Stem Cell Research Facts

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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 stem cell research facts.
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Introduction

Mouse embryonic stem cells were first discovered in 1981. Since then, they have been an invaluable tool of modern biology and medical research. They have provided models to study diseases, they have brought about the discovery of many genes associated with diseases and they have been used to cure certain human disorders in animal models. After 20 years of exciting research, the mouse embryonic stem cell has helped to establish the value of these cells in *regenerative medicine*, which is the creation of cells or organs to replace tissues lost to disease or injury. The discovery of *human* embryonic stem cells in 1998 triggered important ethical controversy and debate, yet scientists are convinced that they hold enormous potential for clinical applications. Many diseases plaguing the modern world may be improved, or even cured, with therapies using human stem cells. Whether human embryonic stem cells or adult stem cells are used in future therapies will depend on the type of disease or injury. There are specific advantages for each stem cell type. Thanks to the ease of growing them in the laboratory, human embryonic stem cells may one day become the source of artificial organs. Or scientists might one day be able to mobilize one's own adult stem cells to repair tissue damage caused by trauma, disease, and even aging. To reach such goals, both human embryonic and adult stem cells will have to be extensively studied. The complementary information acquired from studying both stem cell types is the key to unlocking their full potential.

1. What Are Stem Cells?

A stem cell is the base building block of an entire family of cells that make up any organ. A common trait of stem cells is that they can maintain themselves indefinitely in a stem cell state, which is referred to as "self-renewal," while also producing — through division — more specialized cells. For example, the blood stem cell can produce all the cells in the blood, including the red blood cells, white blood cells and platelets.

2. Who Needs Stem Cells?

Harnessing the power of human stem cells will revolutionize our health, our lives, and our society. In principle, any affliction involving the loss of cells, including many diseases, injuries and even aging, could be treated with stem cells. In the United States alone, more than 100 million people could benefit from therapies derived from stem cell research.

3. Adult Stem Cells

Adult stem cells are more specialized stem cells living in the majority of tissues and organs in our bodies and generate the mature cell types within that tissue or organ. In tissues where adult stem cells have been found, they are extremely rare and very difficult to isolate. Once isolated, adult stem cells grow poorly in culture, and it is difficult to obtain enough of these cells for use in clinical trials. In addition, access to the tissues harboring these cells is problematic since most human tissue is not easily available. Two readily available sources of human adult stem cells are the bone marrow and the umbilical cord blood. In both these tissues are blood stem cells, as well as other rare types of stem cells, which can produce bone, muscle, blood vessels, heart cells and possibly more.

4. Adult Stem Cells in the Clinic

The majority of stem cell clinical trials now underway use blood stem cells from the bone marrow or umbilical cord blood to treat blood disorders or

diseases, such as leukemia, different types of anemia, systemic lupus, and certain other autoimmune diseases or deficiencies. A handful of clinical trials are evaluating the use of one's own bone marrow stem cells to repair heart tissue and to improve blood flow or to help to repair bone and cartilage. Other adult stem cells being explored for use in the clinic include stem cells in the eye and the skin. Adult stem cells are also thought to play a role in tissue transplants that have been performed for several years. For example, insulin-producing cells for type I diabetes, fetal neurons for Parkinson's disease, and skin for bladder reconstruction have been transplanted successfully. It is possible that in cases where long-term regeneration has been achieved, stem cells contained in these tissues have contributed to regeneration. The widespread use of adult stem cell-derived therapies and treatments is complicated by several factors. First, available human tissue is scarce, with only 6000 donors/year for more than 100 million Americans that could benefit from cellular therapy. Second, immune rejection caused by not using one's own cells or tissue is a problem. On the other hand, using one's own cells or tissue may become a problem for older patients, as evidence has been accumulating that adult stem cells age during the life of the body and lose their potential. Thus, stem cells isolated from a young adult may have a greater potential to produce numerous daughter cells than the cells of an older person.

5. Embryonic Stem Cells

Human embryonic stem cells are like a blank slate and can produce all the cells of the body. They are obtained from the ICM (inner cell mass) of the blastocyst.The blastocyst is a very early stage of human development, which forms about 5 days after fertilization of an egg. It is approximately 1/10 the size of the head of a pin, almost invisible to the eye, and it has not yet implanted into the uterus.

Once the blastocyst has implanted and a normal pregnancy can be detected, it is too late to derive human embryonic stem cells from the embryo. At the blastocyst stage, organ formation has not started and more specialized cells are not yet present, not even the beginning of the nervous system. To obtain human embryonic stem cells, blastocysts created in culture for *in vitro fertilization* (IVF) treatment by combining sperm and egg in a dish, are used. If they are not implanted into the uterus, the blastocysts are either discarded or frozen for later fertility cycles. They can also be donated to other patients or to research. If not donated, they will stay in the freezer as long as the storage fees are paid, otherwise they will be discarded. Because the cells obtained from the blastocyst have not yet specialized, they are considered highly valuable. They can generate cells that go on to form all the body's tissues and organs.

6. Why Are Embryonic Stem Cells So Valuable?

While grown in a dish, human embryonic stem cells can maintain their *"stem-cellness"* and provide an unlimited supply of more stem cells, as well as specialized cells that can be used for experiments and for the development of therapies. Apart from their potential to treat or cure diseases, human embryonic stem cells also provide a model to study very early human development and some of the disorders that lead to birth defects and childhood cancers. Many of these disorders develop in early pregnancy and are impossible to study in humans. Also, human embryonic stem cells also can be used to examine the genes that are turned "on" or "off" as stem cells generate more specialized cell types, permitting a unique understanding of the genetics of human development. The specialized cells derived from human embryonic stem cells also can be used to study the effectiveness of potential new drugs to treat diseases. This provides a human cellular model and can reduce animal experimentation and drug development costs. Additionally, embryonic stem cells can be derived from human blastocysts with specific genetic abnormalities. These types of blastocysts are identified through genetic diagnosis during IVF treatment, to screen out genetically abnormal blastocysts, and are usually discarded. The stem cells from them can provide a unique resource to understand genetic diseases and to develop cures. Human embryonic stem cells also could be used to understand the origin or causes of various diseases such as Alzheimer's disease or Parkinson's disease, which are currently unknown. Stem cells derived through *nuclear transfer* (more info below) from patients with such afflictions would provide special tools to study these diseases and possibly develop drugs for treatments.

7. Embryonic Stem Cells in the Clinic

Embryonic stem cells have not yet been used in treating humans. But numerous animal studies have shown that many of the specialized cells derived from them can indeed integrate into damaged tissues and function properly. Thus, diseases such as myocardial infarction, severe immune deficiency, diabetes, Parkinson's disease, spinal cord injury, and demyelination have been successfully treated in animal models. But the pathway from animal models to the clinic is still complex and burdened with obstacles to be overcome. First, not all specialized cells derived from human embryonic stem cells have been shown to integrate into animal tissue and function properly. This can be due to the poor quality

of the specialized cells derived in culture, or to a lack of adequate communication between the human cells and the animal environment in which they are placed. Then there is the problem of scaling up to yield enough of the specialized cells to treat a human, since this requires many more cells than to treat a tiny mouse. Such cells will have to be produced under specific conditions to ensure safety for use in patients. Most human embryonic stem cells are still grown on a layer of mouse feeder cells, a potential source of contamination. Last, there's the problem of immune rejection by the patient. While the drugs used in the organ transplantation field to suppress immune rejection have been improved over the years, rejection is still a major problem.

8. Literatures

Abe, Y., I. Choi, et al. (2002). "Hemophagocytic syndrome: a rare complication of allogeneic nonmyeloablative hematopoietic stem cell transplantation." Bone Marrow Transplant **29**(9): 799-801.

We report two cases of patients with malignant lymphoma who presented with early onset of hemophagocytic syndrome after nonmyeloablative allogeneic peripheral blood stem cell transplantation. Fever and skin eruption developed early after transplantation, and neurological symptoms preceded cytopenia and worsened progressively. Activated macrophages with hemophagocytosis were found in bone marrow of the two patients at day 15 and 56, respectively. The fact that no obvious infectious agents associated with hemophagocytic syndrome were detected, and that serum soluble interleukin-2 receptor concentrations were elevated in the early phase after transplantation, reflecting the activation of donor-derived T cells, suggests that this complication resulted from an alloimmune response.

Abe, Y., S. Yashiki, et al. (2002). "Eradication of virus-infected T-cells in a case of adult T-cell leukemia/lymphoma by nonmyeloablative peripheral blood stem cell transplantation with conditioning consisting of low-dose total body irradiation and pentostatin." Int J Hematol **76**(1): 91-3.

We describe the case of a 55-year-old man with adult T-cell leukemia/lymphoma (ATL) in first remission who underwent nonmyeloablative allogeneic peripheral blood stem cell transplantation with conditioning consisting of 4 courses of pentostatin and low-dose total body irradiation. Complete chimerism in peripheral blood was achieved on day 42 without severe myelosuppression. Concomitantly, the proviral DNA load for human T-cell leukemia virus I (HTLV-I) in peripheral blood mononuclear cells decreased below detectable limits and was still undetectable on day 270. This fact indicates that eradication of ATL cells is feasible by induction of an alloimmune response without high-dose chemoradiotherapy.

Adamson, J. W. (1997). "Cord blood stem cell banking and transplantation." Stem Cells **15 Suppl 1**: 57-9; discussion 59-61.

Cord blood banking for the purpose of stem cell transplantation is a rapidly growing area of medical interest. Based on the fact that cord blood contains large numbers of stem and progenitor cells, transplantation of cord blood for marrow reconstitution was first attempted in 1988. The success of this initial transplant between related donor and patient rapidly led to the establishment of efforts to collect, store and eventually transplant unrelated cord blood samples. A collection and storage program established by the New York Blood Center has led to more than 400 such transplants. The results demonstrate acceptable rates of engraftment and little graft-versus-host disease compared to the results employing adult marrow. As a consequence of these observations, considerable effort is being made to establish cord blood banks around the world.

Adamson, J. W., P. J. Fialkow, et al. (1976). "Polycythemia vera: stem-cell and probable clonal origin of the disease." N Engl J Med **295**(17): 913-6.

Two women with polycythemia vera and heterozygosity (GdB/GdA) at the X-chromosome-linked locus for glucose-6-phosphate dehydrogenase were studied to determine the nature of the cellular origin of their polycythemia. In contrast to unaffected tissue, such as skin fibroblasts, which consisted of both B and A types, the glucose-6-phosphate dehydrogenase of the patients' erythrocytes, granulocytes and platelets was only of Type A. These results provide direct evidence for the stem-cell nature of polycythemia vera and strongly imply a clonal origin for this disease. The fact that no descendants of the presumed normal stem cells were found in circulation suggests that bone-marrow proliferation in this disorder is influenced by local (intramarrow) regulatory factors.

Ahn, N. S., H. Hu, et al. (2005). "Molecular mechanisms of the 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced inverted U-shaped dose responsiveness in anchorage independent growth and cell proliferation of human breast epithelial cells with stem cell characteristics." Mutat Res **579**(1-2): 189-99.

Although 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has a variety of carcinogenic and noncarcinogenic effects in experimental animals, its

role in human carcinogenicity remain controversial. A simian virus 40-immortalized cell line from normal human breast epithelial cells with stem cells and luminal characteristics (M13SV1) was used to study whether TCDD can induce AIG positive colony formation and cause increased cell numbers in a inverted U-shaped dose-response manner. TCDD activated Akt, ERK2, and increased the expression of CYP1A1, PAI-2, IL-lb mRNA, and ERK2 protein levels. TCDD was able to increased phosphorylation and expression of ERK2 in same dose-response manner as AIG positive colony formation. Thus, TCDD induced tumorigenicity in M13SV1, possibly through the phosphorylation of ERK2 and/or Akt. Further, cDNA microarray with 7448 sequence-verified clones was used to profile various gene expression patterns after treatment of TCDD. Three clear patterns could be delineated: genes that were dose-dependently up-regulated, genes expressed in either U-shape and/or inverted U-shape. The fact that these genes are intrinsically related to breast epithelial cell proliferation and survival clearly suggests that they may be involved in the TCDD-induced breast tumorigenesis.

Aksoy, S. (2005). "Making regulations and drawing up legislation in Islamic countries under conditions of uncertainty, with special reference to embryonic stem cell research." J Med Ethics **31**(7): 399-403.

Stem cell research is a newly emerging technology that promises a wide variety of benefits for humanity. It has, however, also caused much ethical, legal, and theological debate. While some forms of its application were prohibited in the beginning, they have now started to be used in many countries. This fact obliges us to discuss the regulation of stem cell research at national and international level. It is obvious that in order to make regulations and to draw up legislation at national or international levels it helps to know the perspectives of different cultures and faith traditions. In this article the issue is explored from an Islamic perspective. Firstly, some basic information is given about Islam to explain how laws are drawn up and regulations made in this tradition. Secondly, the principles on which the laws and regulations are based are applied to stem cell research, and finally the permitted and prohibited methods of stem cell research are described. The discussions throughout the paper demonstrate that while some ethicists argue that stem cell research is unethical in the Islamic tradition, tradition permits it as long as such research is aimed at improving human health.

Almstrup, K., S. B. Sonne, et al. (2006). "From embryonic stem cells to testicular germ cell cancer--should we be concerned?" Int J Androl **29**(1): 211-8.

Since the discovery of testicular carcinoma in situ (CIS) -- the precursor cell for the vast majority of germ cell tumours -- it has been proposed that CIS cells could be derived from transformed primordial germ cells or gonocytes. Here, we review recent discoveries not only substantiating that initial hypothesis but also indicating that CIS cells have a striking phenotypic similarity to embryonic stem cells (ESC). Many cancers have been proposed to originate from tissue-specific stem cells [so-called 'cancer stem cells' (CSC)] and we argue that CIS may be a very good example of a CSC, but with exceptional features due to the retention of embryonic pluripotency. In addition, considering the fact that pre-invasive CIS cells are transformed from early fetal cells, possibly due to environmentally induced alterations of the niche, we discuss potential risks linked to the uncontrolled therapeutic use of ESC.

Arai, S. and H. G. Klingemann (2003). "Role of immunotherapy in stem cell transplantation." Int J Hematol **77**(1): 22-8.

Relapse of the underlying malignancy continues to be a major problem after both autologous and allogeneic stem cell transplantation. Over the years, it has been recognized that immune-mediated graft-versus-tumor effects are crucially involved in eliminating minimal disease and controlling its recurrence after stem cell transplantation. This recognition has led to a number of studies that have attempted to stimulate a cellular immune response in the recipient, especially after allogeneic transplantation. Immunotherapy after autologous transplantation has to take into consideration the fact that patients' immune cells frequently are compromised and tolerance to the host tumor may have developed. Hence, trials involving the administration of cytokines (such as with interleukin and interferon) have shown limited benefits. This situation is different for allogeneic transplantation for which the infusion of donor lymphocytes has shown disease regression, especially in patients with chronic leukemias. However, such treatment is effective only if the patient has limited disease, and severe graft-versus-host disease frequently has to be accepted as a complication. This fact has led investigators to pursue the generation of specific lymphocytes that can recognize tumor antigens but not necessarily induce graft-versus-host disease. Such studies are in the early stages, and although some promising results have been observed, it is unclear at this point if the antitumor effect can be separated sufficiently from the graft-versus-host disease mediated by allogeneic lymphocytes. More recently, it has been shown that natural killer (NK) cells can have an antitumor effect in myeloid malignancies, particularly if the cells are

allogeneic and do not recognize self-HLA antigens. At this point, it appears that engineered T-lymphocytes and allogeneic NK cells may be useful in preventing or treating relapse after allogeneic transplantation. It remains to be seen if such novel cellular therapies can also be implemented after autologous transplantation via the use of engineered allogeneic immune cells.

Au, W. Y., S. K. Ma, et al. (2003). "The occurrence of Philadelphia chromosome (Ph) negative leukemia after hematopoietic stem cell transplantation for Ph positive chronic myeloid leukemia: implications for disease monitoring and treatment." Leuk Lymphoma **44**(7): 1121-9.

Chronic myeloid leukemia (CML) is a clonal neoplastic disorder, characterized by t(9;22)(q34;q11) that results in the formation of the Philadelphia chromosome (Ph) and the BCR/ABL fusion gene. Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for CML. Much of its therapeutic efficacy is attributed to a graft-versus-leukemia (GVL) effect exerted by donor-derived lymphoid cells against the Ph positive (Ph+) clone. Post-HSCT monitoring by cytogenetic and molecular detection of the Ph+ clone is necessary, so that pre-emptive immunologic or pharmacologic treatment may be administered at an early stage of relapse. However, under rare circumstances a second Ph negative (Ph-) leukemia may evolve post-HSCT. The pathogenetic possibilities included leukemia arising from donor-derived hematopoietic stem cells (HSCs), or transformation of residual recipient-derived Ph- HSCs that have survived the conditioning chemotherapy and radiotherapy. Recipient-derived Ph- leukemia may be related to genetic alterations that precede the onset of CML, or myelotoxic effects of the HSCT conditioning regimen. The diagnosis of Ph- relapses requires detailed investigations by conventional karyotyping, fluorescence in-situ hybridization (FISH), and molecular analysis; as well as chimerism studies that help to document the donor or recipient origin of the leukemia. Although uncommonly reported in the past, Ph- relapses may in fact be more frequent if leukemic relapses post-HSCT are more thoroughly evaluated with these investigations. The recognition of Ph- relapses are important in several ways. Ph- relapses cannot be identified by monitoring investigations targeting the Ph+ clone, so that the early detection of Ph- leukemia is usually not possible. Furthermore, Ph- relapses will not respond to therapeutic strategies effective against the Ph+ CML clone.

Aversa, F., A. Terenzi, et al. (2002). "Haploidentical stem cell transplantation for acute leukemia." Int J Hematol **76 Suppl 1**: 165-8.

PREMISE: Since March 1993, 133 patients with high-risk acute leukemia (66 AML, 67 ALL) have received a megadose of T-cell depleted hematopoietic stem cells. The 1993-95 conditioning protocol included TBI, thiotepa, ATG and CY for 36 patients who received an inoculum made up of lectin-separated bone marrow and PBPCs. After 1995, to minimise the extra-hematological toxicity of the conditioning and eliminate GvHD, we substituted fludarabine for CY in the conditioning and PBPCs were depleted of T-cells by a positive selection of the CD34+ cells using CellPro (n=44 patients) or, since January 1999, CliniMacs (n = 53 patients). A later modification to the protocol in January 1999 was the suspension of post transplant G-CSF. WORK IN PROGRESS: We report here the results in the last 53 acute leukemia patients all of whom were transplanted under our modified protocol. Ages ranged from 9 to 62 years with a median of 38 years for the 33 patients with AML and 23 for the 20 with ALL. All were at high risk because 25 were actually in relapse at transplant, 16 were in second or later CR and even the 12 patients in CR1 were at high risk because of the unfavourable prognostic features. Overall 52/53 patients (98%) engrafted. The TBI-Fludarabine-based conditioning was well tolerated even in the 14 patients between 45 and 62 years of age. There was no veno-occlusive disease of the liver and the incidence of severe mucositis was low. Even though no post-transplant immunosuppressive therapy was given, acute GvHD grade > or = II occurred in only 4 cases and only one progressed to chronic GvHD. Overall, 16 patients (30%) have died of non-leukemic causes. Relapses occurred mainly in patients who were already in relapse at transplant (12/25). Only 3 of the 28 who were in any CR at transplant have so far relapsed. As our group has already shown, donor-vs-recipient NK cell alloreactivity exerts a specific graft-vs-AML effect in the absence of GvHD. In fact, leukemia relapse was largely controlled in AML recipients whose donor was NK alloreactive, with only 2 out of 16 relapsing. To date, 13 of 18 AML (72%) and 5 of 10 ALL (50%) who were in any CR at transplant, survive disease-free while 4 of the 15 patients (16%) in relapse at transplant survive. The probability of event-free survival for patients transplanted in CR is 60% in the 18 AML patients and 38% in the 10 ALL. The probability of EFS was significantly better in the 16 AML patients whose transplant included donor vs recipient NK cell alloreactivity than in those whose transplant did not (70% vs 7%). In conclusion, given our current results, the most suitable candidate for the full haplotype mismatched transplant should be in early stage disease and selection of an NK alloreactive donor is recommended.

Baghestanian, M., J. H. Jordan, et al. (2002). "Activation of human mast cells through stem cell factor receptor (KIT) is associated with expression of bcl-2." Int Arch Allergy Immunol **129**(3): 228-36.

BACKGROUND: Mast cells (MCs) are multifunctional effector cells of the immune system. These cells originate from pluripotent hemopoietic progenitors. In contrast to basophils and other leukocytes, MCs exhibit a remarkably long life span (years) in vivo. Although a role for stem cell factor (SCF) and SCF receptor (KIT) in long-term survival of MCs has been proposed, the underlying biochemical mechanisms remain unknown. MATERIALS AND METHODS: We have examined expression of 'survival-related' molecules of the bcl-2 family including bcl-2 and bcl-x(L), in primary human MCs and the human MC line HMC-1. Primary MCs were isolated from dispersed lung tissue by cell sorting using an antibody against KIT. mRNA expression was analyzed by RT-PCR and Northern blotting. Results: As assessed by RT-PCR, purified unstimulated lung MCs (>98% pure) exhibited KIT- and bcl-x(L) mRNA, but did not express bcl-2 mRNA. However, exposure of lung MCS to SCF (100 ng/ml) for 8 h resulted in expression of bcl-2 mRNA. Corresponding results were obtained by immunocytochemistry. In fact, exposure of MC to SCF resulted in expression of the bcl-2 protein whereas unstimulated MCs displayed only the bcl-x(L) protein without expressing the bcl-2 protein. The human MC leukemia cell line HMC-1, which contains a mutated and intrinsically activated SCF receptor, showed constitutive expression of both bcl-2 and bcl-x(L) at the mRNA and protein level. CONCLUSION: Our data show that human MCs can express members of the bcl-2 family. It is hypothesized that bcl-x(L) plays a role in KIT-independent growth of MCs, whereas bcl-2 may be involved in KIT-dependent functions of MCs.

Ballas, C. B., S. P. Zielske, et al. (2002). "Adult bone marrow stem cells for cell and gene therapies: implications for greater use." J Cell Biochem Suppl **38**: 20-8.

There is excitement generated almost daily about the possible uses of stem cells to treat human disease. Much of the interest of late is generated by embryonic stem cells (ESCs). As exciting as ESCs may be, they are quite controversial for moral reasons, given their source. They are also scientifically controversial since they are much less well understood than the original, long-standing, and clinically successful hematopoietic stem cell (HSC). HSCs have the distinct advantage of being reasonably well characterized and have been proven in the clinic. They can be isolated by simple procedures directly from the bone marrow or from peripheral blood after being stimulated (mobilized). They can then be manipulated and delivered to a patient, often producing a cure. Their biology provides the paradigm by which all other stem cells are judged, and they have little in the way of moral controversy surrounding them given they are isolated from adults who have consented to the procedure. Another putative stem cell has gained momentum in the last few years; the mesenchymal stem cell (MSC). MSCs appear to have much in common with HSCs. They were originally characterized from bone marrow, are capable of differentiating along multiple lineages and, at least in vitro, have significant expansion capability. Unlike HSCs, they have not yet been definitively shown to function as stem cells, despite their ability to differentiate into various mesenchymal cell types under the right culture conditions. Still, there is mounting evidence these cells may be useful, if not as true stem cells then at least as vehicles for emerging cell and gene therapies, especially in the field of tissue engineering. While this is an important endpoint, it is more important to thoroughly understand stem cell biology. That understanding can then be applied toward the ultimate goal of using these cells not just for various forms of therapy, but rather as a tool to discover the mechanisms and means to bring about directed repair and regeneration of damaged or diseased tissues and organs. The excitement of HSCs and MSCs has been muted somewhat by the excitement surrounding ESCs, primarily due to the fact HSCs and MSCs are viewed as limited to specific cell types while ESCs could potentially be applied to any cell type. Recent information indicates HSCs, MSCs, and other cells in general may have more universal differentiation abilities than previously thought.

Bao, L., K. Dunham, et al. (2008). "Expansion of cytomegalovirus pp65 and IE-1 specific cytotoxic T lymphocytes for cytomegalovirus-specific immunotherapy following allogeneic stem cell transplantation." Biol Blood Marrow Transplant **14**(10): 1156-62.

