Inhibition of GM-CSF receptor function by stable RNA interference in a NOD/SCID mouse hematopoietic stem cell transplantation model." Oligonucleotides **13**(5): 353-63.

RNA interference (RNAi) describes a highly conserved mechanism of sequence-specific posttranscriptional gene silencing triggered by double-stranded RNA (dsRNA). Whereas RNAi is applied to study gene function in different organisms and in variant cell types, little is known about RNAi in human hematopoietic stem and progenitor cells and their myeloid progeny. To address this issue, short hairpin RNAs (shRNA) were designed to target the common beta-chain of the human receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5 (betaGMR). These receptors regulate proliferation, survival, differentiation, and functional activity of hematopoietic cells. In addition to markedly inhibiting mRNA and protein expression, anti-beta-GMR shRNAs were also found to inhibit receptor function in a cell culture model. Furthermore, lentiviral gene transfer of shRNA expression cassettes into primary normal CD34+ cells selectively inhibited colony formation of transduced progenitors when stimulated with GM-CSF/IL-3 but not when stimulated with cytokines that do not signal via beta-GMR. Finally, anti-beta-GMR shRNAs had no detectable effect on engraftment or lineage composition of lentivirally transduced human CD34+ cells transplanted into NOD/SCID mice. However, the growth defect of transduced colony-forming cells under stimulation with GM-CSF/IL-3 remains unchanged in bone marrow cells harvested from individual NOD/SCID mice 6 weeks after transplantation. These data indicate that lentiviral gene transfer of shRNA expression cassettes may be used to induce long-term RNAi in human hematopoietic stem and progenitor

cells for functional genetics and potential therapeutic intervention.

Schlaeger, T. M., H. K. Mikkola, et al. (2005). "Tie2Cre-mediated gene ablation defines the stem-cell leukemia gene (SCL/tal1)-dependent window during hematopoietic stem-cell development." Blood **105**(10): 3871-4.

The stem-cell leukemia gene (SCL/tal1) is essential for the formation of all blood lineages. SCL is first expressed in mesodermal cells that give rise to embryonic blood cells, and continues to be expressed in fetal and adult hematopoietic stem cells (HSCs). However, SCL is not required for the maintenance of established long-term repopulating (LTR) HSCs in the adult. The time point at which HSC development becomes SCL independent has not been defined. Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2 (Tie2) expression appears in hemogenic and vasculogenic sites shortly after SCL. We therefore used the Tie2Cre mouse to inactivate SCL early during embryonic and fetal hematopoiesis. Tie2Cre completely inactivated SCL in yolk sac, the aortagonad-mesonephros (AGM) region, and fetal liver hematopoietic cells and circulating blood cells. However, the fetal liver was colonized by functional LTR-HSCs. Yet SCL remained crucial for proper differentiation of both primitive and definitive red cells and megakaryocytes. These results indicate that the SCL-dependent phase of HSC development ends before Tie2Cre-mediated gene ablation becomes effective.

Shimamoto, T., K. Ohyashiki, et al. (1995). "The expression pattern of erythrocyte/megakaryocyte-related transcription factors GATA-1 and the stem cell leukemia gene correlates with hematopoietic differentiation and is associated with outcome of acute myeloid leukemia." Blood **86**(8): 3173-80.

To understand the clinical implications of transcription factors and their biologic roles during cellular differentiation in the hematopoietic system, we examined the expression of GATA-1, GATA-2, and stem cell leukemia (SCL) gene in human leukemia cell lines and various leukemia patients using the reverse transcriptase-polymerase chain reaction. Cell lines exhibiting megakaryocytic or erythrocytic phenotypes had GATA-1, GATA-2, and SCL gene transcripts, while monocytic cell lines had no detectable GATA-1, GATA-2, or SCL gene mRNA. In some myeloid cell lines, GATA-1 expression, but not SCL gene expression, was detected; GATA-1 expression in HL-60 cells was downregulated during the process of monocytic differentiation. We next examined GATA-1, GATA-2, and SCL gene expression in 110 leukemia samples obtained from 76 patients with acute myeloid leukemia (AML), 19 with acute lymphoblastic leukemia (ALL), and 15 with chronic myeloid leukemia in blast crisis (CML-BC). SCL gene expression was usually accompanied by GATA-1 expression and was preferentially detected in patients with leukemia exhibiting megakaryocytic or erythrocytic phenotypes, while patients with monocytic leukemia were clustered in the group with no detectable GATA-1 expression. None of the patients with ALL or CML-lymphoid-BC expressed SCL. De novo AML patients with SCL gene expression had a lower complete remission (CR) rate and had a significantly poorer prognosis. Among the patients with AML not expressing SCL, a high percentage of patients with CD7+ AML and CD19+ AML had detectable GATA-1, while patients with GATA-1-negative AML had the best CR rate (87.5%). Our results suggest that the expression pattern of transcription factors reflects the lineage potential of leukemia cells, and GATA-1 and SCL gene expression may have prognostic value for the outcome of patients with AML.

Shojaei, F., J. Trowbridge, et al. (2005). "Hierarchical and ontogenic positions serve to define the molecular basis of human hematopoietic stem cell behavior." Dev Cell **8**(5): 651-63.