Adoptive immunotherapy with antigen-specific cytotoxic T lymphocytes (CTLs) has proven effective in restoring cellular immunity to cytomegalovirus (CMV) and preventing viral reactivation after allogeneic stem cell transplantation (SCT). In an effort to develop a cost-effective, relatively rapid method of CMV CTL expansion, we investigated the use of a pool of overlapping CMV peptides. Because the possibility exists of vaccinating CMV-seronegative donors, and these individuals may have T cell responses predominantly against IE-1,

commercially available peptide mixes for pp65 as well as IE-1 were used to stimulate CTLs from 10 seropositive donors. Of these 10 donors, 4 responded to pp65 only, 1 did not respond to either pp65 or IE-1, 4 responded to both pp65 and IE-1, and 1 responded to IE-1 only. These CMV- specific T cells included a mixture of CD4(+) and CD8(+) effectors, and specific cytotoxicity correlated with interferon-gamma production. The costs associated with a 28-day maintenance course of intravenous ganciclovir, cidofovir, foscarnet, and valganciclovir, as well as the preparation and shipping a single dose of CTLs, were determined. The price of generating CMV CTLs using this method was comparable to or less expensive than a 28-day maintenance course for these agents, not including the costs associated with drug administration, supportive care, and the treatment of drug-related complications. Considering the relative ease, low cost, and the fact that CTL administration can result in CMV-specific immune reconstitution, this option should be considered for patients with CMV reactivation or for prophylaxis in patients at high risk for infection.

Baraniak, A. P., E. L. Lasda, et al. (2003). "A stem structure in fibroblast growth factor receptor 2 transcripts mediates cell-type-specific splicing by approximating intronic control elements." Mol Cell Biol **23**(24): 9327-37.

Alternative splicing of fibroblast growth factor receptor 2 (FGFR2) occurs in a cell-type-specific manner with the mutually exclusive use of exon IIIb or exon IIIc. Specific inclusion of exon IIIb is observed in epithelial cells, whereas exon IIIc inclusion is seen in mesenchymal cells. Epithelium-specific activation of exon IIIb and repression of exon IIIc are coordinately regulated by intronic activating sequence 2 (IAS2) and intronic splicing activator and repressor (ISAR) elements in FGFR2 pre-mRNA. Previously, it has been suggested that IAS2 and a 20-nucleotide core sequence of ISAR form a stem structure that allows for the proper regulation of FGFR2 alternative splicing. Replacement of IAS2 and the ISAR core with random sequences capable of stem formation resulted in the proper activation of exon IIIb and repression of exon IIIc in epithelial cells. Given the high degree of phylogenetic conservation of the IAS2-ISAR core structure and the fact that unrelated stem-forming sequences could functionally substitute for IAS2 and ISAR elements, we postulated that the stem structure facilitated the approximation of intronic control elements. Indeed, deletion of the entire stem-loop region and juxtaposition of sequences immediately upstream of IAS2 with sequences immediately downstream of the ISAR core maintained proper cell-type-specific inclusion of exon IIIb. These data demonstrate that IAS2 and the ISAR core are dispensable for the cell-type-specific activation of exon IIIb; thus, the major, if not the sole, role of the IAS2-ISAR stem in exon IIIb activation is to approximate sequences upstream of IAS2 with sequences downstream of the ISAR core. The downstream sequence is very likely a highly conserved GCAUG element, which we show was required for efficient exon IIIb activation.

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.

Behfar, A., L. V. Zingman, et al. (2002). "Stem cell differentiation requires a paracrine pathway in the heart." Faseb J **16**(12): 1558-66.

Members of the transforming growth factor beta1 (TGF-beta) superfamily--namely, TGF-beta and BMP2--applied to undifferentiated murine embryonic stem cells up-regulated mRNA of mesodermal (Brachyury) and cardiac specific transcription factors (Nkx2.5, MEF2C). Embryoid bodies generated from stem cells primed with these growth factors demonstrated an increased potential for cardiac differentiation with a significant increase in beating areas and enhanced myofibrillogenesis. In an environment of postmitotic cardiomyocytes, stem

cells engineered to express a fluorescent protein under the control of a cardiac promoter differentiated into fluorescent ventricular myocytes beating in synchrony with host cells, a process significantly enhanced by TGF-beta or BMP2. In vitro, disruption of the TGF-beta/BMP signaling pathways by latency-associated peptide and/or noggin prevented differentiation of stem cells. In fact, only host cells that secrete a TGF-beta family member induced a cardiac phenotype in stem cells. In vivo, transplantation of stem cells into heart also resulted in cardiac differentiation provided that TGF-beta/BMP2 signaling was intact. In infarcted myocardium, grafted stem cells differentiated into functional cardiomyocytes integrated with surrounding tissue, improving contractile performance. Thus, embryonic stem cells are directed to differentiate into cardiomyocytes by signaling mediated through TGF-beta/BMP2, a cardiac paracrine pathway required for therapeutic benefit of stem cell transplantation in diseased heart.

Berglund, A., G. Enblad, et al. (2000). "Long-term follow-up of autologous stem-cell transplantation for follicular and transformed follicular lymphoma." Eur J Haematol **65**(1): 17-22.

Despite the fact that follicular lymphomas are both chemo- and radiosensitive, the disease is generally non-curable. These lymphomas often undergo transformation to a more malignant state. In order to improve the prognosis, high-dose treatment with stem cell support has been tested, but its role in the treatment of this disease is still unclear. Fourteen men and eight women with a median age of 45 yr (34-59) were treated with high-dose therapy with autologous stem cell transplantation between 1987 and 1996. The patients were selected to undergo intensive therapy because of an estimated short survival (median < 3 yr), even though they had chemosensitive disease and adequate performance status. Eleven patients' lymphomas had transformed, and the other eleven patients had one or more unfavourable prognostic signs such as advanced stage, bulky disease, multiple relapses, or short remission duration. The conditioning regimen has varied over the period, but BEAC (Becenum, etoposide, cytarabine, cyclophosphamide) or etoposide/cyclophosphamide with or without total body irradiation (TBI) was used in most patients. Nine patients had their stem cells purged. After a median follow-up time of 74 months overall survival was 81% and disease-free survival 72%. One toxic procedure-related death occured. There was no difference in outcome between patients with a transformed lymphoma compared to those without transformation. The patients treated with TBI had a significantly worse outcome. Toxicity was also much higher in TBI-treated patients, including four cases of secondary malignancy (three myelodysplastic syndrome (MDS) cases and one patient with breast carcinoma). This retrospective study, with the longest follow-up time so far reported, shows a promising 6-yr DFS of 72% in a group of follicular lymphoma patients with a bad prognosis. The outcome of patients with transformed lymphoma compared to historical controls is especially encouraging. The high incidence of MDS is worrying. The role of TBI should be questioned because this and other studies have not shown any advantage of using TBI. In the absence of randomised trials the role of high-dose treatment for patients with follicular lymphoma is still not defined.

Beyhan, Z., A. E. Iager, et al. (2007). "Interspecies nuclear transfer: implications for embryonic stem cell biology." Cell Stem Cell **1**(5): 502-12.

Accessibility of human oocytes for research poses a serious ethical challenge to society. This fact categorically holds true when pursuing some of the most promising areas of research, such as somatic cell nuclear transfer and embryonic stem cell studies. One approach to overcoming this limitation is to use an oocyte from one species and a somatic cell from another. Recently, several attempts to capture the promises of this approach have met with varying success, ranging from establishing human embryonic stem cells to obtaining live offspring in animals. This review focuses on the challenges and opportunities presented by the formidable task of overcoming biological differences among species.

Bichindaritz, I., M. F. Siadak, et al. (1998). "CARE-PARTNER: a computerized knowledge-support system for stem-cell post-transplant long-term follow-up on the World-Wide-Web." Proc AMIA Symp: 386-90.

Evidence-based practice in medicine promotes the performance of medicine based upon proven and validated practice. The CARE-PARTNER system presented here is a computerized knowledge-support system for stem-cell post-transplant long-term follow-up (LTFU) care on the WWW, which means that it monitors the quality of the knowledge both of its own knowledge-base and of its users. Its aim is to support the evidence-based practice of the LTFU clinicians and of the home-town physicians who actually care for the transplanted patients. Currently, three fundamental characteristics of CARE-PARTNER are accountable for its knowledge-support function: the quality of its knowledge-base, its availability on the WWW, and its learning from experience capability. As a matter of fact, the integration of a case-based reasoner in the reasoning framework enables the system to introspectively study

its results, and to learn from its successes and failures, thus confronting the quality of the guidelines and pathways it reuses to the reality and complexity of the clinical cases.

Bitan, M., R. Or, et al. (2005). "Successful engraftment following allogeneic stem cell transplantation in very high-risk patients with busulfan as a single agent." Haematologica **90**(8): 1089-95.

BACKGROUND AND OBJECTIVES: Busulfan is the most commonly used myeloablative alkylating agent, but is considered a poor anti-lymphocyte agent. Since engraftment of allogeneic stem cells depends not only on adequate immunosuppression but also on successful hematopoietic competition, and considering the fact that residual lymphocytes of host origin may play a beneficial role in preventing graft-versus-host disease (GVHD), we used low doses of oral busulfan as a single agent for conditioning prior to stem cell transplantation (SCT) in recipients of transplants from a variety of donors. DESIGN AND METHODS: Fifteen heavily pretreated high-risk patients (age 25-66, median 42 years) with hematologic malignancies were conditioned with busulfan alone, 4mg/kg/day for 2, 3, or 4 consecutive days. No additional pre- or post-transplant immunosuppressive agents were used in order to exploit the capacity of donor lymphocytes to induce graft-versus-malignancy (GVM) effects. RESULTS: Conditioning was well tolerated, trilineage engraftment was documented in all patients and none exhibited immune-mediated rejection. Time to recovery of absolute neutrophil count >0.5x10(9)/L and 1.0x10(9)/L was 12 - 38 (median 15) days and 12 - 41 (median 15) days, respectively. The time to platelet recovery >or=20 and >or=50x10(9)/L ranged from 0 to 26 (median 11) days, and from 0 to 83 (median 14) days, respectively. Acute GVHD (<or=grade I) occurred in 13/15 patients. Three patients benefited from long-term survival. INTERPRETATION AND CONCLUSIONS: We suggest that using busulfan alone for the preparation of patients for SCT may be sufficient for engraftment, in very high-risk heavily pre-treated patients.

Blaha, M., P. Mericka, et al. (2006). "Prevention of infection transmission during stem cell transplantation." Folia Microbiol (Praha) **51**(6): 609-13.

Group of 152 patients (investigated before autologous transplantation) and 35 healthy donors for allogeneic transplantation was examined for the risk of infection transmission that can be associated with the infusion of cryopreserved peripheral blood progenitor cells to the patient and/or cross-contamination of stored grafts. No laboratory signs of active infection were found in 22 donors (63 %) and in 91 patients (60%). The most common was active infection by herpes viruses--50 cases in patients, 21 cases in donors; hepatitis B was found in only two cases. The rate of clinically unsuspected (but dangerous) infections in donors and patients thus remains relatively high in spite of the fact that the system of donor search and the whole transplantation procedure have improved in the last years. The system of safety assurance is extremely important and the whole palette of preventive tests according to EBMT (European Blood and Marrow Transplantation Group) and ISHAGE (International Society for Hemotherapy and Graft Engineering) is fully justified.

Bojko, P., M. Scharifi, et al. (2002). "Comparison of processing four and five times the patients' blood volume during peripheral blood stem cell collection and analysis of CD34+38--and CD34+49d+ subsets during apheresis." J Cancer Res Clin Oncol **128**(1): 19-28.

PURPOSE: We investigated whether increasing the patients' processed blood volume (BV) during peripheral blood stem cell collection (PBSCC) from four to five times leads to a greater yield of CD34+ cells. We also studied the kinetics of CD34+38- and CD34+49d+ subsets and compared the amount of transfused cells with engraftment. METHODS: All patients (n=20) received chemotherapy followed by G-CSF for PBSC mobilization. Samples from the patients' peripheral blood and the PBSC harvests were taken after processing 1-, 4-, and 5 times the patients' calculated BV. RESULTS: The mean total yields of CD34+, CD34+38-, and CD34+49d+ cells were 15.69-, 1.13- and 4.17 x 10(6)/kg body weight, respectively. The mean increase for these subsets between 4- and 5 BV was 10%, 8%, and 21%, respectively. Based on the mean number of 2.25 (range 2-3) planned courses of high-dose chemotherapy (HDC) per patient, the mean yield of CD34+ cells per kg body weight and intended course of HDC after 4- and 5 BV was 6.31- and 6.97 x 10(6) (P=0.014). Twenty HDC were evaluable for engraftment. There was some correlation between the number of transfused CD34+ and CD34+38- cells and WBC engraftment (r = -0.66 and --0.69; P<0.01) and CD34+ cells and platelet engraftment (r = -0.56; P= 0.013). No toxicity occurred during PBSCC, although the mean platelet count dropped by 50% which must be kept in mind regarding the additional application of anti-coagulants and the fact that most patients had large indwelling catheters. CONCLUSION: Processing 4 BV is sufficient to collect >5 x 10(6) CD34+ cells/kg body weight and intended course of

HDC in most patients, although extension to 5 BV further increases the total yield of CD34+ cells.

Bosnali, M., B. Munst, et al. (2009). "Deciphering the stem cell machinery as a basis for understanding the molecular mechanism underlying reprogramming." Cell Mol Life Sci **66**(21): 3403-20.

Stem cells provide fascinating prospects for biomedical applications by combining the ability to renew themselves and to differentiate into specialized cell types. Since the first isolation of embryonic stem (ES) cells about 30 years ago, there has been a series of groundbreaking discoveries that have the potential to revolutionize modern life science. For a long time, embryos or germ cell-derived cells were thought to be the only source of pluripotency--a dogma that has been challenged during the last decade. Several findings revealed that cell differentiation from (stem) cells to mature cells is not in fact an irreversible process. The molecular mechanism underlying cellular reprogramming is poorly understood thus far. Identifying how pluripotency maintenance takes place in ES cells can help us to understand how pluripotency induction is regulated. Here, we review recent advances in the field of stem cell regulation focusing on key transcription factors and their functional interplay with non-coding RNAs.

Brazel, C. Y., M. H. Ducceschi, et al. (2001). "The FLT3 tyrosine kinase receptor inhibits neural stem/progenitor cell proliferation and collaborates with NGF to promote neuronal survival." Mol Cell Neurosci **18**(4): 381-93.

The FLT3 receptor tyrosine kinase (FLT3) was originally identified on hematopoietic stem cells (HSCs) and its ligand (FL) induces HSC proliferation. As stem cells originating from various tissues are more similar than once thought, the goal of this study was to determine whether neural stem cells express FLT3 and proliferate in response to FL. In fact, a subset of neural stem/progenitor cells does express FLT3, but contrary to our expectations, FL inhibited EGF and FGF-2 stimulated proliferation. Since FLT3 is expressed weakly by proliferative neuroepithelia but strongly by subsets of neurons in the CNS and PNS, we tested its ability to support neuronal survival. FL synergized with NGF to promote the survival of cultured DRG neurons, although it lacked any neurotrophic activity alone. We conclude that FL serves as an adjunct trophic factor in the nervous system, which differs from its role in the hematopoietic system.

Bryder, D. and S. E. Jacobsen (2000). "Interleukin-3 supports expansion of long-term multilineage repopulating activity after multiple stem cell divisions in vitro." Blood **96**(5): 1748-55.

Although long-term repopulating hematopoietic stem cells (HSC) can self-renew and expand extensively in vivo, most efforts at expanding HSC in vitro have proved unsuccessful and have frequently resulted in compromised rather than improved HSC grafts. This has triggered the search for the optimal combination of cytokines for HSC expansion. Through such studies, c-kit ligand (KL), flt3 ligand (FL), thrombopoietin, and IL-11 have emerged as likely positive regulators of HSC self-renewal. In contrast, numerous studies have implicated a unique and potent negative regulatory role of IL-3, suggesting perhaps distinct regulation of HSC fate by different cytokines. However, the interpretations of these findings are complicated by the fact that different cytokines might target distinct subpopulations within the HSC compartment and by the lack of evidence for HSC undergoing self-renewal. Here, in the presence of KL+FL+megakaryocyte growth and development factor (MGDF), which recruits virtually all Lin(-)Sca-1(+)kit(+) bone marrow cells into proliferation and promotes their self-renewal under serum-free conditions, IL-3 and IL-11 revealed an indistinguishable ability to further enhance proliferation. Surprisingly, and similar to IL-11, IL-3 supported KL+FL+MGDF-induced expansion of multilineage, long-term reconstituting activity in primary and secondary recipients. Furthermore, high-resolution cell division tracking demonstrated that all HSC underwent a minimum of 5 cell divisions, suggesting that long-term repopulating HSC are not compromised by IL-3 stimulation after multiple cell divisions. In striking contrast, the ex vivo expansion of murine HSC in fetal calf serum-containing medium resulted in extensive loss of reconstituting activity, an effect further facilitated by the presence of IL-3. (Blood. 2000;96:1748-1755)

Buno, I., P. Nava, et al. (2005). "A comparison of fluorescent in situ hybridization and multiplex short tandem repeat polymerase chain reaction for quantifying chimerism after stem cell transplantation." Haematologica **90**(10): 1373-9.

BACKGROUND AND OBJECTIVES: Despite the great utility of chimerism analysis after allogeneic stem cell transplantation, a gold standard method for its quantification has not yet been defined. The objective of the present investigation was to compare the sensitivity (detection limit) and the quantification accuracy of fluorescent in situ hybridization with specific probes for the sex chromosomes (XY-FISH) and multiplex short tandem repeat polymerase chain reaction (STR-PCR) revealed by capillary electrophoresis for the quantification of

chimerism after stem cell transplantation. DESIGN AND METHODS: A first experiment was performed on two sets of artificial cell mixtures from two sex-mismatched healthy donors mixed in different proportions (% male: 100, 75, 50, 25, 10, 5, 3, 1, 0.1, 0). In a second experiment, 58 samples obtained from 10 selected patients with different clinical courses and chimerism evolution after sex-mismatched stem cell transplantation, which had been studied by XY-FISH, were retrospectively analyzed by STR-PCR. In a third experiment, 60 unselected prospective samples belonging to 15 patients (5 of whom had also been included in the retrospective study) were analyzed by both XY-FISH and STR-PCR. RESULTS: Both techniques showed high quantification accuracy and were highly reproducible. The sensitivity of both approaches reached 1% under standard conditions. Moreover, the use of long injection times for the capillary electrophoresis (30 and 50s vs. the standard 10s) resulted in an increase of sensitivity of the STR-PCR assay up to 0.1%, which has interesting clinical implications. INTERPRETATION AND CONCLUSIONS: Considering the high sensitivity and quantification accuracy of multiplex STR-PCR and the fact that this assay is sex-independent and can be applied to virtually all patients, STR-PCR could be considered as the method of choice for chimerism quantification after stem cell transplantation when high sensitivity is not a requirement.

Byar, K. L., J. E. Eilers, et al. (2005). "Quality of life 5 or more years post-autologous hematopoietic stem cell transplant." Cancer Nurs **28**(2): 148-57.

This cross-sectional study used a mailed survey to evaluate the quality of life (QOL) of individuals at least 5 years post-autologous stem cell transplant and to determine instrument preference. Instruments selected were the Medical Outcomes Study-Short Form (MOS-SF-36) as the generic measure and the City of Hope-Quality of Life-Bone Marrow Transplant (COH-BMT) and the Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) as transplant-specific measures. Subjects received the MOS-SF-36 and were randomized to receive (1) COH-BMT, (2) FACT-BMT, or (3) COH-BMT and FACT-BMT. Ninety-two subjects returned completed forms, for a 56% response rate. A study-specific form indicated subjects preferred the BMT-specific instruments. The health of the majority of subjects (85%) was similar to or somewhat better than what it was the previous year. Their MOS-SF-36 scores for physical functioning, role-physical, bodily pain, and general health subscales were lower than the values for the general population, but those for the other subscales were not significantly different. When compared to the data reported by Hann and colleagues for posttransplant in breast cancer, study subjects scored significantly lower on all scales except General Health and Mental Health. COH-BMT scores compared with those reported by Whedon and Ferrel (Semin Oncol Nurs. 1994;10:42-57) were higher for Physical Well-Being, Spiritual Well-Being, and Global QOL. FACT-BMT results compared with those reported by McQuellen et al (Bone Marrow Transplant. 1997;19:357-368) showed that Physical, Social/Family, Emotional, and Functional Scores were similar; only BMT scores were significantly different. Research is needed to determine when QOL plateaus and whether instrument preference changes over time. Awareness of long-term effects that affect QOL can guide program revisions and facilitate decisions regarding the need for supportive rehabilitative services.

Castagnola, E., V. Fontana, et al. (2007). "A prospective study on the epidemiology of febrile episodes during chemotherapy-induced neutropenia in children with cancer or after hemopoietic stem cell transplantation." Clin Infect Dis **45**(10): 1296-304.

BACKGROUND: The purpose of our study was to evaluate the incidence and clinical characteristics of febrile episodes during neutropenia following chemotherapy in children with cancer. PATIENTS AND METHODS: A prospective, 3-year single-center observational study of periods of neutropenia was performed. Epidemiology and clinical diagnoses of febrile episodes occurring during the neutropenic periods were evaluated, taking into consideration different categories of anticancer treatment based on the type of tumor and phase of therapy. RESULTS: A total of 703 febrile episodes were observed during 614 (34%) of 1792 neutropenic periods (34%), for a total of 28,001 days at risk, accounting for a rate of 0.76 episodes per 30 days at risk. The highest proportions of neutropenic periods with primary febrile episodes were observed after autologous hemopoietic stem cell transplantation (58%), aggressive treatment for acute leukemia or non-Hodgkin lymphoma (48%), and allogeneic hemopoietic stem cell transplantation (44%); the lowest proportion (9%) was observed during maintenance chemotherapy for acute leukemia (P<.001). The most frequent clinical diagnosis was fever of unknown origin (in 79% of cases), followed by bacteremia (10%); invasive mycosis was diagnosed in only 2% of cases. CONCLUSIONS: The overall incidence of febrile neutropenia and severe infectious complications in children with cancer is low, with differences according to the aggressiveness of chemotherapy. This fact must be considered when designing clinical trials on the management of infectious complications in children with cancer.

Christensen, K., M. Kristiansen, et al. (2000). "X-linked genetic factors regulate hematopoietic stem-cell kinetics in females." Blood **95**(7): 2449-51.

X inactivation makes females mosaics for 2 cell populations, usually with an approximate 1:1 distribution. Skewing of this distribution in peripheral blood cells is more common among elderly women. The depletion of hematopoietic stem cells followed by random differentiation may explain the acquired skewing with age. However, an animal model suggests that selection processes based on X-linked genetic factors are involved. We studied peripheral blood cells from 71 monozygotic twin pairs aged 73 to 93 years and from 33 centenarians, and we found that with age, 1 of the cell populations becomes predominant for most women. We also observed a strong tendency for the same cell line to become predominant in 2 co-twins. This suggests that X-linked genetic factors influence human hematopoietic stem cell kinetics. The fact that females have 2 cell lines with different potentials could be one of the reasons women live longer than men.

Chung, Y., I. Klimanskaya, et al. (2006). "Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres." Nature **439**(7073): 216-9.

The most basic objection to human embryonic stem (ES) cell research is rooted in the fact that ES cell derivation deprives embryos of any further potential to develop into a complete human being. ES cell lines are conventionally isolated from the inner cell mass of blastocysts and, in a few instances, from cleavage stage embryos. So far, there have been no reports in the literature of stem cell lines derived using an approach that does not require embryo destruction. Here we report an alternative method of establishing ES cell lines-using a technique of single-cell embryo biopsy similar to that used in pre-implantation genetic diagnosis of genetic defects-that does not interfere with the developmental potential of embryos. Five putative ES and seven trophoblast stem (TS) cell lines were produced from single blastomeres, which maintained normal karyotype and markers of pluripotency or TS cells for up to more than 50 passages. The ES cells differentiated into derivatives of all three germ layers in vitro and in teratomas, and showed germ line transmission. Single-blastomere-biopsied embryos developed to term without a reduction in their developmental capacity. The ability to generate human ES cells without the destruction of ex utero embryos would reduce or eliminate the ethical concerns of many.

Cilley, J. and J. N. Winter (2006). "Radioimmunotherapy and autologous stem cell transplantation for the treatment of B-cell lymphomas." Haematologica **91**(1): 114-20.

Relapse continues to be the primary cause of treatment failure in patients with non-Hodgkin's lymphomas (NHL) undergoing high-dose therapy and autologous stem cell transplantation. The anti-CD20 radioimmunoconjugates, Y-90 ibritumomab tiuxetan (Zevalin; Biogen Idec, Inc., Cambridge, MA, USA) and I-131 tositumomab (Bexxar; Corixa, Seattle, WA; and Glaxo Smith Kline; Philadelphia, PA, USA) have been associated with high response rates, durable remissions and limited toxicity apart from myelosuppression, making them ideal candidates for use in autotransplantation. Tested first as single agents in relapsed patients with indolent and transformed NHL, and then at much higher doses with stem cell support, these agents have now been combined with high-dose chemotherapy prior to autologous stem cell transplant. Radioimmunoconjugates have been used to replace total body irradiation (TBI) in some studies and to augment standard chemotherapy regimens in others. Thus far the results are promising, with combinations of radioimmunoconjugates and chemotherapy producing long-lasting responses in high-risk patients with no more toxicity than that caused by standard conditioning regimens. These results are notable in light of the fact that the dose of radiation delivered to the tumor is 10-fold higher than the dose achievable with TBI. Whether this increase in radiation dose to the targeted lymphoma translates into more durable remissions and an improvement in overall survival requires further investigation.

Conlan, M. G., W. D. Haire, et al. (1991). "Prothrombotic hemostatic abnormalities in patients with refractory malignant lymphoma presenting for autologous stem cell transplantation." Bone Marrow Transplant **7**(6): 475-9.

Forty-six patients with refractory malignant lymphoma (Hodgkin's and non-Hodgkin's) admitted for autologous marrow or peripheral blood stem cell transplantation (ASCT) were evaluated for the presence of hemostatic abnormalities known to be associated with a hypercoagulable state in other patient populations. All patients had received numerous chemotherapeutic agents in the past and often radiation therapy as well. Hemostatic abnormalities were found to be common in these patients. The most frequent finding was hyperfibrinogenemia, present in 35% of patients. Decreased protein C activity was present in 32% of patients. Protein C antigen was low in only one individual and protein S was normal or increased in all patients. Low levels of antithrombin III were

present in 16%. Plasminogen activator inhibitor was elevated in 20%. Anticardiolipin antibodies were present in 29% of patients; other evidence of a lupus anticoagulant was present in only eight patients. The frequency of each hemostatic abnormality was similar for patients with Hodgkin's disease (HD) and those with non-Hodgkin's lymphoma (NHL) despite the fact that significantly more patients with HD had received irradiation and/or previous splenectomy than patients with NHL. We conclude that multiple prothrombotic abnormalities of hemostasis are present in patients with refractory lymphoma referred for ASCT. Whether these are the result of lymphoma or the result of therapy cannot be determined from this study.

Cowan, M. J. and M. Golbus (1994). "In utero hematopoietic stem cell transplants for inherited diseases." Am J Pediatr Hematol Oncol **16**(1): 35-42.

PURPOSE: The treatment of choice for many inherited diseases is bone marrow transplantation (BMT). Limitations to using marrow transplants for inherited diseases include (a) the toxicity associated with high doses of chemotherapy necessary to obtain engraftment; (b) the complications associated with graft-versus-host disease (GVHD); (c) the fact that only 20-25% of children will have a human leukocyte antigen (HLA)-matched donor; and (d) the concern that, at least for some inherited diseases, significant organ damage, especially to the nervous system, has occurred by the time the child is diagnosed and evaluated for possible BMT. In utero transplantation of hematopoietic stem cells (HSCs) offers the possibility of overcoming many of these limitations. PATIENTS AND METHODS: One of the biggest hurdles to a successful transplant is the ability of the recipient to reject the donor marrow. Except in patients with severe combined immunodeficiency disease (SCID), overcoming this hurdle requires high doses of chemotherapy. Early in gestation, the fetus is significantly immunoincompetent. Before 14-15 weeks of gestation, the human fetus appears to be similar to a child with SCID in its inability to reject allogeneic cells. Potential sources for HSCs are HLA-matched sibling marrow, fetal liver, parental bone marrow, and cord blood. RESULTS: With fetal liver, only cells from fetuses < 10-12 weeks are acceptable because of the high risk of GVHD. With parental marrow, the cells must be T cell depleted in order to minimize the risk for GVHD. Problems in using fetal liver include the inability to obtain sufficient numbers of cells and inadequate supplies of donor tissue. The source and supply of parental bone marrow is almost unlimited, but, because of the need for T-cell depletion, bone marrow from a parent may have a lower engraftment rate in the child. CONCLUSIONS: Studies in fetal murine and Rhesus models using fetal liver or T cell-depleted bone marrow from adult animals suggest that engraftment can be successfully obtained, providing the transplant is performed sufficiently early in gestation. To date, at least a dozen in utero human transplants have been attempted worldwide in fetuses diagnosed with a variety of inherited diseases. Because of the small number of transplanted fetuses and the variety of diseases and differing transplant conditions, it is difficult to draw any firm conclusions regarding ultimate efficacy of the procedure and its risk. However, it does appear that the age of gestation of the recipient, the dose of cells infused, and possibly the route of administration of the HSCs will be critical factors in determining success rates for this approach. The successful application of in utero transplantation would allow treatment of a variety of inherited diseases early in gestation while eliminating many of the risks associated with conventional BMT.

Dao, M. and J. Nolta (1999). "Molecular control of cell cycle progression in primary human hematopoietic stem cells: methods to increase levels of retroviral-mediated transduction." Leukemia **13**(10): 1473-80.

Pluripotent hematopoietic stem cells (HSC) are the ideal targets for gene transfer because they can repopulate a sublethally irradiated recipient, giving rise to all lineages of blood cells. Thus, introduction of a corrected gene into HSC (stem cell gene therapy) should ensure persistent transmission of the gene. To date, the most efficient mode of gene delivery is via Moloney murine leukemia virus (MoMuLV)-based retroviral vectors which stably integrate into the genome of the target cell. The quiescent nature of HSC and the fact that MoMuLV-based retroviral vectors can only integrate into dividing cells are major obstacles in gene therapy. While increasing efforts have been directed toward identifying growth factors which facilitate division of primary hematopoietic progenitor and stem cells, little is known about the molecular mechanisms which these cells use to enter cell cycle. In this review, we will discuss the correlation between the hematopoietic inhibitory and growth factors and their impact on the regulation of the cell cycle components.

Davis, B. R., D. B. Brown, et al. (2000). "Micro-injection-mediated hematopoietic stem cell gene therapy." Curr Opin Mol Ther **2**(4): 412-9.

Over the past decade, significant attention has been devoted to the development of viral vectors (i.e., retrovirus, lentivirus, adeno-associated virus) and conditions capable of transducing hematopoietic stem cells. After several years of disappointing results, recent reports in humans and other primates, most

particularly the French report of successful treatment of X-linked severe combined immune deficiency (SCID) [1.], indicate that viral approaches will be successful in treating specific hematopoietic diseases. However, it is clear that alternate non-viral methods of gene delivery and genetic modification offer significant advantages, and may in fact be the only effective approach for treating certain blood diseases. In this review, we focus on glass needle-mediated micro-injection as a method for the delivery of genetic material into blood stem cells, with an emphasis on molecules capable of either compensating gene deletions/mutations or directly repairing gene mutations.

De Marzo, A. M., A. K. Meeker, et al. (1998). "Prostate stem cell compartments: expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells." Am J Pathol **153**(3): 911-9.

The stem cells of rapidly renewing tissues give rise to transiently proliferating cells, which in turn give rise to postmitotic terminally differentiated cells. Although the existence of a transiently proliferating compartment has been proposed for the prostate, little molecular anatomical evidence for its presence has been obtained to date. We used down-regulation of the cyclin-dependent kinase inhibitor p27Kip1 to identify cells capable of entering the proliferative phase of the cell cycle and, therefore, competent to fulfill the role of the transiently proliferating compartment. We examined the expression of p27Kip1 in relation to its role in the development of prostatic carcinoma. Formalin-fixed paraffin-embedded specimens from matched samples of normal-appearing prostate tissue, benign prostatic hyperplasia, high-grade prostatic intraepithelial neoplasia, primary adenocarcinomas, and pelvic lymph node metastases were evaluated by comparative immunohistochemistry against p27Kip1. In normal-appearing prostate epithelium, moderate to strong nuclear staining of p27Kip1 was present in greater than 85% of the terminally differentiated secretory cells. The normal basal cell compartment, believed to contain prostatic stem cells, showed distinctive p27Kip1 expression; acini in epithelial benign prostatic hyperplasia tissue contained more p27Kip1-negative basal cells than acini from non-benign prostatic hyperplasia tissue. A third layer of cells was identified that was sandwiched between the basal cells and the luminal cells, and this layer was consistently p27Kip1 negative. This intermediate layer was accentuated in the periurethral region, as well as in prostate tissue that had been subjected to prior combined androgen blockade. We hypothesize that, on appropriate additional mitogenic stimulation, cells in this layer, and other p27Kip1-negative basal cells, are competent for rapid entry into the cell cycle. Consistent with the fact that cancer cells are capable of cell division, all cases of high-grade prostatic intraepithelial neoplasia and invasive carcinoma also showed down-regulation of p27Kip1 as compared with the surrounding normal-appearing secretory cells. In pelvic lymph node metastases, p27Kip1 expression was also reduced. In summary, our results suggest that lack of nuclear p27Kip1 protein may delineate a potential transiently proliferating subcompartment within the basal cell compartment of the human prostate. In addition, these studies support the hypothesis that reduced expression of p27Kip1 removes a block to the cell cycle in human prostate epithelial cells and that dysregulation of p27Kip1 protein levels may be a critical early event in the development of prostatic neoplasia.

de Medeiros, B. C., W. N. Rezuke, et al. (2000). "Kaposi's sarcoma following allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia." Acta Haematol **104**(2-3): 115-8.

Unlike solid organ transplantation, Kaposi's sarcoma (KS) occurs rarely following hematopoietic stem cell transplantation (HSCT). In fact, only 5 cases of KS have been reported after allogeneic or autologous HSCT. The usual treatment combines a substantial decrease in, or elimination of, immunosuppressive therapy along with local measures such as surgical excision, cryotherapy or radiation therapy. A 46-year-old woman with chronic myelogenous leukemia who had received an allogeneic HSCT previously from an HLA-identical sibling, presented on day +814 with human herpes virus-8-associated KS involving her left lower extremity. She had been on continuous immunosuppressive therapy since her transplant because of chronic graft-versus-host disease. The intensity of immunosuppressive therapy was decreased once a diagnosis of KS had been established. However, the nodular lesions continued to progress in size and number. Therefore, a course of irradiation was administered to sites of bulk disease on her legs. Furthermore, thalidomide was initiated along with a topical retinoid, alitretinoin 0.1% gel applied twice daily to the nonirradiated lesions. This approach yielded a partial response in both irradiated and nonirradiated lesions over the course of the following 7 months. Both thalidomide and alitretinoin 0.1% gel appear to be beneficial in HSCT-associated KS and exhibit tolerable side effects.

De Smedt, A., M. Steemans, et al. (2008). "Optimisation of the cell cultivation methods in the embryonic stem cell test results in an increased

differentiation potential of the cells into strong beating myocard cells." Toxicol In Vitro **22**(7): 1789-96.

In order to support drug research in the selection process for non-embryotoxic pharmaceutical compounds, a screening method for embryotoxicity is needed. The murine embryonic stem cell test (EST) is a validated in vitro test based on two permanent mouse cell lines and delivering results in 10-days. Implementation of this test within our laboratory, revealed variability in the differentiation potential of the embryonic stem cells and, as a consequence, a lot of assays needed to be rejected due the fact the acceptance criteria were not reached. In order to gain a better yield of contracting myocardial cells, we used (1) a stringent control of the cell growth during subcultivation and a standardised hanging drop culture method and (2) a non-enzymatic cell harvest instead of a trypsin/EDTA cell harvest. Implementing of these cell culture modifications resulted in a decreased variability in the size of embryonic bodies, an increase of the number of acceptable tests and a significant increase of the differentiation potential of embryonic cells into strong beating myocardium, which made scoring less time consuming. Testing of 6 reference compounds in the optimized EST showed that the cell culture modifications did not changed the in vitro classification.

Doran, M. R., B. D. Markway, et al. (2009). "Surface-bound stem cell factor and the promotion of hematopoietic cell expansion." Biomaterials **30**(25): 4047-52.

In vivo, stem cell factor (SCF) exists in both a bound and soluble isoform. It is believed that the bound form is more potent and fundamentally required for the maintenance of hematopoietic stem cells (HSCs). This theory is supported by the observation that steel-Dickie mice lacking the bound isoform of SCF are unable to maintain hematopoiesis and by the fact that bound SCF displayed on the surface of transgenic cells is better able to maintain c-kit activation than soluble SCF. Further work has shown that recombinant SCF molecules, which include a surface-binding domain, are more potent than their soluble equivalent. It is generally assumed that such an elegant approach is necessary to provide the correct molecular orientation and avoid the pitfalls of random cross-linking or the denaturation associated with the adsorption of proteins to surfaces. However, in this work we demonstrate that SCF physisorbed to tissue culture plastic (TCP) is not only bioactive, but more potent than the soluble equivalent. By contrast, cross-linking of SCF via free amines is shown to compromise its bioactivity. These observations demonstrate that simple surface modification solutions cannot be discounted and with the advent of low-cost pharmaceutical grade proteins, they should not be.

Doubek, M., F. Folber, et al. (2009). "Autologous hematopoietic stem cell transplantation in adult acute lymphoblastic leukemia: still not out of fashion." Ann Hematol **88**(9): 881-7.

The role of autologous hematopoietic stem cell transplantation (autoHSCT) in adult acute lymphoblastic leukemia (ALL) is still unclear. We retrospectively analyzed the results of the autoHSCT and maintenance therapy, with oral 6-mercaptopurine and methotrexate, in comparison to conventional-dose chemotherapy in the consolidation treatment of adult ALL and lymphoblastic lymphoma (LBL). The patients, with HLA identical sibling donor, underwent allogeneic transplantation, while the others were treated with autoHSCT and maintenance therapy with oral 6-mercaptopurine and methotrexate, or by conventional-dose chemotherapy (patient's decision, no autologous hematopoietic stem cells harvest). Sixty consecutive adult patients (median age 35.2 years; range 17.3 to 70.7) with ALL (n = 52), LBL (n = 7), and acute biphenotypic leukemia (n = 1) were treated in our center from 1997 to 2007. Patients treated with chemotherapy alone (n = 35) had a shorter median progression-free survival (PFS) compared to patients who underwent autoHSCT plus maintenance therapy (n = 18), 8.4 and 46.8 months, respectively (p = 0.017). Patients treated with chemotherapy alone had also a shorter median overall survival (OS) compared to patients treated with autoHSCT: 13.0 vs. 46.8 months (p = 0.046). The differences remained statistically significant even after excluding patients with Ph positivity. We can conclude that, in our case, autoHSCT followed by maintenance chemotherapy is a good option for adult patients with ALL and, in standard-risk and high-risk patients, provides more favorable OS and PFS rates compared to patients treated by chemotherapy alone. However, we are aware of the fact that our analysis may have been distorted by the fact that the analysis is retrospective, that treatment with autoHSCT was based on patient's decision, and that chemotherapy may have been administered to negatively selected patients.

Duarte, R. F., N. Schmitz, et al. (2008). "Haematopoietic stem cell transplantation for patients with primary cutaneous T-cell lymphoma." Bone Marrow Transplant **41**(7): 597-604.

There is no standard of care for patients with advanced forms of mycosis fungoides, Sezary syndrome and other less common subtypes of primary cutaneous T-cell lymphoma. Expected median survival for such patients with conventional therapy is only 1-4 years. As a result of such dismal prognosis,

alternative strategies based on autologous and allogeneic transplantation have been explored, and a relatively small number of case reports and small series communicated to date have provided evidence for the potential role of haematopoietic transplantation in these patients. High-dose radio-chemotherapy and autologous rescue has been shown to induce complete responses in the majority of patients. Disappointingly though, these responses were very short-lived in nearly all cases. On the contrary, the use of allogeneic transplantation has provided solid evidence for an allogeneic GVL effect in these malignancies. In fact, more than two-thirds of the allogeneic transplant recipients reported in the literature experienced long-term durable remissions of more than 3 years, which would appear superior to the expected median survival for such patients. This review summarizes the experience published to date in this setting and highlights main areas that would merit further investigation.

Duncan, I. D. (2005). "Oligodendrocytes and stem cell transplantation: their potential in the treatment of leukoencephalopathies." J Inherit Metab Dis **28**(3): 357-68.

Cell transplantation is being extensively explored as a means of treating many human degenerative diseases. The leukodystrophies are examples of neurological disorders where new therapeutic strategies, either cellular or molecular, will be required to repair the central nervous system (CNS) of affected patients. Much hope is being pinned on the use of human embryonic stem (ES) cells as the exogenous source of neurons and glia to replace dysfunctional or dying cells in the CNS. In the case of leukoencephalopathies, the goal is to generate oligodendrocytes or other myelinating cells such as Schwann cells from ES cells, to myelinate or remyelinate CNS axons on transplantation. Experimental data suggests that mouse ES cells have this capacity, but at present differentiation of oligodendrocytes in sufficient numbers from human ES cells is not possible. It may in fact be more feasible to isolate oligodendrocytes from human neural stem cells derived from the fetal brain, but the source of these is in short supply and, like that of ES cells, is ethically controversial. None the less, it appears certain that either of these two sources will eventually give rise to sufficient numbers of neural stem cells or oligodendrocyte progenitors that have greater capacity for repair than such cells derived from the adult brain. Once the primary technical issues concerning human ES cell differentiation have been overcome, the most likely first clinical target will be Pelizaeus-Merzbacher disease. However, widespread dissemination of cells throughout the CNS may be required for functional improvement; hence diseases such as adrenoleukodystrophy may also be considered as therapeutic targets.

Echavarria, M., F. Herrera, et al. (2003). "Cyclic recovery of adenovirus in a stem cell transplant recipient: an inverse association with graft-versus-host disease." Bone Marrow Transplant **31**(4): 301-3.

Adenovirus (AdV) infections have been increasingly recognized as significant pathogens that may cause severe morbidity and mortality among stem cell transplant (SCT) recipients. AdV can cause localized infections such as hemorrhagic cystitis (HC), pneumonia, hepatitis and also disseminated disease that can lead to death. We report a case of severe hemorrhagic cystitis in a SCT recipient who died 83 days after transplant. In this patient, AdV recovery was not constantly detected. In fact, fluctuations of the AdV detection in leukocytes and urine were observed by culture and PCR. When analyzing this viral cyclic recovery with different signs or symptoms in the patient, we observed an inverse association with the presence of acute graft-versus-host disease (GVHD). Whether these fluctuations represent donor-derived reactivity, indirectly manifested by the presence of GVHD, requires further study. This is the first case describing a dynamic pattern of AdV replication in leukocytes and urine samples from a patient with severe HC and the temporal correlation with GVHD.

Elkabetz, Y. and L. Studer (2008). "Human ESC-derived neural rosettes and neural stem cell progression." Cold Spring Harb Symp Quant Biol **73**: 377-87.

Neural stem cells (NSCs) are defined by their ability to self-renew while retaining differentiation potential toward the three main central nervous system (CNS) lineages: neurons, astrocytes, and oligodendrocytes. A less appreciated fact about isolated NSCs is their narrow repertoire for generating specific neuron types, which are generally limited to a few region-specific subtypes such as GABAergic and glutamatergic neurons. Recent studies in human embryonic stem cells have identified a novel neural stem cell stage at which cells exhibit plasticity toward generating a broad range of neuron types in response to appropriate developmental signals. Such rosette-stage NSCs (R-NSCs) are also distinct from other NSC populations by their specific cytoarchitecture, gene expression, and extrinsic growth requirements. Here, we discuss the properties of R-NSCs within the context of NSC biology and define some of the key questions for future investigation. R-NSCs may represent the first example of a NSC population capable of recreating the full cellular diversity of the

developing CNS, with implications for both basic stem cell biology and translational applications in regenerative medicine and drug discovery.

Elo, A., J. Immanen, et al. (2009). "Stem cell function during plant vascular development." Semin Cell Dev Biol **20**(9): 1097-106.

While many regulatory mechanisms controlling the development and function of root and shoot apical meristems have been revealed, our knowledge of similar processes in lateral meristems, including the vascular cambium, is still limited. Our understanding of even the anatomy and development of lateral meristems (procambium or vascular cambium) is still relatively incomplete, let alone their genetic regulation. Research into this particular tissue type has been mostly hindered by a lack of suitable molecular markers, as well as the fact that thus far very few mutants affecting plant secondary development have been described. The development of suitable molecular markers is a high priority in order to help define the anatomy, especially the location and identity of cambial stem cells and the developmental phases and molecular regulatory mechanisms of the cambial zone. To date, most of the advances have been obtained by studying the role of the major plant hormones in vascular development. Thus far auxin, cytokinin, gibberellin and ethylene have been implicated in regulating the maintenance and activity of cambial stem cells; the most logical question in research would be how these hormones interact during the various phases of cambial development.

Epstein, J. (1997). "Myeloma stem cell phenotype. Implications for treatment." Hematol Oncol Clin North Am **11**(1): 43-9.

The phenotypic heterogeneity of myeloma cells in fact delineates a differentiation process that appears to be an integral part of the disease process. Immature myeloma cells interact with their microenvironment differently than do the more mature cells. As a result of this interaction, the immature cells display different responses to chemotherapy than do the mature cells. Addressing this issue by tailoring treatment to target immature as well as mature myeloma cells may change dramatically the outcome of treatment. The ability to define the myeloma clone by molecular genetic techniques has markedly increased the ability to detect clonal cells. This technique provides a most sensitive tool for monitoring elimination of tumor cells; however, the role of the early clonal B cells identified through the use of ASO-PCR in the disease process needs to be clarified. Currently, a great deal of effort is directed towards development of treatment protocol that will eliminate all clonal cells, and a method of purging clonal cells from harvested mobilized peripheral stem cells. Understanding the biologic significance of early clonal B cells in myeloma will allow for a more rational approach to curative treatment.

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

The general utility of a novel, fluorescence-based procedure for assessing gene transfer and expression has been demonstrated using hematopoietic stem and progenitor cells. Lineage-depleted hematopoietic cells were isolated from the bone marrow or fetal livers of acid sphingomyelinase-deficient mice, and retrovirally transduced with amphotropic or ecotropic vectors encoding a normal acid sphingomyelinase (ASM) cDNA. Anti-c-Kit antibodies were then used to label stem- and progenitor-enriched cell populations, and the Bodipy fluorescence was analyzed in each group after incubation with a Bodipy-conjugated sphingomyelin. Only cells expressing the functional ASM (ie, transduced) could degrade the sphingomyelin, thereby reducing their Bodipy fluorescence as compared with nontransduced cells. The usefulness of this procedure for the in vitro assessment of gene transfer into hematopoietic stem cells was evaluated, as well as its ability to provide an enrichment of transduced stem cells in vivo. To show the value of this method for in vitro analysis, the effects of retroviral transduction using ecotropic versus amphotropic vectors, various growth factor combinations, and adult bone marrow versus fetal liver stem cells were assessed. The results of these studies confirmed the fact that ecotropic vectors were much more efficient at transducing murine stem cells than amphotropic vectors, and that among the three most commonly used growth factors (stem cell factor [SCF] and interleukins 3 and 6 [IL-3 and IL-6]), SCF had the most significant effect on the transduction of stem cells, whereas IL-6 had the most significant effect on progenitor cells. In addition, it was determined that fetal liver stem cells were only approximately twofold more "transducible" than stem cells from adult bone marrow. Transplantation of Bodipy-selected bone marrow cells into lethally irradiated mice showed that the number of spleen colony-forming units that were positive for the retroviral vector (as determined by polymerase chain reaction) was 76%, as compared with 32% in animals that were transplanted with cells that were nonselected. The methods described within this manuscript are particularly useful for evaluating

hematopoietic stem cell gene transfer in vivo because the marker gene used in the procedure (ASM) encodes a naturally occurring mammalian enzyme that has no known adverse effects, and the fluorescent compound used for selection (Bodipy sphingomyelin) is removed from the cells before transplantation.

Faber, E., V. Koza, et al. (2007). "Reduced-intensity conditioning for allogeneic stem cell transplantation in patients with chronic myeloid leukemia is associated with better overall survival but inferior disease-free survival when compared with myeloablative conditioning - a retrospective study of the Czech National Hematopoietic Stem Cell Transplantation Registry." Neoplasma **54**(5): 443-6.