The molecular basis governing functional behavior of human hematopoietic stem cells (HSCs) is largely unknown. Here, using in vitro and in vivo assays, we isolate and define progenitors versus repopulating HSCs from multiple stages of human development for global gene expression profiling. Accounting for both the hierarchical relationship between repopulating cells and their progenitors, and the enhanced HSC function unique to early stages of ontogeny, the human homologs of Hairy Enhancer of Split-1 (HES-1) and Hepatocyte Leukemia Factor (HLF) were identified as candidate regulators of HSCs. Transgenic human hematopoietic cells expressing HES-1 or HLF demonstrated enhanced in vivo reconstitution ability that correlated to increased cycling frequency and inhibition of apoptosis, respectively. Our report identifies regulatory factors involved in HSC function that elicit their effect through independent systems, suggesting that a unique orchestration of pathways fundamental to all human cells is capable of controlling stem cell behavior.

Sugimoto, K., M. Murata, et al. (2008). "Decreased risk of acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation in patients with the 5,10-methylenetetrahydrofolate reductase 677TT genotype." Int J Hematol **87**(5): 451-8.

Polymorphism in 5,10-methylenetetrahydrofolate reductase (MTHFR), a central enzyme in folate metabolism, has been shown to affect the sensitivity of patients to folate-based drugs such as methotrexate. In this study, we investigated whether a common single nucleotide polymorphism at position 677 in the donor or recipient's MTHFR gene affects the risk for acute graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-identical sibling donors when the recipient receives prophylactic treatment with methotrexate for GVHD. MTHFR genotypes were determined in 159 recipients with a hematological disease and their donors using polymerase chain reaction-restriction fragment length polymorphism analysis of genomic DNA. The 677TT genotype, which encodes an enzyme with approximately 30% of the activity of the wild-type (677CC), was observed in 13% of patients and in 8% of normal donors. Multivariate analyses demonstrated a significant association between 677TT genotype in patients and a lower incidence of grade I-IV acute GVHD (relative risk, 0.35; 95% confidence interval, 0.13-0.95; P = 0.040). There was no association between the incidence of acute GVHD and the donor MTHFR genotypes. These results suggest that greater immunosuppression by methotrexate due to low MTHFR enzyme activity decreases the risk of acute GVHD in recipients of allogeneic HSCT.

Sumida, T., T. Maeda, et al. (1990). "Expression of IgH promoter/enhancer Ly-1 transgene in hematopoietic chimeric mice generated by embryonic stem cell line." Int Arch Allergy Appl Immunol **93**(2-3): 155-64.

We have produced hematopoietic chimeric mice from an embryonic stem (ES) cell line carring Ly-1 cDNA under the control of IgH promoter and enhancer. Various amounts of serum IgM (5-86% of total IgM) in chimeric mice were of ES origin and 30-60% of IgM-positive B cells from the chimeric mice analyzed were reconstituted from ES cells. Using these chimeric mice, the expression of the Ly-1 transgene on lymphoid tissues was examined by polymerase chain reaction assay with primers specific for the transgene, and by cell sorter analysis. Transcription of the Ly-1 transgene was detected in spleen cells, thymocytes and lymph node cells; however, the expression of the Ly-1 molecule was observed only on lipopolysaccharide (LPS)-stimulated splenic IgM-positive B cells but not on resting splenic B cells. There was no significantly increased expression of Ly-1 on splenic T cells and thymocytes. Thus, our findings demonstrate that conventional splenic B cells could express the Ly-1 transgene on their surface in vivo after LPS stimulation. Also discussed is the ES-derived chimeric hematopoietic system.

Sun, Y., D. Zhao, et al. (2007). "Human leukocyte antigens A and B Loci genotyping by reference strand-mediated conformation analysis in hematopoietic stem cell transplantation donor selection." Int J Hematol **86**(1): 77-83.

Research has been carried out to evaluate the reference strand-mediated conformation analysis (RSCA) typing method on human leukocyte antigen (HLA)-A and -B loci matching in donor selection for hematopoietic stem cell transplantation (HSCT). Twenty standard DNA samples from the RSCA program of the 13th International Histocompatibiliy Working Group, and 124 DNA samples extracted from peripheral blood cells of 39 patients and 85 related potential donors were typed both by RSCA and polymerase chain reaction sequence-specific primer (PCR-SSP) for their HLA-A and -B genes. The ambiguous results were further confirmed by sequence-based typing (SBT). HLA-A and -B genotypes assigned by RSCA correlated well with PCR-SSP and the repetitive rate of RSCA was 100%. In our study, 33/84 (39%) and 67/90 (72.8%) RSCA genotyping results of HLA-A and -B loci could be typed to allelic level, respectively. RSCA can detect mismatches that are not routinely identified by the PCR-SSP method and can detect the chimerism status after patients have gone through HLA-mismatched HSCT. Eight ambiguous samples were confirmed by SBT and the results indicated that RSCA was more accurate than low-resolution PCR-SSP and its database should be improved. RSCA is reproducible, has a high resolution, is able to detect chimerism follow-up after HLA-mismatched HSCT, and is a useful approach for donor selection with some insufficiencies to be improved.

Taghon, T., K. Thys, et al. (2003). "Homeobox gene expression profile in human hematopoietic multipotent stem cells and T-cell progenitors: implications for human T-cell development." Leukemia **17**(6): 1157-63.