Allogeneic stem cell transplantation (AlloSCT) has been currently recommended in the treatment of patients with chronic myeloid leukemia (CML) as a second option after imatinib failure or in selected group of patients with high-risk CML and low risk for transplant-related mortality. The actual role of reduced-intensity conditioning (RIC) before AlloSCT in CML patients has not been yet conclusively established. The Czech National Hematopoietic Stem Cell Transplantation Registry has conducted a retrospective analysis of all patients (n=29) transplanted after RIC from the Registry database containing 295 patients with CML transplanted in the Czech Republic in years 1988-2005 and compared them with patients at comparable age (median age 48.3 and 50.6 years, respectively; p=0.587) transplanted during the same period of time using conventional myeloablative conditioning (n=26). Survival advantage of patients transplanted after RIC has been confirmed by log rank test (p=0.036) despite the fact that the relapse rate was significantly higher in RIC group (44.8% versus 0%). Both groups did not differ significantly in the use of voluntary unrelated donors, type of the grafts and in incidence of acute graft versus host disease (GVHD). However, there were trends for higher risk of CML and higher use of unrelated donors in the myeloablative group while peripheral stem cell grafts and chronic GVHD were observed more frequently in the RIC group. Transplant-related mortality was the leading cause of death in both groups of patients. Our results should be interpreted with caution because they may be influenced by small groups of subjects and also the impact of patients with high EBMT risk score on inferior survival in the myeloablative group cannot be fully eliminated. More retrospective and prospective studies are needed to elucidate the actual role of RIC before AlloSCT for CML.

Fassas, A. and G. L. Mancardi (2008). "Autologous hemopoietic stem cell transplantation for multiple sclerosis: is it worthwile?" Autoimmunity **41**(8): 601-10.

High-dose immunosuppressive chemotherapy or total body irradiation followed by autologous transplantation of hemopoietic stem cells (ASCT) was introduced in the treatment of active, progressing, and therapy-resistant multiple sclerosis (MS) in 1995. Since then, more than 300 patients have undergone this sort of treatment worldwide and the European Group for Blood and Marrow Transplantation (EBMT) published on two occasions, in 2002 and in 2006, the results of collective analyses performed in 85 and in 183 cases, respectively. In most communications the results were reported favorable with some cases showing spectacular recoveries and also probabilities of long-lasting disease stability, between 60 and 80% at three years after transplant. Of great interest was the fact that magnetic resonance imaging studies invariably showed that the inflammation in the central nervous system resolved and gadolinium-enhancing lesions were completely abolished or markedly reduced. These results appear superior to those yielded by standard therapies but this superiority needs to be demonstrated by comparative studies, such as the EBMT-launched ASTIMS trial. Moreover, ASCT is a rather toxic procedure associated with a mortality risk of 2-3%. Therefore, it is not a treatment for the general population of MS patients but only for selected cases that do not respond to standard therapies and worsen rapidly, i.e. in situations where benefits are expected to counterbalance morbidity and mortality risks. Nevertheless, certain issues seem to have cleared up: ASCT should be used early, during the inflammatory phase of the disease; very high-intensity pre-transplant conditioning regimens increase toxicity but do not seem to increase efficacy compared to intermediate-intensity regimens; the results are dramatic and life-saving in resistant, so-called "malignant" cases; ASCT does not only cause debulking of autoreactive clones but it also brings about qualitative immunological changes that might eventually establish immunologic self-tolerance; the progression of brain atrophy appears to slow down with time; with the implementation of proper patient-selection criteria, the risks of morbidity and mortality can be minimized.

Fehrer, C. and G. Lepperdinger (2005). "Mesenchymal stem cell aging." Exp Gerontol **40**(12): 926-30.

Stem cells are located throughout the adult body of higher organisms, supporting a continuous renewal and repair of tissues. Unique abilities of stem cells are self-renewal and multipotential differentiation. It is, therefore, of critical importance

for an organism to maintain and control quantity and quality of stem cells within a given pool. Otherwise, when something goes awry within a stem cell, it is likely to have far-reaching effects. Mesenchymal stem cells (MSC) derived from various sources such as bone marrow or fat have been expanded in culture and differentiated in vitro into several lineages such as adipocytes, osteocytes or chondrocytes. In particular, aged human MSC show a decline in differentiation potential as well as in proliferation rate. The latter most likely reflects the fact that aged MSC suffer from eroded telomeres. Besides the individual age of the cell, stem and progenitor cell functions are influenced by the cellular environment, i.e. the niche and the architecture of the tissue, they reside in. This contribution reviews current knowledge about MSC aging (in vitro or in vivo), and respective difficulties for tissue engineering and stem cell therapy.

Frei, E., 3rd, G. Ara, et al. (2000). "Double high-dose chemotherapy with stem cell rescue (HD-SCR) in patients with breast cancer - effect of sequence." Cancer Chemother Pharmacol **45**(3): 239-46.

INTRODUCTION: A preliminary analysis of our double high-dose chemotherapy with stem cell rescue (HD-SCR) clinical trial for breast cancer, and preclinical cross-resistant studies, suggested that melphalan (M) adversely affected response to subsequent chemotherapy, i.e., that the sequence of alkylating agents (AAs) might affect response. We, therefore, constructed and examined preclinical models to determine whether prior exposure to M, in fact, adversely affected response to other therapy. PURPOSE: The purpose of the study was to determine whether the sequence of AAs, specifically the prior use of M, adversely affected response to subsequent treatment. METHODS: The methods employed were the following: (1) Human tumor cell lines rendered resistant by in vitro sequential exposure to five different AAs were developed. The resistant cell lines were examined for cross-resistance to alkylating and other agents. (2) In vivo studies in the p388 mouse leukemia for resistance and cross-resistance among the AAs. (3) In vivo studies of the effect of sequence of AAs on response in mice bearing EMT6 breast cancer. (4) The double transplant model was developed in the mouse and the sequence of high-dose AAs was studied. (5) Biochemical and reverse transcriptase-polymerase chain reaction (RT-PCR) studies of the various resistant tumor cell lines. RESULTS: (1) The in vitro human tumor cells resistant to M were cross-resistant in 57% of tests to other AAs. In contrast, resistance for other AAs crossed to other agents in only 10 to 20% of tests. (2) The in vivo studies of p388 indicated that resistance to M commonly crossed to other AAs and many non-AAs. (3) The results for the mouse breast cancer (EMT6) studies of the sequence of AAs again indicated that M employed first markedly reduced responsiveness to subsequent treatment, particularly with AAs. (4) The double transplant model: again, M first markedly reduced response to other agents. (5) The in vitro resistant human tumor cell lines, particularly the breast cancer cell line MCF7, were found to contain high concentrations of glutathione S1 transferase gamma, which is consistent with that mechanism being responsible for resistance. CONCLUSION: The sequence of alkylating agent treatment may substantially influence response. Melphalan, particularly, produces resistance that commonly crosses to the other AAs. Mechanistic studies indicate significant changes in glutathione S1 transferase, a known mechanism for broadly based resistance to AAs.

Frostesjo, L., I. Holm, et al. (1997). "Interference with DNA methyltransferase activity and genome methylation during F9 teratocarcinoma stem cell differentiation induced by polyamine depletion." J Biol Chem **272**(7): 4359-66.

When ornithine decarboxylase, the initial and highly regulated enzyme in polyamine biosynthesis, is irreversibly inactivated by alpha-difluoromethylornithine, F9 teratocarcinoma stem cells are depleted of putrescine and spermidine and as a result differentiate into a cell type which phenotypically resembles the parietal endoderm cells of the early mouse embryo. Simultaneously the level of decarboxylated S-adenosylmethionine (dcAdoMet), the aminopropyl group donor in spermidine and spermine synthesis, increases dramatically, as the aminopropyl group acceptor molecules (putrescine and spermidine) become limiting. When this excessive accumulation of dcAdoMet is prevented by specific inhibition of the AdoMet decarboxylase activity, the differentiative effect is counteracted, despite the fact that the extent of polyamine depletion remains almost identical. Therefore, it may be concluded that dcAdoMet plays an important role in the induction of differentiation. Moreover, this key metabolite acts as a competitive inhibitor of DNA methyltransferase and is therefore capable of interfering with the maintenance methylation of newly replicated DNA. During the course of F9 cell differentiation, the highly methylated genome is gradually demethylated, and its pattern of gene expression is changed. Our present findings, that the DNA remains highly methylated and that the differentiative process is counteracted when the build-up of dcAdoMet is prevented, provide strong evidence for a causative relation between the level of

dcAdoMet and the state of DNA methylation as well as cell differentiation.

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

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

Goldstone, A. H. (1998). "The case for and against high-dose therapy with stem cell rescue for early poor prognosis Hodgkin's disease in first remission." Ann Oncol **9 Suppl 5**: S83-5.

After 10 years we are still not clear whether dose escalation with stem cell transplantation is relevant for some patients with poor prognosis Hodgkin's disease in first remission. Some of the problems relating to the controversy relate to the fact that the definition of high risk Hodgkin's disease in terms of prognostic factors is only now in 1998 being delineated properly. It is also possible that some of the dose escalation in lymphoma has taken place without an adequate amount of conventional therapy beforehand. It may be possible that dose escalation should be added to an adequate amount of conventional chemotherapy not integrated in a conventional regimen thus shortening it. Newer studies from the German Hodgkin's Disease Study Group, i.e. HD9, may be suggesting that conventional chemotherapy is producing good results in poor prognosis patients and thus negating the need for dose escalation and stem cell transplantation.

Graziano, A., R. d'Aquino, et al. (2008). "Scaffold's surface geometry significantly affects human stem cell bone tissue engineering." J Cell Physiol **214**(1): 166-72.

In this study, we have observed dental pulp stem cells (SBP-DPSCs) performances on different scaffolds, such as PLGA 85:15, hydroxyapatite chips (HA) and titanium. Stem cells were challenged with each engineered surface, either in plane cultures or in a rotating apparatus, for a month. Gingival fibroblasts were used as controls. Results showed that stem cells exerted a different response, depending on the different type of textured surface: in fact, microconcavities significantly affected SBP-DPSC differentiation into osteoblasts, both temporally and quantitatively, with respect to the other textured surfaces. Actually, stem cells challenged with concave surfaces differentiated quicker and showed nuclear polarity, an index of secretion, cellular activity and matrix formation. Moreover, bone-specific proteins were significantly expressed and the obtained bone tissue was of significant thickness. Thus, cells cultured on the concave textured surface had better cell-scaffold interactions and were induced to secrete factors that, due to their autocrine effects, quickly lead to osteodifferentiation, bone tissue formation, and vascularization. The worst cell performance was obtained using convex surfaces, due to the scarce cell proliferation on to the scaffold and the poor matrix secretion. In conclusion, this study stresses that for a suitable and successful bone tissue reconstruction the surface texture is of paramount importance.

Gritti, A. and L. Bonfanti (2007). "Neuronal-glial interactions in central nervous system neurogenesis: the neural stem cell perspective." Neuron Glia Biol **3**(4): 309-23.

Essentially, three neuroectodermal-derived cell types make up the complex architecture of the adult CNS: neurons, astrocytes and oligodendrocytes. These elements are endowed with remarkable morphological, molecular and functional heterogeneity that reaches its maximal expression during development when stem/progenitor cells undergo progressive changes that drive them to a fully differentiated state. During this period the transient

expression of molecular markers hampers precise identification of cell categories, even in neuronal and glial domains. These issues of developmental biology are recapitulated partially during the neurogenic processes that persist in discrete regions of the adult brain. The recent hypothesis that adult neural stem cells (NSCs) show a glial identity and derive directly from radial glia raises questions concerning the neuronal-glial relationships during pre- and post-natal brain development. The fact that NSCs isolated in vitro differentiate mainly into astrocytes, whereas in vivo they produce mainly neurons highlights the importance of epigenetic signals in the neurogenic niches, where glial cells and neurons exert mutual influences. Unravelling the mechanisms that underlie NSC plasticity in vivo and in vitro is crucial to understanding adult neurogenesis and exploiting this physiological process for brain repair. In this review we address the issues of neuronal/glial cell identity and neuronal-glial interactions in the context of NSC biology and NSC-driven neurogenesis during development and adulthood in vivo, focusing mainly on the CNS. We also discuss the peculiarities of neuronal-glial relationships for NSCs and their progeny in the context of in vitro systems.

Guan, Y. J., X. Wang, et al. (2007). "Increased stem cell proliferation in the spinal cord of adult amyotrophic lateral sclerosis transgenic mice." J Neurochem **102**(4): 1125-38.

Harnessing the regenerative potential of the central nervous system to repopulate depleted cellular populations from endogenous stem cells would be a novel approach for the treatment of neurological diseases resulting from cell death. Consequently, understanding if and how the central nervous system is capable of such regeneration would determine if such an approach is feasible. In this report, we provide evidence of widespread regenerative response in the spinal cord of amyotrophic lateral sclerosis transgenic mice. However, this regenerative response appears to be largely unproductive. We demonstrate that there is significantly increased gliogenesis, but an absence of convincing neurogenesis. The fact that the neurodegenerative process stimulates a regenerative response suggests that the adult spinal cord has at least limited ability for regeneration. Further studies will determine if this endogenous regenerative process can be enhanced and directed so as to slow or even reverse the natural progression of this devastating disease.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" Curr Stem Cell Res Ther **4**(3): 168-77.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of

pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

Henon, P. R. (1993). "Peripheral blood stem cell transplantation: critical review." Int J Artif Organs **16 Suppl 5**: 64-70.

Autologous blood stem cell transplantations have been increasingly performed worldwide for almost ten years in place of autologous bone marrow transplantation and even of allogenic bone marrow transplantation. Several crucial issues were the subjects of impassioned controversies. Some of them are now satisfactorily answered while others still remain unresolved. First, it is now possible to conclude today that peripheral blood stem cells (PBSC) are undoubtedly capable of restoring short term hematopoiesis when reinfused after myeloablative therapy as well as even more rapidly than bone marrow stem cells, provided that they have been previously collected in sufficient amounts. On the opposite, it is still impossible to firmly prove that their very immature CD34+ cell subset, although in vitro functionally and phenotypically almost identical to their marrow counterpart, is actually responsible for sustained long term hematopoietic recovery, even if it is likely that these cells play a key role. Most of the time, using chemotherapy alone or a combination of chemotherapy and cytokine(s), mobilizing regimens allow collection of appropriate yields of PBSC with only a small number of apheresis cycles, provided that a sufficient number of residual stem cells remains to be stimulated, when, on the contrary, collection in steady-state is time-consuming and does not provide further accelerated post transplant hematopoietic recovery. It was initially hypothesized that PBSC could have a lower likelihood of tumoral contamination compared with bone marrow. In fact, biological as well as clinical data are discordant and probably depend largely on the type of disease, its evolutive history and its way of dissemination.(ABSTRACT TRUNCATED AT 250 WORDS)

Intawicha, P., Y. W. Ou, et al. (2009). "Characterization of embryonic stem cell lines derived from New Zealand white rabbit embryos." Cloning Stem Cells **11**(1): 27-38.

The purposes of this study were to examine technical details in deriving and maintaining rabbit embryonic stem (rES) cell lines and to analyze their characteristics. When STO cells were used as feeder cells, no rES cell lines were established using either intact blastocysts or inner cell masses (ICMs). On the mouse embryonic fibroblasts (MEF) feeder, rES cell lines were efficiently (24%) derived. Addition of leukemia inhibitory factor (LIF) to the cells cultured on the MEF feeders further increased the derivation efficiency (57%) of rES cells. The fact that LIF induced serine-phosphorylation of STAT3 suggested LIF-dependent maintenance of rES cells. Most of the rES cell lines expressed AP, SSEA-4, Oct4, TRA-1-60, and TRA-1-81. Western blot or RT-PCR analysis also confirmed the expression of Oct4, Nanog, and Sox2. When induced to form EBs in vitro or injected to the severe combined immunodeficiency (SCID) mice, the rES cells generated embryoid bodies (EBs) and teratomas with three germ layers expressing the marker genes including MAP2, Desmin, and GATA4, respectively. In conclusion, rabbit ES cell lines can be efficiently established using our current protocols with LIF supplement. These ES cells express pluripotent stem cell markers and retain their capability to differentiate into different tissue cells. Furthermore, rES cells depend on LIF for self-renewal, likely via the JAK-STAT pathway.

Ito, K., R. Bernardi, et al. (2009). "A novel signaling network as a critical rheostat for the biology and maintenance of the normal stem cell and the cancer-initiating cell." Curr Opin Genet Dev **19**(1): 51-9.

Recent advances from our own group and others have defined a novel PML/PTEN/Akt/mTOR/FoxO signaling network, and highlighted its critical importance in oncogenesis as well as in the functional regulation of normal stem cell and cancer-initiating cell (CIC) biology. These findings are of great importance in cancer therapy in view of the fact that this network is amenable to pharmacological modulation at multiple levels. The integrated analysis of these data allows us to propose a new provocative working model whereby the aberrant superactivation of Akt/mTOR signaling elicits built-in cellular fail-safe mechanisms that could be effectively utilized for cancer treatment to extinguish the CICs pool. In this review, we will discuss these recent findings, this working model, and their therapeutic implications.

Ivanovic, Z. (2009). "Hypoxia or in situ normoxia: The stem cell paradigm." J Cell Physiol **219**(2): 271-5.

Although O(2) concentrations are considerably lowered in vivo, depending on the tissue

and cell population in question (some cells need almost anoxic environment for their maintenance) the cell and tissue cultures are usually performed at atmospheric O(2) concentration (20-21%). As an instructive example, the relationship between stem cells and micro-environmental/culture oxygenation has been recapitulated. The basic principle of stem cell biology, "the generation-age hypothesis," and hypoxic metabolic properties of stem cells are considered in the context of the oxygen-dependent evolution of life and its transposition to ontogenesis and development. A hypothesis relating the self-renewal with the anaerobic and hypoxic metabolic properties of stem cells and the actual O(2) availability is elaborated ("oxygen stem cell paradigm"). Many examples demonstrated that the cellular response is substantially different at atmospheric O(2) concentration when compared to lower O(2) concentrations which better approximate the physiologic situation. These lower O(2) concentrations, traditionally called "hypoxia" represent, in fact, an in situ normoxia, and should be used in experimentation to get an insight of the real cell/cytokine physiology. The revision of our knowledge on cell/cytokine physiology, which has been acquired ex vivo at non physiological atmospheric (20-21%) O(2) concentrations representing a hyperoxic state for most primate cells, has thus become imperious.

Jacobs, S. R., P. B. Jacobsen, et al. (2007). "Evaluation of the functional assessment of cancer therapy cognitive scale with hematopoietic stem cell transplant patients." J Pain Symptom Manage **33**(1): 13-23.

The current study evaluated a newly developed self-report measure of cognitive complaints with cancer patients, the Functional Assessment of Cancer Therapy Cognitive Scale (FACT-Cog). Six or 12 months following hematopoietic stem cell transplantation, participants completed a psychosocial assessment that included the FACT-Cog and a neuropsychological assessment. Using a criterion of two or more times a week, an average of 12 of a total of 50 items were endorsed as complaints on the FACT-Cog. FACT-Cog total, domain, and subscale scores were significantly correlated with measures of depression, fatigue, anxiety, and physical and mental well-being. FACT-Cog scores, with the exception of one subscale, Other People Noticed Deficits, were not significantly correlated with cognitive performance. In general, the FACT-Cog and a commonly used measure of cognitive complaints (European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire-C30 Cognitive Functioning Scale) demonstrated similar psychometric properties. However, the FACT-Cog assesses broader aspects of cognitive complaints, thereby providing greater information about the types of cognitive complaints patients are experiencing.

Jeras, M. (2002). "The role of in vitro alloreactive T-cell functional tests in the selection of HLA matched and mismatched haematopoietic stem cell donors." Transpl Immunol **10**(2-3): 205-14.

Acute graft vs. host (GVH) disease and graft rejection are most frequently caused by undetected or disregarded genetically based disparities between the donor and recipient of bone marrow derived haematopoietic stem cells (HSC). Incompatibilities in extremely polymorphic human leukocyte antigens (HLA), and in certain cases also minor histocompatibility antigens, represent the most important driving force of such unwanted events, threatening the successful outcome of haematopoietic stem cell transplantation (HSCT). The complexity of HLA polymorphism can be precisely and elegantly detected at the genomic level by several polymerase chain reaction (PCR) based techniques that have strongly backed up its predecessor, the far less informative classical serological typing. By applying these modern technologies, we gain the deepest insight into HLA allelic specificities and thus the possibility to, for example, trace and recruit unrelated histocompatible donors for a given patient. In the case when exclusively related intrafamilial HSC donors are being considered, we are confined to the fact that only 25-30% of patients can expect a completely HLA identical donor to be found within core or extended family members. The number of related as well as unrelated donors can be increased if certain HLA mismatches are accepted. When doing so, the precise definition of disparate histocompatibilty antigens between the patient and a possible donor should be carried out. But this does not give us the information about the functional immunogenicity of such differences. Therefore, in vitro functional assays, quantitating the alloreactive potential of lymphocyte T subsets, the central immunocompetent cells, are more than necessary. By evaluating mixed lymphocyte reaction (MLR), the analysis of helper T cell precursor (HTLp) and cytotoxic T cell precursor (CTLp) frequencies, the allogeneic impact of class II and class I HLA mismatches between a donor and graft recipient can be assessed and permissive disparities defined.

Kozak, T. and I. Rychlik (2002). "Developments in hematopoietic stem-cell transplantation in the treatment of autoimmune diseases." Isr Med Assoc J **4**(4): 268-71.

Intractable forms of autoimmune diseases follow a rapid course, with a significantly shortened life expectancy sometimes comparable to that of malignant diseases. Immunoablative therapy, including high dose cytotoxic agents and hematopoietic autologous stem-cell rescue, was recently introduced as an aggressive approach to treat autoimmune diseases that have a rapid course and are resistant to conventional therapy. The most frequent indication for this type of treatment is multiple sclerosis, seconded by systemic sclerosis. The results of immunoablative treatment with documented responses in both diseases are encouraging. The data are mature enough to begin comparative randomized studies of immunoablative versus conventional treatment to validate the benefit of the aggressive approach. A randomized trial involving SSc was recently launched (ASTIS) and a trial involving MS is in preparation. Considerably less experience with immunoablative treatment has been gained in systemic lupus erythematosus, rheumatoid arthritis, and other disorders with an autoimmune pathophysiology. Autologous hematopoietic stem cell transplantation in humans offers more long-lasting immunosuppression than reeducation of lymphocytes. In fact, allogeneic transplantation may replace the whole immune system. However, this attractive approach is still associated with considerable morbidity and mortality and is not yet justified for treatment of autoimmune diseases. Non-myeloablative allogeneic transplantation and sub-myeloblative high dose cyclophosphamide without stem cell support are alternative approaches that could be explored in pilot studies.

Kruger, W. H., R. J. Hornung, et al. (2001). "Practices of infectious disease prevention and management during hematopoietic stem cell transplantation: a survey from the European group for blood and marrow transplantation." J Hematother Stem Cell Res **10**(6): 895-903.

Protocols for the prevention of infections after allogeneic or autologous hemopoietic stem cell transplantations are usual. A questionnaire was sent out to the members of the European Group for Bone and Marrow Transplantation (EBMT) in the spring of 1999. A total of 308 questionnaires from 180 centers were returned. Both allogeneic and autologous transplantation was reported from 128 centers, and allogeneic or autologous transplantation alone from four and 48 centers, respectively. Hemopoietic stem cell transplantation is still a domain of university hospitals. Intensive measures of isolation are usual. Allotransplantation is commonly performed in single rooms with HEPA-filtered air on special wards. However, even in the autologous setting, extensive measures of isolation are commonly used. This observation could be explained by historical developments and by the fact that nearly all centers for allogeneic transplantation perform both allogeneic and autologous transplantations, and thus similar measures are used in both settings. Other measures are usual but heterogeneous due to lack of clinical trials in this field. Drug prophylaxis during transplantation is mostly carried out with quinolones, TMP/SMZ, fluconazole, acyclovir, and pentamidine. Differences in drug prophylaxis after engraftment and in the use of different venous accesses do reflect the requirements after engraftment and discharge of patients from the transplant unit. The intensity of measures in autologous stem cell reinfusion does not reflect the development during the last decade. For cost effectiveness and convenience, it is necessary to abolish senseless measures. It is necessary to investigate anti-infectious strategies separately for allogeneic transplantation and other modalities of anti-cancer treatment in future.

Laino, G., A. Graziano, et al. (2006). "An approachable human adult stem cell source for hard-tissue engineering." J Cell Physiol **206**(3): 693-701.