Class I homeobox (HOX) genes comprise a large family of transcription factors that have been implicated in normal and malignant hematopoiesis. However, data on their expression or function during T-cell development is limited. Using degenerated RT-PCR and Affymetrix microarray analysis, we analyzed the expression pattern of this gene family in human multipotent stem cells from fetal liver (FL) and adult bone marrow (ABM), and in T-cell progenitors from child thymus. We show that FL and ABM stem cells are similar in terms of HOX gene expression, but

significant differences were observed between these two cell types and child thymocytes. As the most immature thymocytes are derived from immigrated FL and ABM stem cells, this indicates a drastic change in HOX gene expression upon entry into the thymus. Further analysis of HOX-A7, HOX-A9, HOX-A10, and HOX-A11 expression with specific RT-PCR in all thymocyte differentiation stages showed a sequential loss of 3' region HOX-A cluster genes during intrathymic T-cell development and an unexpected expression of HOX-A11, previously not recognized to play a role in hematopoiesis. Also HOX-B3 and HOX-C4 were expressed throughout thymocyte development. Overall, these data provide novel evidence for an important role of certain HOX genes in human T-cell development.

Tanaka, Y., T. Era, et al. (2007). "Forced expression of Nanog in hematopoietic stem cells results in a gammadeltaT-cell disorder." Blood **110**(1): 107-15.

Nanog is a key molecule involved in the maintenance of the self-renewal of undifferentiated embryonic stem (ES) cells. In this work we investigate whether Nanog can enhance self-renewal in hematopoietic stem cells. Contrary to our expectation, no positive effect of Nanog transduction was detected in bone marrow reconstitution assays. However, recipients of Nanog-transduced (Nanog) hematopoietic stem cells (HSCs) invariantly develop a unique disorder typified by an atrophic thymus occupied by Nanog-expressing gammadeltaT-cell receptor-positive (TCR(+)) cells (Nanog T cells). All thymi are eventually occupied by Nanog T cells with CD25(+)CD44(+) surface phenotype that home selectively to the thymus on transfer and suppress normal thymocyte development, which is partly ascribed to destruction of the microenvironment in the thymus cortex. Moreover, this initial disorder invariantly develops to a lymphoproliferative disorder, in which Nanog T cells undergo unlimited proliferation in the peripheral lymphoid tissues and eventually kill the host. This invariable end result suggests that Nanog is a candidate oncogene for gammadeltaT-cell malignancy.

Tanaka, Y., Y. Kanda, et al. (2002). "Monitoring cytomegalovirus infection by antigenemia assay and two distinct plasma real-time PCR methods after hematopoietic stem cell transplantation." Bone Marrow Transplant **30**(5): 315-9.

We compared a CMV virus load determined by real-time PCR with an antigenemia value to analyze the correlation between these two methods. We also compared the values for virus load determined by the two distinct real-time PCR methods, which amplify the US17 region and immediate-early (IE) gene of CMV, respectively, to evaluate the reliability of these methods. Two hundred and sixty-five samples were obtained weekly from 29 patients, who had engraftment after unrelated bone marrow transplantation or HLA-mismatched related blood stem cell transplantation. CMV infection was detected in 115 samples from 22 patients by US17-PCR and 69 samples from 20 patients by the antigenemia assay. Fifty-eight samples were positive for both assays, but 57 and 11 samples were positive only for US17-PCR and antigenemia, respectively. A good correlation of the results of US17-PCR and antigenemia was demonstrated ($r = 0.61$). All antigenemia-positive samples and randomly selected antigenemia-negative samples were subjected to IE-PCR. The results of IE-PCR showed a good correlation with those of antigenemia ($r = 0.64$). Furthermore, the best correlation was observed between US17-PCR and IE-PCR ($r = 0.83$). In conclusion, both real-time PCR methods showed a good correlation with the antigenemia assay, and could be used to monitor CMV infection after hematopoietic stem cell transplantation.

Tashiro, K., T. Kinashi, et al. (1991). "Germline transcripts of the immunoglobulin heavy-chain and T cell receptor genes in a murine hematopoietic stem cell line LyD9 and its derivative cell lines." Immunol Lett **28**(2): 147-54.

We compared germline transcript levels of immunoglobulin heavy chain and T cell receptor (TcR) genes in a murine hematopoietic stem cell line, LyD9, and its derivative cell lines. LyD9 cells can be induced to differentiate into at least three lineages, namely, B lymphocyte, macrophage, and granulocyte lineages. Although C mu transcripts were found in stem cells to B lymphocytes, other myeloid-committed cells also expressed significant amounts of C mu transcripts. Germline TcR transcripts did not show good correlation with differentiation potential and stages of hematopoietic cells. During this search we identified a novel germline transcript containing the JH-C microliter sequence in LyD9 and some of its derivative cells. Expression of mRNAs for immunoglobulin- and TcR-associated molecules (lambda 5, MB1 and CD3 delta) was widespread except for lambda 5 mRNA. Among three mRNAs encoding putative recombinase proteins, RAG-1 and RAG-2 mRNAs were not expressed in any cell lines tested, while RBP-2 mRNA was expressed ubiquitously.

Thomsen, S., B. Vogt, et al. (1998). "Lack of functional Pit-1 and Pit-2 expression on hematopoietic stem cell lines." Acta Haematol **99**(3): 148-55.

Hematopoietic stem cells (HSC) are an important target for retroviral gene transfer. However, transduction efficiency in these HSC is extremely low compared to fibroblasts or more mature hematopoietic cells. This infection block was analyzed in the HSC line FDC-Pmix. The infection frequency with the amphotropic murine leukemia virus (MLV-A) is more than 100-fold lower in FDC-Pmix cells as compared to fibroblasts. Pseudotyping with the env of the 10A1 strain (MLV-10A1), which uses both the amphotropic receptor (Pit-2) and the receptor for gibbon ape leukemia virus (Pit-1), did not improve the infection efficiency. Vectors pseudotyped with VSV G protein were found to overcome the infection block in FDC-Pmix, confirming that the block is at the level of virus binding and possibly penetration. Accordingly, we could not detect virus binding of MLV-A or MLV-10A1 to FDC-Pmix cell lines. Northern blot analysis was performed to detect whether the defect is at the level of transcription. Surprisingly, similar levels of Pit-2 receptor transcripts were detected in all cell types. The overexpression of rat Pit-2 DNA in CHO but not in FDC-Pmix cells improved amphotropic infection frequency after introducing rat Pit-2 DNA into the cells. Taken together these results show that the inefficient infection of FDC-Pmix is due to a lack of functional receptors. Either the receptor protein is incorrectly processed in these cells or a cofactor is missing in FDC-Pmix cells that is necessary for efficient binding and/or penetration.

Trobridge, G., B. C. Beard, et al. (2005). "Hematopoietic stem cell transduction and amplification in large animal models." Hum Gene Ther **16**(12): 1355-66.

Progress in retroviral gene transfer to large animal hematopoietic stem cells (HSCs) has led to efficient, reproducible long-term marking in both canine and nonhuman primate models. Successes for HSC gene therapy have occurred in the severe combined immunodeficiency setting, in which transduced cells have a selective advantage. However, for most diseases, the therapeutic transgene does not confer a sufficient survival advantage, and increasing the percentage of gene-marked cells in vivo will be necessary to observe a therapeutic effect. In vivo amplification should expand the potential of HSC gene therapy, and progress in this area has benefited greatly from the use of large animal models where efficacy and toxicity have often not correlated with results in murine models. To date, the best results have been observed with O(6)-methylguanine-DNA methyltransferase (MGMT) selection, with which increases in gene-marked repopulating cells have been maintained long-term, likely because of the toxicity of 1,3-bis-(2-chloroethyl)-1-nitrosourea and temozolomide to quiescent HSCs. Using MGMT selection, long-term marking levels exceeding 50% can now be routinely attained with minimal toxicity. There is cause to be optimistic that HSC gene therapy with in vivo amplification will soon allow the treatment of several genetic and infectious diseases.

Uchida, N., D. He, et al. (1997). "The unexpected G0/G1 cell cycle status of mobilized hematopoietic stem cells from peripheral blood." Blood **89**(2): 465-72.

Treatment with a combination of cytokines and chemotherapy can effectively stimulate the release of hematopoietic stem cells (HSC) into the peripheral blood (PB), which can then be harvested for transplantation. The cell cycle status of the harvested HSC from mobilized PB (MPB) is of interest because of the impact that cell cycling may have on optimizing the conditions for ex vivo expansion, retrovirus-mediated gene transfer, and the engraftment of transplanted tissues. Therefore, we characterized the cell cycling status of mobilized HSC from mice and humans. The murine HSC, which express the phenotype c-kit+ Thy-1.1lo Lin-/lo Sca-1+, were purified from PB, bone marrow (BM), and spleen after the mice were treated with the mobilizing regimen of granulocyte colony-stimulating factor (G-CSF) or a combination of cyclophosphamide (CTX) and G-CSF. Human HSC (CD34+ Thy-1+ Lin-) and progenitor cells (CD34+ Thy-1-Lin-) were isolated from the BM of untreated healthy volunteers and from MPB of healthy volunteers and patients treated with G-CSF or a combination of CTX and GM-CSF. Cell cycle status was determined by quantitating the amount of DNA in the purified cells after staining with the dye Hoechst 33342. Fluorescence-activated cell sorting analysis of the progenitor cells from the murine and human samples showed an unexpected finding, ie, virtually none of the cells from the MPB was cycling. The G0/G1 status of HSC from MPB was surprising, because a significant proportion of HSC from BM are actively proliferating and, after mobilization, the HSC in the spleen and BM were also actively cycling.

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 de Rijn, M., S. Heimfeld, et al. (1989). "Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family." Proc Natl Acad Sci U S A **86**(12): 4634-8.

Recently, hematopoietic stem cells were purified to homogeneity from mouse bone marrow. The protein structure of Sca-1, the cell surface antigen used in the isolation of hematopoietic stem cells, is described here. It is shown that the Sca-1 antigen is a member of the Ly-6 antigen family. The anti-Sca-1 antibody was used in immunohistochemistry experiments to define the structures in several tissues that had previously been shown to contain Ly-6 antigens. In thymus, spleen, and kidney, specific staining of parenchymal cells can be demonstrated, whereas only vasculature reacts with anti-Sca-1 in brain, heart, and liver and possibly in lung.

Van Zant, G., P. W. Eldridge, et al. (1983). "Genetic control of hematopoietic kinetics revealed by analyses of allophenic mice and stem cell suicide." Cell **35**(3 Pt 2): 639-45.

The pluripotential hematopoietic stem cell is influenced by at least one gene that differs between DBA/2 and C3H/He, and C57BL/6 inbred mouse strains. This gene(s) manifests itself by its effect on susceptibility to killing of spleen colony forming cells (CFU-S) caused by hydroxyurea (HU). In strains DBA/2 and C3H/He 20% of the CFU-S population is normally in S phase whereas practically none from strain C57BL/6 are synthesizing DNA. On the other hand, in C57BL/6 in equilibrium DBA/2 allophenic mice we observed that the proportion of DBA/2 erythrocytes was higher than the proportion of DBA/2 lymphocytes; the fraction of platelets and neutrophils with the DBA/2 genotype fell between the values for erythrocytes and lymphocytes. Control experiments using mice congenic at the Fv-2 locus confirm that in both situations we are examining effects of a gene(s) other than Fv-2. For the effect on the S phase fraction of CFU-S, we refer to the gene(s) as Stk (stem cell kinetics). We suggest that the observed skewing in composition among the various mature blood cell types in C57BL/6 in equilibrium DBA/2 allophenic mice is caused by allelic variants of the Stk gene. Such variation would favor the formation of DBA/2 erythrocytes, platelets, and neutrophils over those of the C57BL/6 genotype.