Stem cells were obtained from deciduous dental pulp of healthy subjects, aged 6-10 years. This stem cell population was cultured, expanded, and specifically selected, detecting using a FACsorter, c-kit, CD34, and STRO-1 antigen expression. Then, c-kit+/CD34+/STRO-1+ cells were replaced in the culture medium added of 20% FBS, leading to osteoblast differentiation. In fact, these cells, after a week, showed a large positivity for CD44, osteocalcin, and RUNX-2 markers. To achieve an adipocytic differentiation, cells, after sorting, were challenged with dexamethason 10(-8) mM in the same culture medium. To obtain myotube fusion, sorted cells were co-cultured in ATCC medium with mouse myogenic C2C12 cells and, after a week, human stem cell nuclei were found to be able to fuse, forming myotubes. Differentiated osteoblasts, as assessed by a large positivity to several specific antibodies, after 30 days of culture and already in vitro, started to secrete an extracellular mineralized matrix, which, 2 weeks later, built a considerable number of 3D woven bone samples, which showed a strong positivity to alkaline phosphatase (ALP), alizarin red, calcein, other than to specific antibodies. These bone samples, after in vivo transplantation into immunosuppressed rats, were remodeled in a lamellar bone containing entrapped osteocytes. Therefore, this study provides strong evidence that human deciduous dental pulp is an approachable "niche" of stromal stem cells, and that it is an ideal source of osteoblasts, as well as of

mineralized tissue, ready for bone regeneration, transplantation, and tissue-based clinical therapies.

Ljungman, P., K. N. Ward, et al. (2001). "Respiratory virus infections after stem cell transplantation: a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation." Bone Marrow Transplant **28**(5): 479-84.

Community-acquired respiratory virus infections are a cause of mortality after stem cell transplantation (SCT). A prospective study was performed at 37 centers to determine their frequency and importance. Additional cases were also collected to allow the analysis of risk factors for severe infection. Forty episodes were collected in the prospective study and 53 additional episodes through subsequent case collection. The frequency of documented respiratory virus infections was 3.5% among 819 allogeneic and 0.4% among 1154 autologous SCT patients transplanted during the study period. The frequency of lower respiratory tract infections (LRTI) was 2.1% among allogeneic and 0.2% among autologous SCT patients. The mortality within 28 days from diagnosis of a respiratory viral infection was 1.1% among allogeneic SCT while no autologous SCT patient died. The deaths of five patients (0.6%) were directly attributed to a respiratory virus infection (three RSV; two influenza A). On multivariate analysis, lymphocytopenia increased the risk for LRTI (P = 0.008). Lymphocytopenia was also a significant risk factor for LRTI in patients with RSV infections. The overall mortality in RSV infection was 30.4% and the direct RSV-associated mortality was 17.4%. For influenza A virus infection, the corresponding percentages were 23.0% and 15.3%. This prospective study supports the fact that community-acquired respiratory virus infections cause transplant-related mortality after SCT.

Martins, C., J. F. Lacerda, et al. (2005). "Autologous stem cell transplantation in acute myeloid leukemia. Factors influencing outcome. A 13 year single institution experience." Acta Med Port **18**(5): 329-37.

We report our results of autologous stem cell transplantation (SCT) in patients with AML during the last 13 years. Between August 1990 and December 2003, 42 patients with acute myeloid leukemia (AML) received an autologous SCT. Patients were classified as standard risk if first complete remission (CR) was induced after one or two chemotherapy regimens and the white blood cell count at presentation was below 50,000/mL (n=12), while patients requiring more than two induction regimens to attain first CR and with CR2 ou more advanced disease and/or had a higher white blood cell count at presentation were defined as high risk (n=30). Twenty one patients were transplanted in first CR. The median patient age was 24 years (range, 2-56 years), and the median time interval from diagnosis to autologous SCT was 9 months (range 3-87 months). The conditioning regimen for SCT consisted of busulfan (BU) 16 mg/kg and melfalan (MEL) 180 mg/m2 (BUMEL) in 17 (40%) patients and busulfan 16 mg/kg and VP-16 60 mg/kg (BUVP16) in 22 (52%) patients. Three patients received a different conditioning regimen with BCNU 300 mg/m2, VP16 2 g/m2 and melphalan 160 mg/m2 (BEM). Twenty five (60%) patients received bone marrow (BM), 11 (26%) patients received peripheral blood stem cells (PBSC) and 6 patients (14%) received BM plus PBSC. With a median follow-up of 7 years, the 13 year overall survival (OS) and diseasefree survival (DFS) of all patients is 52% and 40%, respectively. In univariate analysis, males had a significantly superior DFS than females (55% vs 22%, p=0.003), and patients younger than 15 years of age had significantly superior OS and DFS than older patients (50% vs 35%, p=0.05; and 50% vs 28%, p=0.03, respectively). Patients with FAB M3 subtype also had a superior OS than the other FAB subtypes (100% vs 44%, p=0.05). There was a strong statistical correlation between risk group and survival. In fact, the patients with standard risk had a superior OS and DFS than those with high risk disease (67% vs 23%, p=0.0004; and 50% vs 27%, p=0.01, respectively). When patients with FAB M3 disease were excluded from the analysis, the group with standard risk continue to have a superior OS and DFS (67% vs 13%, p=0.008; and 50% vs 14%, p=0.02, respectively). We conclude that autologous SCT is an effective treatment in AML with the possibility of long survivorship, particularly in patients with standard risk disease.

Mayani, H. (2003). "A glance into somatic stem cell biology: basic principles, new concepts, and clinical relevance." Arch Med Res **34**(1): 3-15.

Somatic stem cells are undifferentiated cells with a high capacity for self-renewal that can give rise to one or more specialized cell types with specific functions in the body. Profound characterization of these cells has been difficult due to the fact that their frequency in different tissues of the body is extremely low; furthermore, their identification is not based on their morphology but on immunophenotypic and functional assays. Nevertheless, significant advances in the study of these cells at both cellular and molecular levels have been achieved during the last decade. The majority of what we know concerning somatic stem cell biology has come from work on hematopoietic stem cells. More recently, however,

there has been a great amount of information on neural and epithelial stem cells. The importance of stem cell research has gone beyond basic biology and is currently contributing to the development of new medical approaches for treatment of hematologic, neurologic, autoimmune, and metabolic disorders (cellular therapy).

Mehta, J. and S. Singhal (2008). "Current status of autologous hematopoietic stem cell transplantation in myeloma." Bone Marrow Transplant **42 Suppl 1**: S28-S34.

High-dose melphalan with autologous hematopoietic SCT (HSCT) improves response rates and survival in myeloma. This is despite the fact that unlike other hematologic malignancies treated with high-dose therapy and autotransplantation, autografted myeloma patients continue to relapse several years after transplantation and the procedure is not curative in the majority of patients. However, patients surviving for several years with essentially normal quality of life may be considered to be 'operationally cured.' Also, unlike with other hematologic malignancies relapsing after an autograft, recurrent disease can be treated with novel agents or repeat high-dose chemotherapy and autologous or allogeneic HSCT--and long-term survival is seen in a number of patients after relapse. Although tandem transplantation is clearly superior to a single autograft, it is unclear if this should be offered to all patients routinely or only to those not attaining CR after one transplant. It is also unclear if novel agents should be used before transplantation or reserved for relapse. Despite their excellent activity, there is no evidence that novel agents such as thalidomide, bortezomib and lenalidomide can replace high-dose chemotherapy and HSCT, and the best strategy is to use all options in all eligible patients at appropriate stages of the disease.

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

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

Melone, M. A., M. Giuliano, et al. (2009). "Genes involved in regulation of stem cell properties: studies on their expression in a small cohort of neuroblastoma patients." Cancer Biol Ther **8**(13): 1300-6.

Cancer stem cells have been isolated from many tumors. Several evidences prove that neuroblastoma contains its own stem cell-like cancer cells. We chose to analyze 20 neuroblastoma tumor samples in the expression of 13 genes involved in the regulation of stem cell properties to evaluate if their misregulation could have a clinical relevance. In several specimens we detected the expression of genes belonging to the OCT3/SOX2/NANOG/KLF4 core circuitry that acts at the highest level in regulating stem cell biology. This result is in agreement with studies showing the existence of malignant stem cells in neuroblastoma. We also observed differences in the expression of some stemness-related genes that may be useful for developing new prognostic analyses. In fact, preliminary data suggests that the presence/absence of UTF1 along with differences in BMI1 mRNA levels could distinguish low grade neuroblastomas from IV stage tumors.

Menasche, P. (2009). "Stem cell therapy for heart failure: are arrhythmias a real safety concern?" Circulation **119**(20): 2735-40.

So far, the major safety issue raised by the use of stem cells for cardiac repair has been the occurrence of ventricular arrhythmias, particularly after skeletal myoblast transplantation. Although one cannot refute a potential intrinsic arrhythmogenicity of stem cells, primarily related to their common lack of electromechanical integration into the recipient myocardium, it is also important to recognize that patients eligible for cell replacement therapy are prone to develop arrhythmias because of their underlying ischemic heart disease. Another confounding factor is the method used for the intramyocardial delivery of

the cells, which can cause enough inflammatory tissue damage to further increase ventricular irritability on top of an already high baseline level. Thus any strategy designed to minimize the risk of stem cell-associated ventricular arrhythmias should take into account, besides the cell-specific ability to appropriately couple with host cardiomyocytes, the method of cell transfer and the nature of the myocardial environment targeted for cell engraftment. A more accurate characterization of the baseline risk of arrhythmias in these patients would thus be helpful for better assessing the respective contribution of the donor cells and the host myocardium to these complications. The risk-to-benefit ratio of stem cell therapy will finally have to be revisited in light of the fact that because this baseline risk is usually high, most of these patients will in any way be fitted with an implantable defibrillator.

Minamino, T. and I. Komuro (2008). "Vascular aging: insights from studies on cellular senescence, stem cell aging, and progeroid syndromes." Nat Clin Pract Cardiovasc Med **5**(10): 637-48.

Epidemiological studies have shown that age is the chief risk factor for atherosclerotic cardiovascular diseases, but the molecular mechanisms that underlie the increase in risk conferred by aging remain unclear. Evidence suggests that the cardiovascular repair system is impaired with advancing age, thereby inducing age-associated cardiovascular dysfunction. Such impairment could be attributable to senescence of cardiovascular tissues at the cellular level as a result of telomere shortening, DNA damage, and genomic instability. In fact, the replicative ability of cardiovascular cells, particularly stem cells and/or progenitor cells, has been shown to decline with age. Recently, considerable progress has been made in understanding the pathogenesis of human progeroid syndromes that feature cardiovascular aging. Most of the genes responsible have a role in DNA metabolism, and mutated forms of these genes result in alterations of the response to DNA damage and in decreased cell proliferation, which might be common features of a phenotype of aging. Here we review the cardiovascular research on cellular senescence, stem cell aging, and progeroid syndromes and discuss the potential role of cellular senescence in the mechanisms underlying both normal aging and premature aging syndromes.

Mocini, D., F. Colivicchi, et al. (2005). "Stem cell therapy for cardiac arrhythmias." Ital Heart J **6**(3): 267-71.

Clinical studies suggest that stem cell transplantation (SCT) is feasible and has the potential for beneficial effects in several cardiac affections, including myocardial infarction and advanced heart failure. However, concern exists about the possible occurrence of serious arrhythmias after SCT, even if such complication has been shown only in case of skeletal myoblast transplantation. SCT might induce arrhythmias by several mechanisms, such as electrotonic stimulation of cardiac cells, electrical heterogeneity of action potentials during stem cell differentiation process, increased nerve sprouting, and local tissue injury induced by intramyocardial injection. As a matter of fact, the use of endothelial progenitor cells from the peripheral blood or of stem cells from bone marrow has not been associated with any significant cardiac rhythm disturbance. Recently, a new opportunity for SCT has emerged: the development of a biological cardiac pacemaker. Both gene therapy and cell therapy have been used in this new perspective. In fact, at present, the transformation of a normal cardiomyocyte in a pacemaker cell can be obtained in animal models by the injection of a plasmid or virus, incorporating the gene encoding for specific proteins. This procedure transforms cardiomyocytes in transgenic cells that may show an overexpression of beta2-adrenergic receptors, or abnormal membrane ion channels. As an alternative, genetically modified mesenchymal stem cells can be delivered within the heart and engraft to develop a biological pacemaker. To date, several studies have been performed in different animal models employing both cell and gene therapy. However, complex problems concerning safety and efficacy require a solution before we can move to the step of clinical evaluation in human beings.

Molero, A. E., S. Gokhan, et al. (2009). "Impairment of developmental stem cell-mediated striatal neurogenesis and pluripotency genes in a knock-in model of Huntington's disease." Proc Natl Acad Sci U S A **106**(51): 21900-5.

The pathogenesis of Huntington's disease (HD) remains elusive. The identification of increasingly early pathophysiological abnormalities in HD suggests the possibility that impairments of striatal medium spiny neuron (MSN) specification and maturation may underlie the etiology of HD. In fact, we demonstrate that HD knock-in (Hdh-Q111) mice exhibited delayed acquisition of early striatal cytoarchitecture with aberrant expression of progressive markers of MSN neurogenesis (Islet1, DARPP-32, mGluR1, and NeuN). Hdh-Q111 striatal progenitors also displayed delayed cell cycle exit between E13.5-15.5 (BrdU birth-dating) and an enhanced fraction of abnormal cycling cells in association with expansion of the pool of intermediate progenitors and over expression of the core pluripotency (PP) factor, Sox2. Clonal analysis further

revealed that Hdh-Q111 neural stem cells (NSCs) displayed: impaired lineage restriction, reduced proliferative potential, enhanced late-stage self-renewal, and deregulated MSN subtype specification. Further, our analysis revealed that in addition to Sox2, the core PP factor, Nanog is expressed within the striatal generative and mantle regions, and in Hdh-Q111 embryos the fraction of Nanog-expressing MSN precursors was substantially increased. Moreover, compared to Hdh-Q18 embryos, the Hdh-Q111 striatal anlagen exhibited significantly higher levels of the essential PP cofactor, Stat3. These findings suggest that Sox2 and Nanog may play roles during a selective window of embryonic brain maturation, and alterations of these factors may, in part, be responsible for mediating the aberrant program of Hdh-Q111 striatal MSN specification and maturation. We propose that these HD-associated developmental abnormalities might compromise neuronal homeostasis and subsequently render MSNs more vulnerable to late life stressors.

Moller, E., G. Stenman, et al. (2008). "POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands." J Pathol **215**(1): 78-86.

The EWSR1 gene is known to play a crucial role in the development of a number of different bone and soft tissue tumours, notably Ewing's sarcoma. POU5F1 is expressed during early development to maintain the totipotent status of embryonic stem and germ cells. In the present study, we report the fusion of EWSR1 and POU5F1 in two types of epithelial tumours: hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands. This finding not only broadens considerably the spectrum of neoplasms associated with EWSR1 fusion genes but also strengthens the evidence for shared pathogenetic mechanisms in the development of adnexal and salivary gland tumours. Reminiscent of the previously reported fusion genes involving EWSR1, the identified transcript is predicted to encode a chimeric protein consisting of the EWSR1 amino-terminal domain and the POU5F1 carboxy-terminal domain. We assessed the transcriptional activation potential of the chimera compared to the wild-type proteins, as well as activation of transcription through the oct/sox composite element known to bind POU5F1. Among other POU5F1 target genes, this element is present in the promoter of NANOG and in the distal enhancer of POU5F1 itself. Our results show that although the chimera is capable of significant transcriptional activation, it may in fact convey a negative regulatory effect on target genes.

Montes, R., G. Ligero, et al. (2009). "Feeder-free maintenance of hESCs in mesenchymal stem cell-conditioned media: distinct requirements for TGF-beta and IGF-II." Cell Res **19**(6): 698-709.

A paracrine regulation was recently proposed in human embryonic stem cells (hESCs) grown in mouse embryonic fibroblast (MEF)-conditioned media (MEF-CM), where hESCs spontaneously differentiate into autologous fibroblast-like cells to maintain culture homeostasis by producing TGF-beta and insulin-like growth factor-II (IGF-II) in response to basic fibroblast growth factor (bFGF). Although the importance of TGF-beta family members in the maintenance of pluripotency of hESCs is widely established, very little is known about the role of IGF-II. In order to ease hESC culture conditions and to reduce xenogenic components, we sought (i) to determine whether hESCs can be maintained stable and pluripotent using CM from human foreskin fibroblasts (HFFs) and human mesenchymal stem cells (hMSCs) rather than MEF-CM, and (ii) to analyze whether the cooperation of bFGF with TGF-beta and IGF-II to maintain hESCs in MEF-CM may be extrapolated to hESCs maintained in allogeneic mesenchymal stem cell (MSC)-CM and HFF-CM. We found that MSCs and HFFs express all FGF receptors (FGFR1-4) and specifically produce TGF-beta in response to bFGF. However, HFFs but not MSCs secrete IGF-II. Despite the absence of IGF-II in MSC-CM, hESC pluripotency and culture homeostasis were successfully maintained in MSC-CM for over 37 passages. Human ESCs derived on MSCs and hESCs maintained in MSC-CM retained hESC morphology, euploidy, expression of surface markers and transcription factors linked to pluripotency and displayed in vitro and in vivo multilineage developmental potential, suggesting that IGF-II may be dispensable for hESC pluripotency. In fact, IGF-II blocking had no effect on the homeostasis of hESC cultures maintained either on HFF-CM or on MSC-CM. These data indicate that hESCs are successfully maintained feeder-free with IGF-II-lacking MSC-CM, and that the previously proposed paracrine mechanism by which bFGF cooperates with TGF-beta and IGF-II in the maintenance of hESCs in MEF-CM may not be fully extrapolated to hESCs maintained in CM from human MSCs.

Morrissey, C. O., P. G. Bardy, et al. (2008). "Diagnostic and therapeutic approach to persistent or recurrent fevers of unknown origin in adult stem cell transplantation and haematological malignancy." Intern Med J **38**(6b): 477-95.

Persistent or recurrent fevers of unknown origin (PFUO) in neutropenic patients on broad-spectrum antibiotics have traditionally been treated

with empirical antifungal therapy (EAFT). The lack of survival benefit seen with the use of amphotericin B deoxycholate (AmB-D) as EAFT has been attributed to its toxicities. More recently, newer, less toxic and more expensive antifungal agents such as the lipid formulations of AmB, the newer azoles (fluconazole, itraconazole and voriconazole) and caspofungin have been analysed in a number of EAFT trials. Compared with AmB-D the newer agents have superior safety but are of equivalent efficacy. This lack of survival advantage is related to the fact that the trigger for commencement of EAFT is late and non-specific. Thus, alternative approaches are required. New sensitive serological and molecular tests for the detection of Aspergillus antigens and genomic DNA have been developed and evaluated in accuracy studies. These tests have been incorporated into management strategies (i.e. pre-emptive strategies) to direct antifungal therapy. The pre-emptive approach has been shown to be safe and feasible but its impact on clinically important patient outcomes such as survival is less clear. Other advances include the introduction of effective, non-toxic mould-active antifungal prophylaxis and patient risk-group stratification. In this paper we provide new evidence-based algorithms for the diagnosis and treatment of PFUO in adult patients undergoing stem cell transplantation and chemotherapy for haematological malignancy which incorporate these newer diagnostic tests and are directed by the risk category of the patient and type of antifungal prophylaxis the patient is receiving.

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

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

Nardelli-Haefliger, D. and M. Shankland (1992). "Lox2, a putative leech segment identity gene, is expressed in the same segmental domain in different stem cell lineages." Development **116**(3): 697-710.

The segmented tissues of the adult leech arise from a set of five, bilaterally paired embryonic stem cells via a stereotyped sequence of cell lineage. Individual segments exhibit unique patterns of cell differentiation, and previous studies have suggested that each stem cell lineage establishes at least some aspects of its own segmental specificity autonomously. In this paper, we describe a putative leech segment identity gene, Lox2, and examine its expression in the various stem cell lineages. Both sequence analysis and the segmental pattern of Lox2 expression suggest a specific homology to the fruitfly segment identity genes Ubx and abdA. In situ hybridization reveals a cellular accumulation of Lox2 RNA over a contiguous domain of 16 midbody segments (M6-M21), including postmitotic neurons, muscles and the differentiating genitalia. Lox2 transcripts were not detected at the stage when segment identities are first established, suggesting that Lox2 gene products may not be part of the initial specification process. Individual stem cell lineages were labeled by intracellular injection of fluorescent tracers, and single cell colocalization of lineage tracer and hybridization reaction product revealed expression of Lox2 RNA in the progeny of four different stem cells. The segmental domain of Lox2 RNA was very similar in the various stem cell lineages, despite the fact that some stem cells generate one founder cell/segment, whereas other stem cells generate two founder cells/segment.

Nardi, N. B. (2005). "All the adult stem cells, where do they all come from? An external source for organ-specific stem cell pools." Med Hypotheses **64**(4): 811-7.

Stem cells can self-renew and maintain the ability to differentiate into mature lineages. Whereas the "stemness" of embryonic stem cells is not discussed, the primitiveness of a stem cell type within adult organisms is not well determined. Data presently available are either inconclusive or controversial regarding two main topics: maintenance or senescente

of the adult stem cell pool; and pluripotentiality of the cells. While programmed senescence or apoptosis following uncorrected mutations represent no problem for mature cells, the maintenance of the stem cell pool itself must be assured. Two different mechanisms can be envisaged for that. In the first mechanism, which is generally accepted, stem cells originate during ontogeny along with the organ which they are responsible for, and remain there during all the lifespan of the organism. Several observations derived from recent reports allow the suggestion of a second mechanism. These observations include: organ-specific stem cells are senescent; adult stem cells circulate in the organism; stem cell niches are essential for the existence and function of stem cells; adult stem cells can present lineage markers; embryo-like, pluripotent stem cells are present in adult organisms, as shown by the development of teratomas, tumors composed of derivatives of the three germ layers; and the fact that the gonads may be a reservoir of embryo-like, pluripotent stem cells in adult organisms. The second mechanism for the maintenance of adult stem cells compartments implies a source external to the organ they belong, consisting of pluripotent, embryo-like cells of unrestricted life span, presenting efficient mechanisms for avoiding or correcting mutations and capable to circulate in the organism. According to this model, primitive stem cells exist in a specific organ in adult organisms. They undergo asymmetrical divisions, which originate one "true" stem cell and another one which enters the pool of adult stem cells, circulating through the entire organism. Upon signals liberated by organ-specific niches, this cell becomes activated to express lineage-specific genes, homes to that particular organ and repopulates its stem cell compartment, differentiating thus in what is seen as the organ-specific stem cell. The gonads are the natural candidates for homing the primitive stem cells in adult organisms. The model proposed in this work for the maintenance of organ-specific stem cell pools from an external source, represented by primitive, embryo-like germinal stem cells present in testes and ovaries, may contribute to the more complete understanding of this complex issue.

Necas, E. and F. Hauser (1982). "Analysis of the effect of hydroxyurea on stem cell (CFU-s) kinetics." Cell Tissue Kinet **15**(1): 39-47.

Hydroxyurea induces profound changes in the pluripotential haemopoietic stem cell (CFU-s) kinetics. The main feature of these changes is a synchronous entry of resting G0 CFU-s into the cell cycle. The analysis of the passage of the CFU-s cohort through the cell cycle has been largely based on the examination of the fraction of CFU-s which synthesize DNA in the S phase of the cell cycle. This analysis has, however, been hampered by the fact that both the sensitivity of the S phase CFU-s to hydroxyurea and their sensitivity in the [3H]thymidine suicide technique vary as the cells pass through the S phase. Methods which overcome these difficulties have been used in the experiments presented in this paper. It was demonstrated that hydroxyurea kills only about 80% of the S phase CFU-s. The sensitivity to hydroxyurea gradually decreases as the cells approach the middle part of the S phase and increases again as the cells enter the late portions of the S phase. The degree of CFU-s synchrony at the point of entry into and exit from, the S phase has been established. Mathematical analysis of the available data suggests that CFU-s pass through the S phase with a mean transit time of 4.79 hr (standard deviation, 1.45 hr). Hydroxyurea, administered in vivo, blocks CFU-s in the late G1 phase. The duration of this G1-S block, induced by a dose of 1000 mg of hydroxyurea per kg body weight, is approximately 2 hr. The CFU-s in the middle of the S phase, which survive hydroxyurea administration, are also blocked in their passage through the S phase. These cells, however, seem to finish the S phase with a delay of approximately 2 hr.

Neff, T., B. C. Beard, et al. (2006). "Survival of the fittest: in vivo selection and stem cell gene therapy." Blood **107**(5): 1751-60.

Stem cell gene therapy has long been limited by low gene transfer efficiency to hematopoietic stem cells. Recent years have witnessed clinical success in select diseases such as X-linked severe combined immunodeficiency (SCID) and ADA deficiency. Arguably, the single most important factor responsible for the increased efficacy of these recent protocols is the fact that the genetic correction provided a selective in vivo survival advantage. Since, for most diseases, there will be no selective advantage of gene-corrected cells, there has been a significant effort to arm vectors with a survival advantage. Two-gene vectors can be used to introduce the therapeutic gene and a selectable marker gene. Efficient in vivo selection strategies have been demonstrated in clinically relevant large-animal models. Mutant forms of the DNA repair-enzyme methylguanine methyltransferase in particular have allowed for efficient in vivo selection and have achieved sustained marking with virtually 100% gene-modified cells in large animals, and with clinically acceptable toxicity. Translation of these strategies to the clinical setting is imminent. Here, we review how in vivo selection strategies can be used to make stem cell gene therapy applicable to the treatment of a wider scope of genetic diseases and patients.

Neri, Q. V., T. Takeuchi, et al. (2009). "Treatment options for impaired spermatogenesis: germ cell transplantation and stem-cell based therapy." Minerva Ginecol **61**(4): 253-9.

Advances in infertility treatment had the most extraordinary breakthrough with the birth of the first in vitro fertilization baby in 1978. Fourteen years later, intracytoplasmic sperm injection has been introduced for the treatment of male factor infertility. Intra cytoplasmic sperm injection in combination with testicular sperm extraction has allowed men with azoospermia to father children. In fact, as long as a fully developed spermatozoon is identified, it can be utilized or can even be duplicated to inseminate several oocytes while providing information on its genomic content. There are, however, men who are suffering from spermatogenic arrest, where no post-meiotic germ cells are retrieved, and therefore, unable to generate their own offspring. More recently, the successful isolation and cultivation of spermatogonial stem cells has allowed the exploration of their biological characteristics and their application in therapeutic approaches following transplantation or in vitro maturation. Finally, men diagnosed with germ cell aplasia can only be treated by donor or de novo generated gametes. In the past several years, we have attempted to manufacture gametes by inducing haploidization of somatic cells and more recently, generating sperm-like cells through embryonic stem cell differentiation.

Nirmalanandhan, V. S., M. S. Levy, et al. (2006). "Effects of cell seeding density and collagen concentration on contraction kinetics of mesenchymal stem cell-seeded collagen constructs." Tissue Eng **12**(7): 1865-72.

Our group has been engineering cell-scaffold constructs to improve tendon repair by contracting mesenchymal stem cells (MSCs) in collagen gels and then evaluating their repair potential in wound sites in rabbits. Because the construct's initial conditions may influence the ultimate repair outcome, this two-part study sought to distinguish which factors most influence contraction kinetics in culture. (1)We optically determined if varying cell-to-collagen ratio significantly affected construct contraction. Temporal changes in construct area were monitored up to 168 h for 4 cell-to-collagen ratios (HK = 0.04, LK = 0.08, HM = 0.4, and LM = 0.8, where H, L = 2.6, 1.3 mg/mL collagen and K,M = 0.1, 1 million cells/mL, respectively).A mathematical model was created with terms that represent the different combinations of cell densities and collagen concentrations in order to predict the contraction kinetics as a function of time. Highly significant differences in construct areas were found among all 4 ratios after 8 h of contraction with the exception of the LK (0.08) vs. HM(0.4) conditions. This similar pattern raised the question of whether cell density or collagen concentration more influenced these events. (2) To isolate these effects, the contraction kinetics of the HM construct were compared to those of a new construct (L5K) with equivalent cell-to-collagen ratio (0.4) but half the cell density (500 K MSCs/mL) and half the collagen concentration (1.3 mg/mL). The L5K construct contracted significantly faster and more completely than the HM construct but no differently than the LM construct. These results indicate that above a threshold value of cell density, percentage reductions in collagen concentration influence contraction kinetics more than equivalent percentage increases in cell seeding density. The fact that our model successfully predicted intermediate time points of contraction suggests its utility for examining other cell and collagen densities. Controlling scaffold as well as cellular initial conditions will be critical in achieving our goal of functional tissue engineering (FTE) a successful tendon repair.

Ohashi, H., C. Kato, et al. (2005). "Leukemic relapse in the central nervous system after allogeneic stem cell transplantation with complete remission in the bone marrow and donor-type chimerism: report of two cases." Am J Hematol **79**(2): 142-6.

We studied two cases with leukemia that relapsed in the central nervous system (CNS) after allogeneic stem cell transplantation. One patient underwent peripheral blood stem cell transplantation (SCT) from a related, yet haplotype-mismatched, donor for chronic myelomonocytic leukemia. She was kept in complete remission (CR) in the bone marrow (BM) for 7 months, until relapse in the cerebrospinal fluid (CSF) was evident. In the other patient, with acute lymphoblastic leukemia, systemic relapse occurred when he was still on immunosuppression 6 months after SCT from an unrelated donor. After induction chemotherapy following cessation of immunosuppression, the BM examination proved CR. During consolidation chemotherapy, however, he developed leukemic dissemination in the CSF, despite the fact that the BM was in CR. Chimerism status in the BM mononuclear cells and fractionated peripheral blood (PB) cells (granulocytes, T-lymphocytes, and the others) was assessed by short tandem repeat analysis. In both patients, the BM cells and all the fractions of the PB cells proved donor-type chimeras. These results seem to suggest that the graft-versus-leukemia effects might not be as effective in the CNS as in the BM, even when complete T-lymphoid chimerism is achieved.

Okuno, K., Y. Horie, et al. (2009). "Epstein-Barr virus associated post-transplant Hodgkin lymphoma in an adult patient after cord blood stem cell transplantation for acute lymphoblastic leukemia." J Clin Exp Hematop **49**(1): 45-51.

Post-transplant lymphoproliferative disorder (PTLD) is one of the most important complications of solid organ transplantation or hematopoietic stem cell transplantation. Most PTLDs are associated with Epstein-Barr virus (EBV) infection. Although post-transplant Hodgkin lymphoma (HL) is included in PTLD, there have been no studies in the literature on adult cases of post-transplant HL after cord blood stem cell transplantation (CBSCT). Three years and eight months after CBSCT, the enlarged cervical lymph node was histologically diagnosed as EBV associated post-transplant HL, which showed immunophenotypes of classical HL and latency type II EBV infection. She underwent chemotherapy, and has survived 4 years and 6 months after CBSCT. Differential diagnosis of post-transplant HL with good prognosis and HL-like PTLD with aggressive behavior is important, and immunohistochemical methods were useful and essential for it. The source of EBV associated HL in this case will be discussed.

Ostronoff, L. K., E. Kremmer, et al. (2008). "Canine stem cell factor augments expression of matrix metalloproteinase-9 by CD34 cells." Cytotherapy **10**(2): 193-202.

BACKGROUND: Canine models have proved to be predictive of clinical findings in human bone marrow (BM) transplantation; consequently, the utilization of dogs is an excellent tool for supporting therapeutic purposes. Considering the role of growth factors in homing and mobilization of hematopoietic progenitors, the aim of this work was to evaluate whether canine stem cell factor (cSCF) contributes to matrix metalloproteinase (MMP)-9 secretion by CD34 cells. METHODS: The study was carried out in a cell population selected by immunomagnetic techniques using the anti-canine CD34 monoclonal antibody (MAb) 3B4 produced by us. Secretion of MMP-9 was evaluated by zymography. RESULTS: Analyzes of canine CD34(+) cells guaranteed that the MAb 3B4 was optimum for selecting a subset population with defined characteristics of primitive hematopoietic cells. The isolated cells were able to proliferate onto irradiated pre-established stroma, giving rise to mature neutrophils. There was also a 20-fold enrichment in the long-term culture-initiating cell content when the isolated population was added to irradiated cultures, with respect to the starting mononuclear cell population. DISCUSSION: We have provided the first evidence that canine BM CD34(+) cells constitutively express MMP-9 and the role of cSCF in up-regulating the secretion of this enzyme. The fact that cSCF augments expression of MMP-9 together with the ability of the isolated CD34(+)cells to proliferate onto irradiated pre-established stroma enables further investigations to determine whether the secretion of MMP-9 mediated by cSCF is one of the factors that enhance migration, homing and repopulation of primitive hemopoietic cells.

Oyan, B., Y. Koc, et al. (2005). "Successful salvage with high-dose sequential chemotherapy coupled with in vivo purging and autologous stem cell transplantation in 2 patients with primary refractory mantle cell lymphoma presenting in the leukemic phase." Int J Hematol **81**(2): 155-8.

We report the results of an aggressive salvage regimen in 2 patients with advanced-stage leukemic-phase mantle cell lymphoma who were refractory to previous conventional therapies. We combined multiple phases of a cytoreductive regimen including rituximab and sequential high-dose treatment with autologous stem cell transplantation (ASCT). The regimen consisted of a debulking phase with fludarabine, idarubicin, high-dose cytarabine, and high-dose methotrexate; a mobilization and in vivo purging phase with rituximab, cyclophosphamide, and granulocyte colony-stimulating factor; high-dose sequential chemotherapy with etoposide, mitoxantrone, and melphalan followed by ASCT; and, finally, posttransplantation consolidation with rituximab for treatment of minimal residual disease. With this regimen, these 2 refractory patients with multiple poor prognostic factors are in complete remission at 41 and 42 months following transplantation. Although the fact that these 2 patients are still in remission beyond 3 years after ASCT is encouraging, we need a longer follow-up to comment on their long-term survival.

Paek, H. J., L. J. Moise, et al. (2005). "Origin of insulin secreted from islet-like cell clusters derived from murine embryonic stem cells." Cloning Stem Cells **7**(4): 226-31.

Islet-like cell clusters (ILCCs) were derived from murine embryonic stem cells using a slightly modified version of the protocol originally described by Lumelsky et al. in 2001. Analysis with enzyme-linked immunosorbent assays (ELISAs) that distinguish human from murine insulin demonstrated that insulin released from these ILCCs, upon initial in vitro glucose challenge, was of non-murine origin and in fact corresponded to the species of insulin, human or bovine, that had been added to the culture media used to derive ILCCs. This finding convincingly supports the hypothesis that ILCCs are not synthesizing insulin de novo, but rather simply

regurgitating insulin taken up during tissue culture. In further experiments, ILCCs were derived in media in which insulin had been replaced by IGF-I with which it shares a common signaling pathway. These ILCCs failed to release any detectable insulin. In contrast, ILCCs produced by various protocols stained positive (dithizone and immunoselective antibodies) for intracellular insulin and, in some cases, C-peptide. Despite the presence of at least some level of de novo, synthesized insulin in ILCCs, the majority of insulin released by ILCCs was sequestered from the exogenous medium.

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.

Paulus, U., C. S. Potten, et al. (1992). "A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation." Cell Prolif **25**(6): 559-78.

The control mechanisms involved in regeneration of murine intestinal crypts after perturbations are presently not well understood. The existence of some feedback signals from the cells on the villus to the cells in the crypt has been suggested. However, some recent experimental data point to the fact that regeneration in the crypt starts very early after perturbation, at a time when the villus cell population has hardly changed. In particular, this early cell proliferative activity is seen specifically at the bottom of the crypt, i.e. in the presumed stem cell zone and furthest from the villus. The objective of this study was to investigate whether a new concept of regulation operating solely at the stem cell level could explain the present mass of accumulated data on the post-irradiation recovery, which is an extensively studied perturbation from the experimental point of view. In order to check its validity, the new concept was formalized as a mathematical simulation model thus enabling comparison with experimental data. The model describes the cellular development from stem cells to the mature villus cells. As a basic feature it is assumed that the self-maintenance and the cell cycle activity of the stem cells are controlled by the number of these cells in an autoregulatory fashion. The essential features of the experimental data (i.e. the recovery with time and the consistency between different types of measurements) can be very well reproduced by simulations using a range of model parameters. Thus, we conclude that stem cell autoregulation is a valid concept which could replace the villus crypt feedback concept in explaining the early changes after irradiation when the damage primarily affects the crypt. The question of the detailed nature of the control process requires further investigation.

Peault, B. (1996). "Hematopoietic stem cell emergence in embryonic life: developmental hematology revisited." J Hematother **5**(4): 369-78.

In utero, hematopoiesis takes place initially in the extraembryonic yolk sac, then switches to the liver, thymus, and, finally, bone marrow. This chronologic sequence and the fact that all blood-forming tissues but the yolk sac sustain hematopoiesis after colonization by stem cells of external origin have led to the hypothesis that the whole prenatal and postnatal blood system is founded by yolk sac-derived stem cells. Experimental data recently obtained from bird and mouse embryo models strongly suggest, however, that definitive hematopoiesis is established from an intraembryonic source of stem cells arising in the vicinity of the developing aorta. In agreement, an abundant population of CD34+ primitive hematopoietic cells has been identified in the equivalent area of the human embryo. These novel findings will contribute to our understanding of blood cell homeostasis and may help to further develop therapeutic protocols making use of fetal hematopoietic cells transplanted in utero or in postnatal life.

Pecora, A. L., H. M. Lazarus, et al. (2002). "Breast cancer cell contamination of blood stem cell products in patients with metastatic breast cancer: predictors and clinical relevance." Biol Blood Marrow Transplant **8**(10): 536-43.

The incidence and clinical relevance of tumor cells contaminating the stem cell products of patients with advanced breast cancer treated with high-dose chemotherapy is uncertain because prior studies used small sample sizes and lacked standardization of the immunocytochemistry (ICC) detection method used. We evaluated blood stem cell and bone marrow samples obtained from 535 women with metastatic breast cancer who received high-dose chemotherapy and unmanipulated mobilized blood stem cell support. Of the patients tested, 20.6% and 26.3% had blood stem cell and bone marrow contamination, respectively. Blood stem cell contamination was significantly more frequent in patients with marrow involvement than in patients without marrow involvement (35% versus 18.4%, respectively; P = .009). In fact, according to multivariate analysis results, marrow involvement was the only significant predictor for blood stem cell product contamination. Patients without marrow involvement who had fewer apheresis procedures were also observed to have a significantly lower incidence rate of blood stem cell contamination than patients who had more procedures (P < or = .008), and patients who received combined chemotherapy and cytokine mobilization therapy had less contamination than patients who received cytokine alone (P = .0001). Combined mobilization therapy appears to be associated with a lower incidence of contamination as a result of fewer apheresis procedures rather than through an antitumor effect of chemotherapy (P < or = .001). Patients with ICC-negative blood stem cell products had significantly longer progression-free survival (PFS) and overall survival (OS) than did patients with ICC-positive blood stem cell products (median PFS, 401 versus 291 days, respectively, P = .007; median OS, 1060 versus 697 days, P = .009) . However, multivariate analysis did not reveal any significant independent predictors of survival outcomes. Thus, further study is needed to determine if contaminating tumor cells in the stem cell products of breast cancer patients ever directly impact survival outcomes or are only indicative of residual in vivo disease in high-dose chemotherapy recipients.

Pedrazzoli, P., J. A. Ledermann, et al. (2006). "High dose chemotherapy with autologous hematopoietic stem cell support for solid tumors other than breast cancer in adults." Ann Oncol **17**(10): 1479-88.

Since the early 1980s high dose chemotherapy with autologous hematopoietic stem cell support was adopted by many oncologists as a potentially curative option for solid tumors, supported by a strong rationale from laboratory studies and apparently convincing results of early phase II studies. As a result, the number and size of randomized trials comparing this approach with conventional chemotherapy initiated (and often abandoned before completion) to prove or disprove its value was largely insufficient. In fact, with the possible exception of breast carcinoma, the benefit of a greater escalation of dose of chemotherapy with stem cell support in solid tumors is still unsettled and many oncologists believe that this approach should cease. In this article, we critically review and comment on the data from studies of high dose chemotherapy so far reported in adult patients with small cell lung cancer, ovarian cancer, germ cell tumors and sarcomas.

Pedron, C., L. Madero, et al. (2000). "Short-term follow-up of the nutritional status of children undergoing autologous peripheral blood stem cell transplantation." Pediatr Hematol Oncol **17**(7): 559-66.

A prospective longitudinal study was conducted to analyze the evolution of the nutritional status of 34 children (12 girls and 22 boys), aged 1.5-15.8 years (median age 9.06), undergoing autologous peripheral blood stem cell transplantation (PBSCT). The nutritional status was evaluated at baseline, days +1 and +7, discharge, and day +30 by dietary or parenteral intake, anthropometric and laboratory measurements, and nitrogen balance. At baseline, changes in anthropometric (53%) and biochemical measurements (83%) are frequent but mild. The mean caloric intake was normal. Children with normal values for the anthropometric parameters all had an intake > 80% (p < .01). No correlation was found between the anthropometric and biochemical parameters. During transplantation, significant changes (p < .001) were found for energy intake, albumin, transferrin, and nitrogen balance. Fibronectin, prealbumin, and retinol-binding protein showed only a few changes. All but prealbumin recovered on day +30. No correlation was found between the nutritional status and toxicity or infection in children undergoing autologus PBSCT. The changes in the nutritional status observed at the start of transplantation correlated with the nutrional intake. Anthropometric and biochemical changes are complementary. The results may be ascribable to the fact that the patients in this series had mild malnutrition.

Perseghin, P., M. Dassi, et al. (2003). "Low efficiency of a newly introduced high-density microparticles method for B cell depletion in multiple myeloma patients undergoing autologous hematopoietic stem cell transplantation." J Hematother Stem Cell Res **12**(5): 537-41.

Autologous peripheral blood stem cell (PBSC) transplantation proved to increase complete remission (CR) and DFS in multiple myeloma (MM) patients. CD34(+) cell selection has been used to reduce possible myeloma cell contamination in the graft, but it has not been showed to offer substantial advantages when compared to unpurged grafts; on the contrary, an increase of infectious complications was observed. We investigated the feasibility of a new negative-selection method in this setting. B cell negative selection was performed by using Eligix B cell HDM method. B cell contamination in the yield and in the final product was investigated by flow cytometry. Three patients with newly diagnosed MM entered the study. CD34(+) cell recovery in the three procedures was 73, 97, and 106%, and CD3(+) cell recovery was 88, 86, and 102%, respectively. CD20(+) cell depletion was 100% in all procedures, while CD19(+) cell depletion was 0.37, 1.21, and 0.07 log, respectively. We found an unexpected unreliability and a low efficiency in this B cell depletion method and suggest the need for further extensive testing before its introduction in the preclinical and clinical settings, at least in MM patients. In fact, reasons of such unsatisfactory results are still controversial: platelet contamination/activation in the preselection product, plasma protein interference, reduced CD19 antigen expression on immature B cells, lack of specificity of anti-CD19 monoclonal antibodies, instable binding between anti-CD19-coated high-density microparticles (HDM) and CD19 antigen may, alone or in combination, be involved in the system's low performance.

Perseghin, P., E. Terruzzi, et al. (2009). "Management of poor peripheral blood stem cell mobilization: incidence, predictive factors, alternative strategies and outcome. A retrospective analysis on 2177 patients from three major Italian institutions." Transfus Apher Sci **41**(1): 33-7.

CD34+ peripheral blood hematopoietic stem cells (HSC) are usually collected following mobilization therapy accomplished by using growth factors (GF) such as rHuG-CSF or rHuGM-CSF with or without chemotherapy. A target dose of yielded CD34+ is usually prescribed by the attending physician depending on different protocols, which may include single or double transplantation. HSC collection usually is performed when at least 20 CD34+ HSC/microL are detected by means of flow cytometry. A cumulative dose of at least 2 x 10(6)/Kg/bw CD34+ HSC has been considered as the threshold to allow a prompt and persistent hematopoietic recovery. Unfortunately, this goal is not achieved by the totality of patients undergoing mobilization regimen. In fact, 5-46% of patients who underwent mobilization therapy fail HSC collection due to very low peripheral blood HSC CD34+ count. Patients' characteristics, including age, sex, stage of the underlying disease (complete or partial remission), diagnosis, previously administered radio/chemotherapy regimens, time-lapse from last chemotherapy before mobilization and mobilization schedule (including dose of GF) were considered as possibly predictive of poor or failed mobilization. We performed a retrospective analysis in 2177 patients from three large Italian academic institutions to assess the incidence of poor mobilizers within our patients' series. Therefore, a patient who fails a first mobilization (and when an HLA-compatible related on unrelated donor is not available) could undergo a second attempt either with different mobilization schedule or by using different GF, such as stem cell factor, growth hormone (GH), or more recently newly introduced drugs such as AMD3100, alone or in combination with rHuG- or -rHuGM-CSF. Thus, we investigated the fate of those who failed a first mobilization and subsequently underwent a second attempt or alternative therapeutic approaches.

Phillips, M. I., Y. L. Tang, et al. (2008). "Stem cell therapy for heart failure: the science and current progress." Future Cardiol **4**(3): 285-98.

Cell therapy, particularly with stem cells, has created great interest as a solution to the fact that there are limited treatments for postischemic heart disease and none that can regenerate damaged heart cells to strengthen cardiac performance. From the first efforts with myoblasts to recent clinical trials with bone marrow-derived stem cells, early reports of cell therapy suggest improvement in cardiac performance as well as other clinical end points. Based on these exciting but tentative results, other stem cell types are being explored for their particular advantages as a source of adult stem cells. Autologous adipose-derived stem cells are multilinear and can be obtained relatively easily in large quantities from patients; cardiac-derived stem cells are highly appropriate for engraftment in their natural niche, the heart. Human umbilical cord blood cells are potentially forever young and allogenic adult mesenchymal stem cells appear not to evoke the graft versus host reaction. Human embryonic stem cells are effective and can be scaled up for supply purposes. The recent discovery of induced pluripotentcy in human adult stem cells, with

only three transcription factor genes, opens a whole new approach to making autologous human pluripotent stem cells from skin or other available tissues. Despite the excitement, stem cells may have to be genetically modified with heme oxygenase, Akt or other genes to survive transplantation in a hypoxic environment. Homing factors and hormones secreted from transplanted stem cells may be more important than cells if they provide the necessary stimulus to trigger cardiac regrowth to replace scar tissue. As we await results from larger and more prolonged clinical trials, the science of stem cell therapy in cardiac disease keeps progressing.

Piliponsky, A. M., G. J. Gleich, et al. (2003). "Non-IgE-dependent activation of human lung- and cord blood-derived mast cells is induced by eosinophil major basic protein and modulated by the membrane form of stem cell factor." Blood **101**(5): 1898-904.

The allergic reaction begins with the antigen-induced aggregation of occupied high-affinity IgE receptors expressed on mast cell surface, their activation, and the release of proinflammatory mediators that cause the "early phase" of this process. In addition, mast cell activation induces the onset of a "late phase" reaction characterized by the tissue infiltration of inflammatory cells, mainly eosinophils. We have hypothesized that during the late phase mast cells interact with and are activated by eosinophils. Here we report that highly purified human lung mast cells became responsive to eosinophil major basic protein (MBP) when in coculture with human lung fibroblasts. In addition, cord blood-derived mast cells maintained in coculture with 3T3 fibroblasts released more histamine and prostaglandin D(2) (PGD(2)) compared with cells maintained in suspension. The fibroblast-derived membrane form of stem cell factor (SCF) was found to be involved in the mast cell increased responsiveness to MBP. In fact, cord blood-derived mast cells cocultured with 3T3 in the presence of antisense for SCF or cocultured with fibroblasts that do not express the membrane form of SCF were inhibited in their histamine-releasing activity toward MBP. In addition, this form of SCF induced the expression of a pertussis toxin-sensitive G(i) protein, G(i3) that interacts with MBP to trigger mast cell non-IgE-dependent activation in a manner similar to other cationic compounds such as compound 48/80. Mast cell responsiveness to eosinophil mediators is a potentially novel evidence for an alternative pathway of allergen-independent activation able to contribute to the perpetuation of allergy.

Pirovano, S., L. D. Notarangelo, et al. (2004). "Mutations of the T-cell receptor constant region after in utero stem cell transplantation." Immunogenetics **56**(3): 214-9.

Like the immunoglobulin genes, the T-cell receptor genes are generated by rearrangements of non-contiguous genomic V, D and J regions, but unlike the immunoglobulin genes, somatic hypermutation is an infrequent event in T-cell receptor genes. Here, we describe the occurrence of spontaneous mutations in the constant regions of the T-cell receptor beta chains of T lymphocytes obtained from two babies who underwent in utero transplantation because of severe combined immunodeficiency. In view of the fact that in babies receiving transplants before birth, hematopoietic chimerism is consistently present, the lymphocytes are likely to be under chronic activation, which may represent a relevant biologic stimulus for generating the observed T-cell receptor hypermutation. This possibility is supported by the finding that the highest number of mutations was identified in clonally expanded T cells. These results provide further support indicating that hypermutation of the T-cell receptor genes may indeed occur, given the necessary conditions.

Poulsom, R., M. R. Alison, et al. (2002). "Adult stem cell plasticity." J Pathol **197**(4): 441-56.