Vaughan, W. P., C. I. Civin, et al. (1988). "Acute leukemia expressing the normal human hematopoietic stem cell membrane glycoprotein CD34 (MY10)." Leukemia **2**(10): 661-6.

Thirty-one cases of acute leukemia with blast cells greater than or equal to 70% positive for the hematopoietic stem cell Ag, CD34 (MY10, HPCA-1), were identified from the University of Nebraska Medical Center and The Johns Hopkins Oncology Center over an 18-month period. Fourteen of the cases were classified as early B-lineage ALL, 3 cases were other ALL subtypes, and 14 of the cases were ANLL. Five of the 17 cases of ALL expressed one or more myeloid-associated surface Ags, 3 ANLL cases expressed CD10 (CALLA, J5), and T-lymphoid Ags were present in 12 of 31 cases (1 T-cell ALL, 3 of 16 B-lineage ALL cases, and 8 of 14 ANLL cases). Eleven of 12 CD34+ ALL cases studied had abnormal karyotypes; only 7 of 12 CD34+ ANLL cases studied had abnormal karyotypes, and 3 of these were CD10+ ANLL. Six cases were Ph1 positive, including the one mature B cell ALL, 4 early B-lineage ALL, and 1 CD10+ ANLL case. Good and poor prognosis subgroups of high frequency of expression of CD34 leukemias could be identified, generally, as would have been predicted by previously defined criteria. Thus, of the 10 Ph1-negative early B-lineage ALL patients, 9 achieved CR (90%). At the other extreme, the CR rate of CD10- ANLL was 4 of 11 (36%). The leukemias characterized by greater than or equal to 70% of cells positive for CD34 form a relatively undifferentiated subset of the leukemias which may show features associated with more than one lineage, and if CD10- and myeloid morphology, may respond poorly to therapy.

Venard, V., J. N. Dauendorffer, et al. (2001). "Infection due to acyclovir resistant herpes simplex

virus in patients undergoing allogeneic hematopoietic stem cell transplantation." Pathol Biol (Paris) **49**(7): 553-8.

Over an eight-month period from October 1997 to May 1998, four patients who had received bone marrow transplant (BMT) from unrelated donor presented with severe mucosal cutaneous infections involving acyclovir resistant herpes simplex virus 1 (HSV-1). The four isolates were acyclovir (ACV) resistant, three of which were also foscarnet resistant as determined by the dye uptake method. The sequencing of the thymidine kinase (TK) gene did not permit to establish a relation between mutations and resistance to ACV. Three patients were considered as clinically cured of their HSV infection by replacement of ACV or foscarnet with either valacyclovir (one case) or cidofovir (two cases) but eventually two of them died of graft vs host disease. One patient died of extensive HSV infection despite administration of cidofovir. This study emphasizes the importance of monitoring the herpes virus resistance to antiviral drugs in bone marrow transplant recipients and the usefulness of the evaluation of novel antiviral drug for treatment of infections due to strains of HSV resistant to ACV and foscarnet that occur in about 5% of immunocompromised patients.

Vescio, R. A., C. H. Hong, et al. (1994). "The hematopoietic stem cell antigen, CD34, is not expressed on the malignant cells in multiple myeloma." Blood **84**(10): 3283-90.

Autologous stem cell transplantation has become an important therapy in multiple myeloma (MM). To develop adequate autograft purging methods, it is necessary to determine whether antigens expressed on early hematopoietic progenitors exist on malignant cells. The Ig heavy chain produced by the MM cells shows evidence of prior somatic mutation without intraclonal diversity. As a result, this sequence can be used as a specific marker to detect all members of the malignant clone. The Ig heavy chain sequence expressed by the MM cells was obtained in five patients with advanced disease. Patient specific oligonucleotide primers were designed based on the complementarity determining regions (CDR) of each MM Ig sequence and used to amplify DNA by polymerase chain reaction for the detection of malignant cells. A highly purified collection of CD34+ cells was obtained after passage of the initial bone marrow cells through an immunoadsorption column and fluorescence-activated cell sorting. Despite an assay sensitivity of 1 tumor cell in 2,500 to 44,000 normal cells, none of the CD34+ samples showed product with the myeloma-specific CDR primers. Therefore, positive selection for cells bearing this antigen should yield a tumor-free autograft capable of providing hematopoietic recovery after myeloablative chemotherapy.

von Kalle, C., B. Fehse, et al. (2004). "Stem cell clonality and genotoxicity in hematopoietic cells: gene activation side effects should be avoidable." Semin Hematol **41**(4): 303-18.

Two serious adverse events involving activation of the LMO2 oncogene through retrovirus vector insertion in the otherwise extremely successful first gene therapy trial for X-linked severe combined immunodeficieny type 1 (SCID-X1) had initially caused widespread concern in the patient and research communities. Careful consideration 1 year after diagnosis of the second case still finds 12 of the treated patients clearly benefiting from gene therapy (freedom from treatment failure, 80%; survival 100%), a situation that should not portend the end of gene therapy for this disease, and is, in fact encouraging. While current approaches are justified to treat patients with otherwise life-threatening disorders, a broad consensus has developed that systematic basic research is required to further understand the pathophysiology of these serious adverse events and to provide new insights, enabling safer and more effective gene therapy strategies. With the continued success of SCID-X1 gene therapy in the majority of patients treated, it is of even greater importance to understand exactly which vector element or combination of elements predispose to toxicity. An in-depth study of the mechanisms behind the activation of the LMO2 and gammac genes will be highly instructive for the development of safer procedures and vectors. We summarize the central observations, ongoing experimental approaches, new concepts, and developments relevant to understanding, interpreting, and eventually overcoming the real and perceived obstacles posed by insertional mutagenesis due to gene transfer vectors.

Vu, T., G. Carrum, et al. (2007). "Human herpesvirus-6 encephalitis following allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **39**(11): 705-9.

Immunosuppressive monoclonal antibodies directed to immune system cells may reduce rejection and graft versus host disease (GvHD) after allogeneic stem cell transplantation (SCT), but can increase the risks of viral infection. Here, we report human herpes virus-6 (HHV-6) encephalitis despite antiviral prophylaxis in 5 of 43 (11.6%) patients receiving alemtuzumab supported conditioning. Encephalitis occurred at 41-103 days (median 60 days) presenting with confusion in all patients, combined with amnesia (n=3) or seizures (n=2). MRI revealed non-specific white matter changes in two and a non-enhancing

medial temporal lobe lesion in three patients. Cerebrospinal fluid (CSF) PCR amplification for HHV-6 was positive in all five patients, (600-2 25 000 (median 4700) copies/ml CSF), while analysis of peripheral blood revealed 100-22 500 (median 1200) viral copies/ml plasma. CSF protein was elevated in four patients, with minimal CSF pleocytosis. Intravenous foscarnet produced neurological improvement at 8-13 (median 11) days and negative plasma PCR at 30-66 (median 50) days. Four patients had complete neurological recovery, but one patient with persistent viral DNA in the CSF succumbed to progressive encephalopathy. Given this high incidence of HHV-6 and the possibility of successful outcome with prompt treatment, a high index of suspicion of this disorder is required in recipients of monoclonal antibody supported allografts.

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., P. Horn, et al. (2008). "Aging of hematopoietic stem cells is regulated by the stem cell niche." Exp Gerontol **43**(11): 974-80.

Adult stem cells provide the basis for regeneration of aging tissue. Their dual ability for self-renewal and multilineage differentiation is controlled by direct interaction with a specific microenvironment -- the so called "stem cell niche". Hematopoietic stem cells (HSC) reside in the bone marrow. It is still under debate if HSC can rejuvenate infinitively or if they do not possess "true" self-renewal and undergo replicative senescence such as any other somatic cell. Furthermore, the question arises to what extent age-related changes in HSC are due to intrinsic factors or regulated by external stimuli. There is growing evidence, that the stem cell niche is most important for the regulation of cellular aging in adult stem cells. It is the stem cell niche that (i) maintains HSC in a quiescent state that reduces DNA damage as well as replicative senescence, (ii) protects from radicals and toxic compounds, (iii) regulates cell intrinsic signal cascades and (iv) modulates gene expression and epigenetic modifications in HSC. Thus, the interplay with the stem cell niche controls HSC function including the aging process of the hematopoiesis.

Wang, G., C. Weiss, et al. (1996). "Retrovirus-mediated transfer of the human O6-methylguanine-DNA methyltransferase gene into a murine hematopoietic stem cell line and resistance to the toxic effects of certain alkylating agents." Biochem Pharmacol **51**(9): 1221-8.

O6-Methylguanine-DNA methyltransferase (MGMT) is an important DNA repair protein that plays a key role in cancer chemotherapy by alkylating agents such as carmustine (BCNU) and Dacarbazine (DTIC). Therapy by BCNU and DTIC is reduced by dose-limiting hematological toxicity as a result of low MGMT repair activity in bone marrow cells. In this study, we have constructed a Moloney murine leukemia virus retroviral vector containing the human mgmt gene. High-titer retrovirus producer cells lines have been generated. Retroviral-mediated transfer of the human mgmt gene into murine multi-potent hematopoietic stem cells, FDCP-1, resulted in the expression of a high level of MGMT activity. In comparison with the control cells that were transduced with the parent vector, the MGMT-expressing clones were considerably more resistant to the cytotoxicity of the methylating agents, such as N-methyl-N'-nitro-N-nitrosoguanidine, N-nitroso-N-methyl-urea, and temozolomide, as well as the chloroethylating agents 1-(2-chloroethyl)-1-nitrosourea and BCNU. The protection provided by MGMT could be eliminated by the MGMT inactivator O6-benzylguanine. Thus, the principal lethal lesions produced by these alkylating agents in the murine hematopoietic stem cells and the MGMT deficiency in these cells can be complemented by retroviral-mediated gene transduction.

Wang, L. J., P. Chou, et al. (2002). "Evaluation of mixed hematopoietic chimerism in pediatric patients with leukemia after allogeneic stem cell transplantation by quantitative PCR analysis of variable number of tandem repeat and testis determination gene." Bone Marrow Transplant **29**(1): 51-6.