Observations made in the last few years support the existence of pathways, in adult humans and rodents, that allow adult stem cells to be surprisingly flexible in their differentiation repertoires. Termed plasticity, this property allows adult stem cells, assumed, until now, to be committed to generating a fixed range of progeny, to switch, when they have been relocated, to make other specialized sets of cells appropriate to their new niche. Reprogramming of some adult stem cells can occur in vivo; the stem cells normally resident in bone marrow appear particularly flexible and are able to contribute usefully to multiple recipient organs. This process produces cells with specialized structural and metabolic adaptations commensurate with their new locations. In a few examples, the degree of support is sufficient to assist or even rescue recipient mice from genetic defects. Some studies provide evidence for the expansion of the reprogrammed cells locally, but in most it remains possible that cells arrive and redifferentiate, but are no longer stem cells. Nevertheless, the fact that appropriately differentiated cells are delivered deep within organs simply by injection of bone marrow cells should make us think differently about the way that organs regenerate and repair. Migratory pathways for stem cells in adult organisms may exist that could be exploited to effect repairs using an individual's own stem cells, perhaps after gene therapy. Logical extensions of this concept

are that a transplanted organ would become affected by the genetic susceptibilities of the recipient, alleles that re-express themselves via marrow-derived stem cells, and that plasticity after bone marrow transplantation would also transfer different phenotypes, affecting important parameters such as susceptibility to long-term complications of diabetes, or the ability to metabolize drugs in the liver. This article reviews some of the evidence for stem cell plasticity in rodents and man.

Prindull, G. (2005). "Hypothesis: cell plasticity, linking embryonal stem cells to adult stem cell reservoirs and metastatic cancer cells?" Exp Hematol **33**(7): 738-46.

Embryonal stem (ES) cells are the earliest ontogenetically identifiable stem cells of the embryo proper for all subsequent mesenchymal stem cells and for highly specialized differentiated cells. This review characterizes, in a working hypothesis, the role of reversible EMT/MET (epithelialmesenchymal transition) as a manifestation of cell plasticity 1) in the development of ES cells to adult stem cells (hematopoietic stem cells) and 2) in metastasizing cancer cells. Animal studies support the concept that EMT/MET is a key manifestation of cell plasticity in the development of ES cells to adult stem cells, and in conversion of localized to metastasizing cancer cells. In fact, ES cells may persist to postnatal life, in cytologically verifiable form and/or within the frame of EMT/MET, as ultimate reservoir for adult stem cells. Furthermore, EMT could possibly serve as a conceptional link between physiologic and pathologic signaling pathways. Clonal confirmation in humans is necessary.

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

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

Quesenberry, P. J., M. Abedi, et al. (2004). "Stem cell plasticity: an overview." Blood Cells Mol Dis **32**(1): 1-4.

The capacity of adult bone marrow cells to convert to cells of other tissues, referred to by many as stem cell plasticity, was the focus of the meeting in Providence entitled "Challenges in the Era of Stem Cell Plasticity". The meeting provided a showcase for the many impressive positive results on tissue restoration including the capacity of purified marrow stem cells to restore heart, skin, and liver function in impaired mice or humans. This area of research has become a center of controversy, although it is not clear why. Calls for clonality, robustness, and function have been shown to be erroneous or premature. A call for clonality (which has been shown nicely in one study) is meaningless on a predefined stem cell population which is intrinsically heterogeneous, as they all are. Robustness means nothing; it all depends on the details of the situation. Function on an organ level is, of course, the goal of many investigators and should not be raised as a limiting consideration. Lastly, fusion has been highlighted as undermining studies with adult stem cells. It, of course, does not. Fusion is simply a means to a final goal, which occurs in certain settings of marrow conversions (transdifferentiation) and not in others. We hypothesize that the conversion phenomena may, in fact, be due to one or several marrow stem cells with broad differentiation potential which can be expressed when the cell is placed in an environment with the appropriate inductive signals. Furthermore, initial events may be relatively rare and significant conversion numbers may be obtained with massive or

ongoing selection. Fusion appears in an initial mechanism in some cases and not in others. Overall, the therapeutic potential of adult marrow stem cells is very intriguing, and successful use therapeutically will probably depend on definition of the most appropriate transplant model and tissue injury.

Quesenberry, P. J., G. Colvin, et al. (2007). "The stem cell continuum: cell cycle, injury, and phenotype lability." Ann N Y Acad Sci **1106**: 20-9.

The phenotype of the hematopoietic stem cell is intrinsically labile and impacted by cell cycle and the effects of tissue injury. In published studies we have shown that there are changes in short- and long-term engraftment, progenitor numbers, gene expression, and differentiation potential with cytokine-induced cell cycle transit. Critical points here are that these changes are reversible and not unidirectional weighing, heavily against a hierarchical model of stem cell regulation. Furthermore, a number of studies have now established that stem cells separated by lineage depletion and selection for Sca-1 or c-kit or low rhodamine and Hoechst staining are in fact a cycling population. Last, studies on Hoechst separated "cycling" stem cells indicates that the observed phenotype shifts relate to phase of cell cycle and are not due to in vitro exposure to cytokines. These data suggest a continuum model of stem cell regulation and further indicate that this model holds for in vivo situations. Observations that marrow cells can convert to various tissue cells under different injury conditions continue to be published despite a small, but influential, number of negative studies. Our studies and those of others indicate that conversions of marrow-derived cells to different tissue cells, such as skeletal muscle and lung, is critically dependent upon multiple variables, the most important of which is the presence of tissue injury. Variables which affect conversion of marrow cells to nonhematopoietic cells after in vivo transplantation include the nature and timing of the injury; marrow mobilization; the marrow cell type infused; the timing of cell infusion and the number of cells infused; the cell cycle state of the marrow cells, and other functional alterations in the marrow cells the treatment of the host mouse separate from specific injury; the mode of cell delivery; and possibly the presence of microvesicles from injured tissue. At least some of the highlighted negative reports on stem cell plasticity appear to be due to a failure to address these variables. Recently, we have observed that irradiated lung releases microvesicles which can enter marrow cells and lead to the marrow cells expressing lung-specific mRNA and protein. This could provide an underlying mechanism for many of the plasticity phenomena. Altogether, marrow appears to represent a highly flexible ever-changing cell system with the capacity to respond to products of injured cells and top repair a broad range of tissues.

Quesenberry, P. J., G. A. Colvin, et al. (2002). "The chiaroscuro stem cell: a unified stem cell theory." Blood **100**(13): 4266-71.

Hematopoiesis has been considered hierarchical in nature, but recent data suggest that the system is not hierarchical and is, in fact, quite functionally plastic. Existing data indicate that engraftment and progenitor phenotypes vary inversely with cell cycle transit and that gene expression also varies widely. These observations suggest that there is no progenitor/stem cell hierarchy, but rather a reversible continuum. This may, in turn, be dependent on shifting chromatin and gene expression with cell cycle transit. If the phenotype of these primitive marrow cells changes from engraftable stem cell to progenitor and back to engraftable stem cell with cycle transit, then this suggests that the identity of the engraftable stem cell may be partially masked in nonsynchronized marrow cell populations. A general model indicates a marrow cell that can continually change its surface receptor expression and thus responds to external stimuli differently at different points in the cell cycle.

Reipert, S., J. A. Hickman, et al. (1996). "DNA inclusions within autolytic cytoplasmic vacuoles of hemopoietic stem cell line FDCP-Mix." J Histochem Cytochem **44**(6): 549-58.

FDCP-Mix, a pluripotent routine hemopoietic stem cell line undergoes internucleosomal cleavage of DNA when induced to apoptosis either by drugs or by withdrawal of growth factor (IL-3), and also displays a pattern of nuclear morphology that is typical for apoptosis. However, increased autolytic activity in the cytoplasm precedes the nuclear changes. For etoposide-treated FDCP-Mix cells, mitochondria were identified as a target for autolytic digestion in large autolytic vacuoles, but during this period an increase in the number of mitochondria was observed. The autolytic vacuoles displayed variations in their content. Large, electron-dense inclusions resembling "condensed chromatin" could regularly be found in FDCP-Mix cells treated with low concentrations of etoposide (<4 microM). Confocal fluorescence microscopy and DNAse-gold labeling were employed to demonstrate the presence of DNA in the formation of the electron-dense inclusions within autolytic vacuoles. The identification of mitochondrial macroautophagy, the evidence for an etoposide-induced proliferation of mitochondria, and the fact that electron-dense inclusions are formed at a stage when the morphology

of the nucleus is still not effected, suggests that the DNA within the autolytic vacuoles may be of mitochondrial origin.

Riekstina, U., I. Cakstina, et al. (2009). "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis." Stem Cell Rev **5**(4): 378-86.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues, e.g., bone marrow, adipose tissue, dermis, hair follicles, heart, liver, spleen, dental pulp. Due to their immunomodulatory and regenerative potential MSCs have shown promising results in preclinical and clinical studies for a variety of conditions, such as graft versus host disease (GvHD), Crohn's disease, osteogenesis imperfecta, cartilage damage and myocardial infarction. MSC cultures are composed of heterogeneous cell populations. Complications in defining MSC arise from the fact that different laboratories have employed different tissue sources, extraction, and cultivation methods. Although cell-surface antigens of MSCs have been extensively explored, there is no conclusive evidence that unique stem cells markers are associated with these adult cells. Therefore the aim of this study was to examine expression of embryonic stem cell markers Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4 in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, dermis and heart. Furthermore, we tested whether human mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions. We found that bone marrow MSCs express embryonic stem cell markers Oct4, Nanog, alkaline phosphatase and SSEA-4, adipose tissue and dermis MSCs express Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4, whereas heart MSCs express Oct4, Nanog, SOX2 and SSEA-4. Our results also indicate that human adult mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions during early passages, as shown by distinct germ layer and embryonic stem cell marker expression patterns. Studies are now needed to determine the functional role of embryonic stem cell markers Oct4, Nanog and SOX2 in adult human MSCs.

Rossi, D. J., J. Seita, et al. (2007). "Hematopoietic stem cell quiescence attenuates DNA damage response and permits DNA damage accumulation during aging." Cell Cycle **6**(19): 2371-6.

The aging of tissue-specific stem and progenitor cells is believed to be central to the pathophysiological conditions arising in aged individuals. While the mechanisms driving stem cell aging are poorly understood, mounting evidence points to age-dependent DNA damage accrual as an important contributing factor. While it has been postulated that DNA damage may deplete stem cell numbers with age, recent studies indicate that murine hematopoietic stem cell (HSC) reserves are in fact maintained despite the accrual of genomic damage with age. Evidence suggests this to be a result of the quiescent (G0) cell cycle status of HSC, which results in an attenuation of checkpoint control and DNA damage responses for repair or apoptosis. When aged stem cells that have acquired damage are called into cycle under conditions of stress or tissue regeneration however, their functional capacity was shown to be severely impaired. These data suggest that age-dependent DNA damage accumulation may underlie the diminished capacity of aged stem cells to mediate a return to homeostasis after acute stress or injury. Moreover, the cytoprotection afforded by stem cell quiescence in stress-free, steady-state conditions suggests a mechanism through which potentially dangerous lesions can accumulate in the stem cell pool with age.

Ruiz-Arguelles, G. J. and D. Gomez-Almaguer (2004). "Breaking dogmata to help patients: non-myeloablative haematopoietic stem cell transplantation." Expert Opin Biol Ther **4**(10): 1693-9.

Various dogmata have been broken as a consequence of the evolution of knowledge in the area of allogeneic haematopoietic stem cell (HSC) transplantation. The following is now clear: for the successful engraftment of allogeneic HSC, bone marrow ablation of the recipient is not required; HSCs create their own space through graft-versus-host reactions; several malignancies can be eradicated by the graft-versus-tumour effect; HSC allografting can be conducted on an out-patient basis; HSC allografting can be done in aged or debilitated individuals; HSC allografting can be achieved without transfusion of blood products; and the costs of the allografting procedures can be substantially diminished. Despite the fact that HSC allografting with reduced intensity conditioning may be related to several disadvantages, such as mixed chimaerism and relapse of the malignancy, breaking these dogmata has resulted in availability of HSC allografting to a larger number of individuals worldwide, thus offering true curative therapeutic options to patients who otherwise would not qualify to be given these opportunities.

Ruiz-Arguelles, G. J., D. Gomez-Almaguer, et al. (2006). "Transient mixed chimerism after stem cell transplantation can induce durable molecular

complete remissions in chronic myelogenous leukemia." Leuk Lymphoma **47**(12): 2590-2.

Three patients with BCR/ABL+ chronic myelogenous leukemia were allografted, two with unrelated compatible placental blood and one from a human leukocyte antigen compatible sibling. The patients engrafted successfully, achieved mixed chimerism and all cleared the BCR/ABL fusion transcript. Despite the fact that the three patients lost the chimerism, they have remained in complete molecular remissions 9 months, 16 months and 5 years, respectively, after the allografts. It is possible that the transient induction of a graft-vs.-leukemia effect was able to control the malignancy despite the fact that the three patients lost the graft; however, other possible explanations are discussed. A longer follow-up of the patients is mandatory to further clarify these observations.

Russo, J., G. A. Balogh, et al. (2006). "The concept of stem cell in the mammary gland and its implication in morphogenesis, cancer and prevention." Front Biosci **11**: 151-72.

The breast attains its maximum development during pregnancy and lactation. After menopause the breast regresses in both nulliparous and parous women containing lobular structures that have been designated lobules type 1. Despite the similarity in the lobular composition of the breast at menopause, the fact that nulliparous women are at higher risk of developing breast cancer than parous women, indicates that Lobules type 1 in these two groups of women might be biologically different, or exhibit different susceptibility to carcinogenesis. Based on these observations it was postulated that the Lobule type 1 found in the breast of nulliparous women and of parous women with breast cancer never went through the process of differentiation, retaining a high concentration of epithelial cells that are targets for carcinogens and therefore susceptible to undergo neoplastic transformation, these cell are called Stem cells 1, whereas Lobules type 1 structures found in the breast of early parous postmenopausal women free of mammary pathology, on the other hand, are composed of an epithelial cell population that is refractory to transformation called Stem cells 2. It was further postulated that the degree of differentiation acquired through early pregnancy has changed the "genomic signature" that differentiates the Lobule type 1 from the early parous women from that of the nulliparous women by shifting the Stem cell 1 to a Stem cell 2 that is refractory to carcinogenesis, making this the postulated mechanism of protection conferred by early full term pregnancy. The identification of a putative breast stem cell (Stem cell 1) has reached in the last decade a significant impulse and several markers also reported for other tissues have been found in the mammary epithelial cells of both rodents and humans. Although still more work needs to be done in order to better understand the role of the Stem cell 2 and its interaction with the genes that confer it a specific signature, collectively, the data presently available provides evidence that pregnancy, through the process of cell differentiation, shifts the Stem cell 1 to Stem cell 2, cells that exhibit a specific genomic signature that could be responsible for the refractoriness of the mammary gland to carcinogenesis.

Safdar, A., G. Rodriguez, et al. (2005). "The safety of interferon-gamma-1b therapy for invasive fungal infections after hematopoietic stem cell transplantation." Cancer **103**(4): 731-9.

BACKGROUND: The restoration of normal immune responses, especially of the T-helper type 1 immune response, is an important predictor of fungal infection outcome in patients with malignant disease who undergo hematopoietic stem cell transplantation (HSCT). The authors sought to evaluate the safety of adjuvant recombinant interferon-gamma-1b as an immune-modulatory therapy HSCT recipients. METHODS: Thirty-two patients received interferon-gamma-1b after undergoing HSCT at the author's institution between 1998 and 2003. A retrospective analysis was undertaken after obtaining permission from the Institutional Review Board. RESULTS: Twenty-six of 32 patients (81%) received allogeneic stem cell grafts. All but 1 patient received interferon-gamma-1b and antifungals to treat infections; the other patients received interferon-gamma-1b to promote autologous graft-versus-tumor effect. Interferon-gamma-1b usually was administered at a dose of 50 mug subcutaneously every other day. The median duration (+/- standard deviation) of interferon-gamma-1b therapy was 6+/-6.5 doses (range, 1-29 doses), and the median cumulative dose was 487+/-453 mug (range, 35-2175 microg). During therapy with interferon-gamma-1b, fever was common (n=9 patients; 28%). In 1 patient (3%), new-onset lymphocytopenia occurred but resolved after cytokine therapy was discontinued; there were no interferon-gamma-1b-related episodes of neutropenia, thrombocytopenia, anemia, or liver dysfunction. Interferon-gamma-1b therapy did not precipitate or exacerbate acute or chronic graft-versus-host disease (GVHD). In fact, in 2 of 7 patients (29%) with acute GVHD and in 3 of 10 patients (30%) with chronic GVHD, significant improvements in GVHD were noted during therapy with interferon-gamma-1b. Among the 26 patients with aspergillosis, 14 patients (54%) died. However, 5 of 10 patients (50%) with presumed pulmonary aspergillosis, 3 of 9 patients (33%) with probable pulmonary aspergillosis, 1 of 2

patients (50%) with definite pulmonary aspergillosis, and 3 of 5 patients (60%) with disseminated aspergillosis responded to antifungals and adjuvant interferon-gamma-1b. CONCLUSIONS: Recombinant interferon-gamma-1b was tolerated without serious adverse reactions in HSCT recipients. A large, prospective, randomized study will be needed to evaluate the efficacy of this cytokine in high-risk HSCT recipients who have invasive mycoses.

Savani, B. N., T. Donohue, et al. (2007). "Increased risk of bone loss without fracture risk in long-term survivors after allogeneic stem cell transplantation." Biol Blood Marrow Transplant **13**(5): 517-20.

We studied bone mineral density (BMD) in 79 long-term survivors of allogeneic stem cell transplantation (SCT) (median follow-up: 78 months; range: 38-160). Seventy patients received a total body irradiation (TBI)-based myeloablative SCT and 9 patients received a non-TBI, reduced-intensity SCT. Fourteen (18%) patients were receiving immunosuppressive therapy (IST) for chronic graft-versus-host disease (cGVHD) beyond 3 years from SCT. Fifty-eight (73.4%) of patients had bone loss (BL): 33 (41.8%) with osteopenia and 25 (31.6%) with osteoporosis. Factors associated with a significantly increased risk of osteoporosis were age and prolonged IST and for overall BL prolonged IST. However, BL was not associated with an increased fracture risk, despite the fact that most patients had not received prophylactic biphosphonates. Our data shows that BL is a long-term posttransplant complication, and emphasize the importance of serial BMD scans, and the treatment of BL with biphosphonates reserved for worsening BL or additional risk factors.

Schrauder, A., S. Saleh, et al. (2009). "Pharmacokinetic monitoring of intravenous cyclosporine A in pediatric stem-cell transplant recipients. The trough level is not enough." Pediatr Transplant **13**(4): 444-50.

In order to monitor CsA serum levels after SCT, trough levels (C0) are widely used. The aim of this study was to estimate the population and individual PK parameters for patients receiving intravenous CsA after SCT. In 27 pediatric patients after SCT receiving CsA (3 mg/kg/day) every 12 h, a total of 289 CsA concentrations was obtained. To describe the PK parameters of CsA, a two-compartment model with first order elimination was used. Covariate analysis identified body weight, age, and the co-administration with itraconazole and tobramycine as factors influencing the Cl. The statistical comparison of AUC, trough level, and C2 indicates a correlation between AUC and C2, but no correlation between the AUC and C0, $r = 0.24$ ($p = 0.146$) vs. $r = 0.526$ ($p = 0.000692$), respectively. Our results underscore the fact that CsA trough levels do not reflect the drug exposure in patients receiving intravenous CsA after SCT. By contrast, CsA blood levels measured 2-6 h after CsA infusion showed a better correlation with the AUC. Our data provide new information to optimize the balancing act between GvHD-prophylaxis, graft vs. leukemia effect, and CsA side-effects after SCT.

Schulmeister, L., K. Quiett, et al. (2005). "Quality of life, quality of care, and patient satisfaction: perceptions of patients undergoing outpatient autologous stem cell transplantation." Oncol Nurs Forum **32**(1): 57-67.

PURPOSE/OBJECTIVES: To further expand the limited body of knowledge of the perceptions of quality of life (QOL), quality of care, and patient satisfaction among patients who receive high-dose chemotherapy with an autologous stem cell transplant (ASCT) on an outpatient basis. DESIGN: Descriptive longitudinal. SETTING: Nine clinical sites associated with a national oncology practice management network in locations across the United States. SAMPLE: 36 patients scheduled to receive high-dose chemotherapy with ASCT selected by nonprobability consecutive sampling. METHODS: Subjects completed the Functional Assessment of Cancer Therapy Bone Marrow Transplant (FACT-BMT) before high-dose chemotherapy, four to six weeks postchemotherapy, and six months postchemotherapy. An independent nurse researcher conducted telephone interviews about the treatment experience, perceptions of quality of care, and satisfaction with care. FACT-BMT data were analyzed using descriptive statistics and multivariate analysis of variance, and qualitative data about perceptions of care were analyzed using Giorgis methodologic reduction. Bivariate associations were made between overall degree of satisfaction with care and QOL as measured by the FACT-BMT. MAIN RESEARCH VARIABLES: Clinical outcome, QOL, patient satisfaction, and patient perceptions of care quality. FINDINGS: Mean FACT-BMT scores were lower one month post-treatment than at baseline and highest six months post-treatment. Subjects with progressive disease reported lower QOL at one and six months post-treatment, noted more complaints, and ranked their satisfaction with care lower than subjects with no evidence of disease. Subjects offered ASCT program improvement recommendations in the areas of communication, information, nursing care, ancillary needs assistance, ancillary agencies, and survivor support. CONCLUSIONS: In this study, the QOL of patients undergoing outpatient high-dose

chemotherapy with ASCT decreased post-treatment but increased to levels higher than those found at pretreatment by six months. A good clinical outcome following high-dose chemotherapy and ASCT was associated with higher QOL and greater satisfaction with care. IMPLICATIONS FOR NURSING: Knowledge of the outpatient ASCT experience and its effect on QOL can be used to further refine the content and timing of educational and supportive interventions for patients undergoing ASCT. Information about patients satisfaction with treatment and perceptions of quality of care provides insight about their expectations and perceived needs and can be used to redesign outpatient ASCT programs.

Schulze, A., H. Schirutschke, et al. (2008). "Altered phenotype of natural killer cell subsets after haploidentical stem cell transplantation." Exp Hematol **36**(4): 378-89.

OBJECTIVE: Haplotype-mismatched CD34(+) selected allogeneic stem cell transplantation (HASCT) has been described as a therapeutic option for patients with acute myeloid leukemia. The success of this regimen is based mainly on natural killer (NK) cell-mediated antileukemia effects. MATERIALS AND METHODS: We prospectively investigated NK-cell (CD56(+)/CD3(-)) reconstitution, including expression of antileukemia effector molecules in patients undergoing HASCT. RESULTS: Although absolute NK-cell numbers rapidly increased, their phenotype notably differed compared to healthy controls. In fact, the "effector" CD56(dim) subset was significantly reduced, as was the NKG2D expression on "regulatory" CD56(bright) cells. Perforin was completely absent on NK cells in one-third of patients. The expression of Fas-ligand (Fas-L) on NK cells as well as soluble Fas-L and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) plasma levels were also significantly lower after HASCT. In contrast, expression of TRAIL on CD56(dim) cells and interleukin-15 plasma levels were upregulated. Because the death rate due to relapse or infectious complications was high in the initial phase of the trial, subsequent patients received an adoptive infusion of donor NK cells followed by interleukin-2 in vivo in order to augment NK-cell function. This led to a distinct upregulation of perforin and Fas-L on the CD56(dim) subset accompanied by increased NK-cell cytotoxicity in vitro. CONCLUSION: The phenotype of reconstituting NK cells after HASCT is significantly altered. Whether the clinical outcomes of patients undergoing this regimen can be improved by a cytokine-based modulation of NK-cell activity needs to be determined.

Schwartz, G. S. and E. J. Holland (1998). "Iatrogenic limbal stem cell deficiency." Cornea **17**(1): 31-7.

PURPOSE: To describe a group of patients with limbal stem cell (SC) deficiency without prior diagnosis of a specific disease entity known to be causative of SC deficiency. METHODS: We performed a retrospective review of the records of all patients with ocular surface disease seen at the University of Minnesota between 1987 and 1996. Patients were categorized according to origin of limbal deficiency. Patients who did not have a specific diagnosis previously described as being causative of limbal deficiency were analyzed. Risk factors, clinical findings, and sequelae were evaluated. RESULTS: Fourteen eyes of 12 patients with SC deficiency not caused by a known diagnosis were described. All eyes had prior ocular surgery involving the corneoscleral limbus. Eleven eyes had been receiving long-term topical medications, and all eyes had concurrent external disease such as pterygium, keratoconjunctivitis sicca, rosacea, herpes simplex virus keratitis, or aphakic or pseudophakic corneal edema. All eyes had superior quadrants affected, corresponding to areas of prior limbal surgery. Sequelae of disease included corneal scarring and neovascularization, with seven eyes having visual acuity of 20/150 or worse. CONCLUSION: Because the epitheliopathy started peripherally and extended centrally in all patients, we believe it represents an SC deficiency. The fact that all patients were affected superiorly, at sites of a prior limbal surgical incision, points to surgical trauma to the SC as the likely major etiologic factor for the deficiency. The surgical trauma to the limbal SC probably made these cells more susceptible to damage from other external disease influences and toxicity from long-term topical medications. Because the SC deficiency is the result of prior ocular surgery and long-term topical medications, we propose the term "iatrogenic limbal stem cell deficiency."