In order to monitor the clinical outcome of pediatric patients with leukemia following allogeneic hematopoietic transplantation, tests of variable number of tandem repeat (VNTR) and sex determination by quantitative polymerase chain reaction (PCR) were performed. PCR results combined with the blast counts from 21 leukemia patients were analyzed. Complete chimerism (100% donor cells) was found in 15 cases with remission, and incomplete chimerism in six cases with relapse. In the majority of cases, complete chimerism was always associated with no detectable blasts, while blasts were often detected in association with incomplete chimerism. There is significant correlation ($P<0.0001$) between the percentage of donor DNA and blast percentage in these patients. Early detection of incomplete chimerism may therefore predict a poor prognosis. In one patient (case 15), a differing percentage of donor DNA was observed between samples of bone marrow and peripheral blood collected on the same day. This may be due to the fact that allogeneic stem cells proliferate at different rates depending on their environment (bone marrow or peripheral blood). In addition, 100% donor cells found in the peripheral blood may not reflect the number of cells in the bone marrow. In case 17, asynchronous engraftment of donor cells was present between the white and red blood cell lineages, indicating that the degree of chimerism may not be the same in all cell lineages. At the time of this report, the significance of this observation is unknown and needs further investigation.

Wang, Y., F. Yates, et al. (2005). "Embryonic stem cell-derived hematopoietic stem cells." Proc Natl Acad Sci U S A **102**(52): 19081-6.

Despite two decades of studies documenting the in vitro blood-forming potential of murine embryonic stem cells (ESCs), achieving stable long-term blood engraftment of ESC-derived hematopoietic stem cells in irradiated mice has proven difficult. We have exploited the Cdx-Hox pathway, a genetic program important for blood development, to enhance the differentiation of ESCs along the hematopoietic lineage. Using an embryonic stem cell line engineered with tetracycline-inducible Cdx4, we demonstrate that ectopic Cdx4 expression promotes hematopoietic mesoderm specification, increases hematopoietic progenitor formation, and, together with HoxB4, enhances multilineage hematopoietic engraftment of lethally irradiated adult mice. Clonal analysis of retroviral integration sites confirms a common stem cell origin of lymphoid and myeloid populations in engrafted primary and secondary mice. These data document the cardinal stem cell features of self-renewal and multilineage differentiation of ESC-derived hematopoietic stem cells.

Wang, Z., G. Li, et al. (2009). "Conditional deletion of STAT5 in adult mouse hematopoietic stem cells causes loss of quiescence and permits efficient nonablative stem cell replacement." Blood **113**(20): 4856-65.

Currently, there is a major need in hematopoietic stem cell (HSC) transplantation to develop reduced-intensity regimens that do not cause DNA damage and associated toxicities and that allow a wider range of patients to receive therapy. Cytokine receptor signals through c-Kit and c-Mpl can modulate HSC quiescence and engraftment, but the intracellular signals and transcription factors that mediate these effects during transplantation have not been defined. Here we show that loss of one allele of signal transducer and activator of transcription 5 (STAT5) in nonablated adult mutant mice permitted engraftment with wild-type HSC. Conditional deletion of STAT5 using Mx1-Cre caused maximal reduction in STAT5 mRNA (> 97%) and rapidly decreased quiescence-associated c-Mpl downstream targets (Tie-2, p57), increased HSC cycling, and gradually reduced survival and depleted the long-term HSC pool. Host deletion of STAT5 was persistent and permitted efficient donor long-term HSC engraftment in primary and secondary hosts in the absence of ablative conditioning. Overall, these studies establish proof of principle for targeting of STAT5 as novel transplantation conditioning and demonstrate, for the first time, that STAT5, a mitogenic factor in most cell types, including hematopoietic progenitors, is a key transcriptional regulator that maintains quiescence of HSC during steady-state hematopoiesis.

Williams, D. A. and M. K. Majumdar (1994). "Analysis of steel factor (stem cell factor) isoforms in the hematopoietic microenvironment." Stem Cells **12 Suppl 1**: 67-74; discussion 75-7.

Hematopoietic cell proliferation and differentiation is dependent in part on the interaction of hematopoietic stem and progenitor cells with cells making up the hematopoietic microenvironment (HM). Direct cell-cell interactions appear to be important in the hematopoietic microenvironment. One mechanism to accomplish such interactions is the expression of membrane-associated growth factors.

Stem cell factor (SCF), the product of the steel gene in mice (also termed mast cell growth factor, c-kit ligand, or Steel factor), is a hematopoietic growth factor demonstrating substantial synergistic activity with a number of other cytokines on primitive hematopoietic stem and progenitor cells. Cloned SCF cDNA encode both a membrane-associated and a secreted growth factor. The physiologic relevance of these isoforms is unknown at present. In order to better understand the physiologic role of these SCF isoforms in normal hematopoiesis, we have established multiple stromal cell lines expressing each isoform. We have used these cell lines to study protein sequences that are required for appropriate post-translational processing of SCF protein in HM-derived stromal cell lines. These lines have also been used to study the interaction of membrane-associated and secreted SCF with murine and human hematopoietic cells. In addition, we have generated transgenic mice expressing each isoform of murine and human SCF. These transgenic mice will be used to study the function of each isoform in hematopoiesis in vivo.

Yamane, A., M. Karasawa, et al. (2001). "X chromosome methylation-based chimerism assay for sex-mismatched hematopoietic stem cell transplantation." Bone Marrow Transplant **28**(10): 969-73.

Analysis of hematopoietic chimerism is important for monitoring engraftment, graft failure, and disease recurrence. Although several techniques are now available, their sensitivity is unsatisfactory. In sex-mismatched stem cell transplantation (SCT) with a female donor, Y chromosome-specific sequences have proven the most sensitive marker. However, in the case of a male donor, no such reliable marker has been available to date. In this study, we report a novel method we developed to detect microchimerism in female recipients who receive SCT from male donors. The X-linked human androgen receptor gene (HUMARA) contains a highly polymorphic CAG trinucleotide repeat. Near this polymorphic site are methyl-sensitive HpaII restriction enzyme sites. After HpaII digestion, unmethylated male HUMARA sequences are completely digested, while methylated female ones remain intact among the male origin cells. This allows a highly efficient detection of a small number of female cells. Combined with the nested PCR technique, the X chromosome methylation-based chimerism assay could attain a 10(-4) level of sensitivity, which is 1000-fold higher than that of conventional assays. The applicability of the method was confirmed in two transplant cases. This highly sensitive method can also be applied to detect minimal residual disease or microchimerism in conditions other than hematopoietic SCT.

Yoshida, T., I. Hazan, et al. (2008). "The role of the chromatin remodeler Mi-2beta in hematopoietic stem cell self-renewal and multilineage differentiation." Genes Dev **22**(9): 1174-89.

The ability of somatic stem cells to self-renew and differentiate into downstream lineages is dependent on specialized chromatin environments that keep stem cell-specific genes active and key differentiation factors repressed but poised for activation. The epigenetic factors that provide this type of regulation remain ill-defined. Here we provide the first evidence that the SNF2-like ATPase Mi-2beta of the Nucleosome Remodeling Deacetylase (NuRD) complex is required for maintenance of and multilineage differentiation in the early hematopoietic hierarchy. Shortly after conditional inactivation of Mi-2beta, there is an increase in cycling and a decrease in quiescence in an HSC (hematopoietic stem cell)-enriched bone marrow population. These cycling mutant cells readily differentiate into the erythroid lineage but not into the myeloid and lymphoid lineages. Together, these effects result in an initial expansion of mutant HSC and erythroid progenitors that are later depleted as more differentiated proerythroblasts accumulate at hematopoietic sites exhibiting features of erythroid leukemia. Examination of gene expression in the mutant HSC reveals changes in the expression of genes associated with self-renewal and lineage priming and a pivotal role of Mi-2beta in their regulation. Thus, Mi-2beta provides the hematopoietic system with immune cell capabilities as well as with an extensive regenerative capacity.

Yu, J. M., R. V. Emmons, et al. (1999). "Expression of interferon-gamma by stromal cells inhibits murine long-term repopulating hematopoietic stem cell activity." Exp Hematol **27**(5): 895-903.

Several lines of evidence suggest that overexpression of interferon gamma (IFN-gamma) in the marrow microenvironment may play a role in the pathogenesis of marrow suppression in aplastic anemia. We previously showed that overexpression of IFN-gamma by marrow stromal cells inhibits human long-term culture initiating cell activity assayed in vitro to a much greater degree than the addition of soluble IFN-gamma. The effect of IFN-gamma on true repopulating stem cells assayed in vivo has not been studied previously. We compared the effect of co-culture of murine marrow cells in the presence of stromal cells transduced with a retroviral vector expressing murine IFN-gamma vs stromal cells transduced with a control neo vector. Using a murine congenic competitive repopulation assay, there was significantly less long-term repopulating stem cell

activity remaining after culture on mIFN-gamma-expressing stroma as compared to control stroma. We also investigated the effect of directly transducing murine bone marrow cells with the mIFN-gamma or control vector. Marrow cells transduced with either vector were transplanted into W/Wv recipient mice. The percentage of vector-containing cells in the mIFN-gamma mice was significantly lower than in the control mice, suggesting that mIFN-gamma-transduced primitive cells may not have survived culture, or that mIFN-gamma directly decreases gene transfer into repopulating cells. Despite no significant differences in white or red blood cells in the mice transplanted with the mIFN-gamma-transduced cells, the number of bone marrow colony-forming unit-C 16 weeks after transplantation was significantly lower in the IFN-gamma group. These data indicate that ectopic or overexpression of mIFN-gamma, especially by marrow microenvironmental elements, may have a marked effect on primitive hematopoiesis as assayed in vivo.

Yuasa, H., Y. Oike, et al. (2005). "Oncogenic transcription factor Evi1 regulates hematopoietic stem cell proliferation through GATA-2 expression." Embo J **24**(11): 1976-87.

The ecotropic viral integration site-1 (Evi1) is an oncogenic transcription factor in murine and human myeloid leukemia. We herein show that Evi1 is predominantly expressed in hematopoietic stem cells (HSCs) in embryos and adult bone marrows, suggesting a physiological role of Evi1 in HSCs. We therefore investigate the role and authentic target genes of Evi1 in hematopoiesis using Evi1-/- mice, which die at embryonic day 10.5. HSCs in Evi1-/- embryos are markedly decreased in numbers in vivo with defective self-renewing proliferation and repopulating capacity. Notably, expression rate of GATA-2 mRNA, which is essential for proliferation of definitive HSCs, is profoundly reduced in HSCs of Evi1-/- embryos. Restoration of the Evi1 or GATA-2 expression in Evi1-/- HSCs could prevent the failure of in vitro maintenance and proliferation of HSC through upregulation of GATA-2 expression. An analysis of the GATA-2 promoter region revealed that Evi1 directly binds to GATA-2 promoter as an enhancer. Our results reveal that GATA-2 is presumably one of critical targets for Evi1 and that transcription factors regulate the HSC pool hierarchically.

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.

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