Selleri, C., P. Ragno, et al. (2006). "The metastasis-associated 67-kDa laminin receptor is involved in G-CSF-induced hematopoietic stem cell mobilization." Blood **108**(7): 2476-84.

The 67-kDa laminin receptor (67LR) is a nonintegrin cell-surface receptor with high affinity for laminin, which plays a key role in tumor invasion and metastasis. We investigated the role of 67LR in granulocyte colony-stimulating factor (G-CSF)-induced mobilization of CD34+ hematopoietic stem cells (HSCs) from 35 healthy donors. G-CSF-mobilized HSCs, including CD34+/CD38- cells, showed increased 67LR expression as compared with unstimulated marrow HSCs; noteworthy, also, is the fact that the level of 67LR expression in G-CSF-

mobilized HSCs correlated significantly with mobilization efficiency. During G-CSF-induced HSC mobilization, the expression of laminin receptors switched from alpha6 integrins, which mediated laminin-dependent adhesion of steady-state human marrow HSCs, to 67LR, responsible for G-CSF-mobilized HSC adhesion and migration toward laminin. In vitro G-CSF treatment, alone or combined with exposure to marrow-derived endothelial cells, induced 67LR up-regulation in marrow HSCs; moreover, anti-67LR antibodies significantly inhibited transendothelial migration of G-CSF-stimulated marrow HSCs. Finally, G-CSF-induced mobilization in mice was associated with 67LR up-regulation both in circulating and marrow CD34+ cells, and anti-67LR antibodies significantly reduced HSC mobilization, providing the first in vivo evidence for 67LR involvement in stem-cell egress from bone marrow after G-CSF administration. In conclusion, 67LR up-regulation in G-CSF-mobilized HSCs correlates with their successful mobilization and reflects its increase in marrow HSCs, which contributes to the egress from bone marrow by mediating laminin-dependent cell adhesion and transendothelial migration.

Serra, P. A., S. Pluchino, et al. (2008). "The MPTP mouse model: cues on DA release and neural stem cell restorative role." Parkinsonism Relat Disord **14 Suppl 2**: S189-93.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause parkinsonism in humans and this fact is a major incentive for using this toxin as an animal model to study the pathogenesis of Parkinson's disease (PD). Although the monkey MPTP model remains the best, most studies have been performed in mice. The so-called acute and sub-acute regimens are commonly used. Both induce tissue striatal dopamine (DA) depletion and nigral neuron death. Tissue striatal DA depletion does not necessarily correlate with impairment of striatal dopaminergic functioning. In freely moving mice, systemic acute or sub-acute MPTP directly induces prolonged release of striatal DA. Such DA release may be considered the first step in MPTP-induced striatal DA depletion. Reportedly, neural stem cells improve symptoms in the MPTP model of PD by interacting with the MPTP-induced pathological nigrostriatal milieu.

Shan, L. (2004). "FluidMAG iron nanoparticle-labeled mesenchymal stem cells for tracking cell homing to tumors."

Personalized diagnosis and treatment with allogenic or autologous cells are becoming a reality in the field of medicine (1, 2). Cytotoxic or engineered T-cells are under clinical trial for the treatment of hematopoietic or other malignant diseases (3). Contrast agent-tagged macrophages are used as cellular probes to image the early inflammatory processes in macrophage-rich conditions such as inflammation, atherosclerosis, and acute cardiac graft rejection (4). The roles of stem cells are under intensive investigation in therapeutic and regenerative medicine, such as regenerating cardiomyocytes, neurons, bone, and cartilage (1). Genetically modified cells are used to treat genetic disorders (5). With promising results from these studies, a critical issue is how to monitor the temporal and spatial migration and the homing of these cells, as well as the engraftment efficiency and functional capability of the transplanted cells in vivo (6, 7). Histopathological techniques have only been used to obtain information on the fate of implanted cells at the time of animal euthanization or via biopsy or surgery. To track the real-time changes of cell location, viability, and functional status, cell imaging techniques have been introduced during the last few years. Cells of interest are labeled with reporter genes, fluorescent dyes, or other contrast agents that transform the tagged cells into cellular probes or imaging agents (2, 6, 7). The ability to monitor superparamagnetic iron oxide particles (SPIO) with magnetic resonance imaging (MRI) has been utilized in animal models as well as in a few clinical settings to investigate the fate of labeled cells (6-10). The advantages of using MRI for cell tracking include the high spatial resolution with high anatomic background contrast, the lack of exposure to ionizing radiation, and the ability to follow the cells for months, although it is difficult to measure the rate of cell division and to determine whether each progeny shares the SPIO in vivo. In addition, cell labeling with SPIO nanoparticles is generally nontoxic and does not affect the cell proliferation and differentiation capacity, although a few studies have reported that the stem cells labeled with SPIO lose part of their differentiation capacity in a SPIO concentration-dependent manner. An important limitation of MRI is the fact that MRI signals cannot indicate whether cells are dead or alive. It is also unknown whether the MRI signal comes from targeted or labeled cells or from macrophages. Basically, SPIO particles are used to label the target cells by systemic application or by injection into the tissue area of interest to monitor target cell migration after phagocytosis. SPIO are more frequently used to label the cells in vitro by incorporating into the cells directly. Furthermore, SPIO are usually encapsulated by organic polymers to increase their stability and biocompatibility and to allow the chemical modification of their surfaces. The fact is that the uptake of different particles varies largely between different cell types and between

particle coatings (6, 7). Msenchymal stem cells (MSCs) represent a heterogeneous subset of pluripotent stromal cells that can be isolated from different adult tissues including adipose tissue, liver, muscle, amniotic fluid, placenta, umbilical cord blood, and dental pulp, although the bone marrow remains the principal source for most preclinical and clinical studies (1, 11, 12). Although MSCs account for only 0.001-0.01% of the total nucleated cells within isolated bone marrow aspirates, they can easily be isolated and expanded in vitro through as many as 40 population doublings after 8-10 weeks of culture (1, 13). These cells exhibit the potential to differentiate into cells of diverse lineages such as adipocytes, chondrocytes, osteocytes, myoblasts, cardiomyocytes, neurons, and astrocytes. In addition, MSCs show tropism or homing to tumors and thus have been used as vehicles for directed cancer delivery (14, 15). The mechanism responsible for the homing of MSCs to tumors is thought to involve chemokine ligands and receptors, as with the recruitment of leukocytes to areas of inflammation. However, unlike with leukocytes, the specific chemokines responsible for MSC migration are poorly characterized (14, 15). Nevertheless, homing to tumors has been confirmed with traditional immunohistochemistry and other methods in many studies. Loebinger et al. labeled MSCs with fluidMAG iron nanoparticles and imaged homing of the labeled MSCs to tumors with MRI (16). FluidMAG nanoparticles are commercially available ferrofluids consisting of an aqueous dispersion of magnetic iron oxides with a hydrodynamic diameter of 200 nm and a starch coating. The investigators showed that as few as 1,000 labeled MSCs were detected 1 month after their co-injection with breast cancer cells that formed subcutaneous tumors. The investigators further demonstrated that intravenously injected labeled cells could be tracked in vivo to home to multiple lung metastases (16).

Shan, L. (2004). "Multimodal, rhodamine B isothiocyanate-incorporated, silica-coated magnetic nanoparticle-labeled human cord blood-derived mesenchymal stem cells for cell tracking."

Personalized diagnosis and treatment with allogenic or autologous cells are becoming a reality in the field of medicine (1, 2). Cytotoxic or engineered T cells are under clinical trial for the treatment of hematopoietic or other malignant diseases (1). Contrast agent-tagged macrophages are used as cellular probes to image the early inflammatory processes in macrophage-rich conditions such as inflammation, atherosclerosis, and acute cardiac graft rejection (2). The roles of stem cells are under intensive investigation in the therapeutic and regenerative medicine such as regenerating cardiomyocytes, neurons, bone, and cartilage (3). Genetically modified cells are used to treat genetic disorders (4). With the promising results from these studies, a critical issue is how to monitor the temporal and spatial migration and the homing of these cells, as well as the engraftment efficiency and functional capability of the transplanted cells in vivo (5, 6). Histopathological techniques have only been used to obtain information on the fate of implanted cells at the time of animal euthanization or via biopsy or surgery. To track the real-time changes of cell location, viability, and functional status, cell imaging techniques have been introduced during the last few years. Cells of interest are labeled with reporter genes, fluorescent dyes, or other contrast agents that transform the tagged cells into cellular probes or imaging agents (5-7). The ability to monitor superparamagnetic iron oxide particles (SPIO) by magnetic resonance imaging (MRI) has been utilized in animal models as well as in a few clinical settings to investigate the fate of labeled cells (5-9). The advantages of using MRI for cell tracking include the high spatial resolution with high anatomic background contrast, the lack of exposure to ionizing radiation, and the ability to follow the cells for months. SPIO particles provide a strong change in signal per unit of metal, in particular on T2- and T2*-weight images. In addition, cell labeling with SPIO nanoparticles is generally nontoxic and does not affect the cell proliferation and differentiation capacity, although a few studies have reported that the stem cells labeled with SPIO lose part of their differentiation capacity in a SPIO concentration-dependent manner (8, 9). An important limitation of MRI is the fact that MRI signals cannot indicate whether cells are dead or alive. It is also unknown whether the MRI signal comes from targeted or labeled cells or from macrophages. Basically, SPIO particles are used to label the target cells by systemic application or by injecting into the tissue area of interest to monitor target cell migration after phagocytosis. SPIO are more frequently used to label the cells in vitro by incorporating into the cells directly. Furthermore, SPIO are usually encapsulated by organic polymers to increase their stability and bio-compatibility, and allow the chemical modification of their surfaces. The fact is that the uptake of different particles varies largely between different cell types (5, 6). Mesenchymal stem cells (MSCs) represent a heterogeneous subset of pluripotent stromal cells that can be isolated from different adult tissues including adipose tissue, liver, muscle, amniotic fluid, placenta, umbilical cord blood, and dental pulp, although the bone marrow remains the principal source for most preclinical and clinical studies (3, 10, 11). Although MSCs account for only 0.01-0.001% of the total nucleated cells within isolated bone marrow aspirates,

they can easily be isolated and expanded in vitro through as many as 40 population doublings in ~8-10 weeks of culture (12). These cells exhibit the potential to differentiate into cells of diverse lineages, such as adipocytes, chondrocytes, osteocytes, myoblasts, cardiomyocytes, neurons, and astrocytes. In addition, MSCs possess remarkable immunosuppressive properties, and they have been shown to be effective against tumor cell growth (13, 14). Yoon et al. generated multimodal, rhodamine B isothiocyanate (RITC)-labeled, silica-coated magnetic nanoparticles (MNPs@SiO2(RITC)) and Park et al. labeled the MSCs with the nanoparticles and successfully tracked the labeled cells transplanted into the subcutaneous tissue and liver of the mice (7, 15). Their results indicate that MNPs@SiO2(RITC) are biocompatible and useful for human MSC labeling and cell tracking with multimodality imaging.

Sharma, V. K., C. Carles, et al. (2003). "Maintenance of stem cell populations in plants." Proc Natl Acad Sci U S A **100 Suppl 1**: 11823-9.

Flowering plants have the unique ability to produce new organs continuously, for hundreds of years in some species, from stem cell populations maintained at their actively growing tips. The shoot tip is called the shoot apical meristem, and it acts as a self-renewing source of undifferentiated, pluripotent stem cells whose descendents become incorporated into organ and tissue primordia and acquire different fates. Stem cell maintenance is an active process, requiring constant communication between different regions of the shoot apical meristem to coordinate loss of stem cells from the meristem through differentiation with their replacement through cell division. Stem cell research in model plant systems is facilitated by the fact that mutants with altered meristem cell identity or accumulation are viable, allowing dissection of stem cell behavior by using genetic, molecular, and biochemical methods. Such studies have determined that in the model plant Arabidopsis thaliana stem cell maintenance information flows via a signal transduction pathway that is established during embryogenesis and maintained throughout the life cycle. Signaling through this pathway results in the generation of a spatial feedback loop, involving both positive and negative interactions, that maintains stem cell homeostasis. Stem cell activity during reproductive development is terminated by a temporal feedback loop involving both stem cell maintenance genes and a phase-specific flower patterning gene. Our current investigations provide additional insights into the molecular mechanisms that regulate stem cell activity in higher plants.

Sherman, A. C., T. G. Plante, et al. (2009). "Prospective study of religious coping among patients undergoing autologous stem cell transplantation." J Behav Med **32**(1): 118-28.

Considerable attention has focused on relationships between religious or spiritual coping and health outcomes among cancer patients. However, few studies have differentiated among discrete dimensions of religious coping, and there have been surprisingly few prospective investigations. Negative or conflicted aspects of religious coping, in particular, represent a compelling area for investigation. This prospective study examined negative religious coping, positive religious coping, and general religious orientation among 94 myeloma patients undergoing autologous stem cell transplantation. Participants were assessed during stem cell collection, and again in the immediate aftermath of transplantation, when risks for morbidity are most elevated. Outcomes included Brief Symptom Inventory anxiety and depression and Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMI) scales. Negative religious coping at baseline predicted worse post-transplant anxiety, depression, emotional well-being, and transplant-related concerns, after controlling for outcome scores at baseline and other significant covariates. Post-transplant physical well-being was predicted by an interaction between baseline positive and negative religious coping. Results suggest that religious struggle may contribute to adverse changes in health outcomes for transplant patients, and highlight the importance of negative or strained religious responses to illness.

Sherman, A. C., S. Simonton, et al. (2009). "Changes in quality-of-life and psychosocial adjustment among multiple myeloma patients treated with high-dose melphalan and autologous stem cell transplantation." Biol Blood Marrow Transplant **15**(1): 12-20.

High-dose melphalan and autologous hematopoietic stem cell transplantation (HSCT) is a standard treatment for myeloma, but very little is known about the psychosocial or quality-of-life difficulties that these patients encounter during treatment. Data regarding older patients is particularly scarce. Using a prospective design, this investigation evaluated 94 patients at stem cell collection and again after high-dose therapy and transplantation. Outcomes included quality-of-life (FACT-BMT) and psychosocial adjustment (ie, Brief Symptom Inventory, Impact of Events Scale, and Satisfaction with Life Scale). Findings were compared with age- and sex-adjusted population norms and with transplantation patient norms. At stem cell collection, physical deficits were common, with most patients scoring 1 standard deviation below population norms

for physical well-being (70.2%) and functional well-being (57.5%), and many reporting at least moderate fatigue (94.7%) and pain (39.4%). Clinically meaningful levels of anxiety (39.4%), depression (40.4%), and cancer-related distress (37.0%) were evident in a notable proportion of patients. After transplantation, there was a worsening of transplant-related concerns ($P < .05$), depression ($P < .05$), and life-satisfaction ($P < .001$); however, pain improved ($P < .01$), and social functioning was well preserved. Overall, the declines in functioning after transplantation were less pronounced than anticipated. Older patients were not more compromised than younger ones; in multivariate analyses, they reported better overall quality of life ($P < .01$) and less depression ($P < .05$) before transplantation. Our findings emphasize the importance of early screening and intervention.

Shinohara, T., K. E. Orwig, et al. (2000). "Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells." Proc Natl Acad Sci U S A **97**(15): 8346-51.

The spermatogonial stem cell initiates and maintains spermatogenesis in the testis. To perform this role, the stem cell must self replicate as well as produce daughter cells that can expand and differentiate to form spermatozoa. Despite the central importance of the spermatogonial stem cell to male reproduction, little is known about its morphological or biochemical characteristics. This results, in part, from the fact that spermatogonial stem cells are an extremely rare cell population in the testis, and techniques for their enrichment are just beginning to be established. In this investigation, we used a multiparameter selection strategy, combining the in vivo cryptorchid testis model with in vitro fluorescence-activated cell sorting analysis. Cryptorchid testis cells were fractionated by fluorescence-activated cell sorting analysis based on light-scattering properties and expression of the cell surface molecules alpha6-integrin, alphav-integrin, and the c-kit receptor. Two important observations emerged from these analyses. First, spermatogonial stem cells from the adult cryptorchid testis express little or no c-kit. Second, the most effective enrichment strategy, in this study, selected cells with low side scatter light-scattering properties, positive staining for alpha6-integrin, and negative or low alphav-integrin expression, and resulted in a 166-fold enrichment of spermatogonial stem cells. Identification of these characteristics will allow further purification of these valuable cells and facilitate the investigation of molecular mechanisms governing spermatogonial stem cell self renewal and hierarchical differentiation.

Slavin, S. (2002). "Maternal-fetal relationship, natural chimerism and bilateral transplantation tolerance as the basis for non-myeloablative stem cell transplantation." Int J Hematol **76 Suppl 1**: 172-5.

Bone marrow transplantation (BMT) which represents an important clinical tool for treatment of patients with a wide variety of malignant and non-malignant diseases, however, the procedure is associated with procedure-related toxicity and mortality as well as unavoidable late complications. Many of the undesirable consequences of BMT are caused directly or indirectly by the intensive conditioning administered during the pre-transplant period. However, if the main goal of the BMT procedure is to enable immunotherapy by alloreactive donor lymphocytes, the conditioning prior to BMT needs to be reconsidered, because transplantation tolerance across major histocompatibility complex (MHC) occurs spontaneously in nature, as evidenced by the fact that pregnant females do not reject their conceptus. In fact, as shown by Owens in the 1940s, placental parabiosis in utero leads to permanent mixed chimerism and bilateral transplantation tolerance. Tolerant recipients were shown to be chimeras with only a small proportion of donor cells. However, without corroborating evidence that transplantation tolerance could be intentionally induced, the approach could not be applied in clinical practice for immunocompetent recipients. Starting in 70s, we documented the feasibility of establishing bilateral transplantation tolerance by mixed chimerism following non-myeloablative conditioning in immu. nologically mature recipients across MHC in mice, rats and dogs. Several studies have shown that reduced intensity conditioning can be very useful for immunoregulation whereas more intensive the pre-grafting immunosuppression resulted in more aggressive the GVHD. These and other findings suggested that lower intensity conditioning may be sufficient for engraftment of donor stem cells, thus suggesting that immunosuppression without myeloablation may be sufficient for prevention of allograft rejection. Following engraftment of donor stem cells, donor lymphocytes infused with bone marrow or mobilized blood stem cells can eradicate residual hematopoietic cells of host origin, occasionally non-hematopoietic tumor cells of host origin as well. Whenever indicated, donor lymphocytes infusion (DLI) can be used at a later stage post BMT to eradicate residual malignant cells of host origin or for the treatment of residual or recurrent disease. Taken together, ongoing clinical studies suggest that high-dose, myeloablative chemoradiotherapy, could be safely replaced with non-myeloablative conditioning (NST).

Slavin, S. (2005). "Allogeneic cell-mediated immunotherapy at the stage of minimal residual disease following high-dose chemotherapy supported by autologous stem cell transplantation." Acta Haematol **114**(4): 214-20.

Cumulative clinical experience suggests that immunotherapy may be an effective tool for eradicating tumor cells resistant to maximum tolerated doses of chemotherapy and radiation. Immunotherapy is much more effective when applied at the stage of minimal residual disease, especially against slowly growing tumors because development of graft-versus-leukemia, lymphoma, myeloma, or in a broader sense graft-versus-tumor effects renders immunotherapy more time consuming. Hence, eradication of rapidly growing bulky tumors may be difficult or impossible to achieve. Considering the fact that optimal immunotherapy may be accomplished in patients treated at the stage of minimal (MRD) disease, in patients with hematological malignancies and chemosensitive solid tumors a stage of MRD may be best achieved following administration of myeloablative high-dose chemotherapy or chemoradiotherapy supported by autologous stem cell transplantation (autoSCT). Taken together, immunotherapy following autoSCT may provide an ideal combination for improving the cure rate of otherwise incurable cancers, especially if tumor cells may respond to cytokine-mediated immunotherapy or cell-mediated cytokine-activated immunotherapy. Following lymphocyte depletion in the course of autoSCT, adoptive transfer of alloreactive or tumor-reactive lymphocytes may be much more effective due to the preponderance of anticancer effector cells on the one hand, and elimination or depletion of the patient's regulatory cells that may downregulate anticancer effector mechanisms.

Smith, S. E., A. Toor, et al. (2006). "The administration of polymerized human hemoglobin (Pyridoxylated) to a Jehovah's Witness after submyeloablative stem cell transplantation complicated by delayed graft failure." Compr Ther **32**(3): 172-5.

A 55-yr-old woman with a history of B-cell lymphoma of the nasopharynx diagnosed in March 1999 eventually underwent submyeloablative allogeneic stem cell transplantation from a sibling donor in December 2002 after conventional treatment options were exhausted. The treatment approach was somewhat altered by the fact that the patient was a practicing Jehovah's Witness and refused blood-blood product transfusion. The course of her treatment was unremarkable until around day 100 posttransplant when she developed graft failure, leading to severe anemia. Blood transfusions were refused. Donor cells were re-infused. During this treatment period, the patient's hemoglobin dropped to a low of 2.7 g/dL, with the patient experiencing severe fatigue, dyspnea on exertion, headaches, and blurred vision. Polymerized human hemoglobin (pyridoxylated) (Poly- Heme, Northfield Laboratories Inc., Evanston, IL) was given under an emergency, compassionate use protocol and successfully bridged the patient's hemoglobin and relieved symptoms during her marrow recovery period.

Sottile, V., M. Li, et al. (2006). "Stem cell marker expression in the Bergmann glia population of the adult mouse brain." Brain Res **1099**(1): 8-17.

Recent evidence suggests that the postnatal cerebellum contains cells with characteristics of neural stem cells, which had so far only been identified in the subventricular zone of the lateral ventricles and the subdentate gyrus of the hippocampus. In order to investigate the identity of these cells in the adult cerebellum, we have analyzed the expression of Sox1, a transcription factor from the SoxB1 subgroup and widely used marker of neural stem cells. In situ hybridization and the use of a transgenic mouse model show that, in the adult cerebellum, Sox 1 is only expressed in the Bergmann glia, a population of radial glia present in the Purkinje cell layer. Furthermore, another neural stem cell marker, Sox2 (also member of the SoxB1 subgroup), is also expressed in the Bergmann glia. We have previously shown that these same cells express Sox9, a member of the SoxE subgroup known for its role in glial development. Here we show that Sox9 is in fact also expressed in other regions harboring adult neural stem cells, suggesting that Sox9 represents a novel stem cell marker. Finally, using a Sox1-null mouse, we show that the formation of this Sox2/Sox9 positive Bergmann glia population does not require the presence of a functional Sox1. Our results identify these radial glia as a previously unreported Sox1/Sox2/Sox9 positive adult cell population, suggesting that these cells may represent the recently reported stem cells in the adult cerebellum.

Sproull, F. and C. N. David (1979). "Stem cell growth and differentiation in Hydra attenuata. II. Regulation of nerve and nematocyte differentiation in multiclone aggregates." J Cell Sci **38**: 171-9.

The differentiation of nerve cells and nematocytes from interstitial stem cells in Hydra has been investigated under conditions of changing stem cell density. Interstitial stem cells were cultured in a feeder layer system consisting of aggregates of nitrogen mustard-inactivated tissue. The aggregates were seeded with varying numbers of stem cells from

10 to 400 per aggregate; between 4 and 7 days later the rates of nerve and nematocyte differentiation were measured. Nerve differentiation was scored by labelling the stem cell population with [3H]-thymidine and counting nests of 4 proliferating nematoblasts. In both cases the numbers of differentiating cells were normalized to the size of the stem cell population. The results indicate that the rate of nematocyte differentiation increases as the concentration of stem cells increases in aggregates; under the same conditions the rate of nerve differentiation remains essentially constant. To calculate the numbers of stem cells entering each pathway per generation, a computer was programmed to simulate the growth and differentiation of interstitial stem cells. Standard curves were prepared from the simulations relating the rates of nerve and nematocyte differentiation to the fraction of stem cells committed to each pathway per generation. The rates of nerve and nematocyte commitment were then estimated from the experimentally observed rates of differentiation using the standard curves. The results indicate that nerve commitment remains constant at about 0.13 stem cells per generation over a wide range of stem cell concentration. Nematocyte commitment, by comparison, increases from 0.15 to 0.21 stem cells per generation as stem cell concentration increases in aggregates. The fact that the ratio of nerve to nematocyte commitment changes under our conditions suggests that stem cell commitment is not a stochastic process but subject to control by environmental stimuli.

Strizzi, L., D. E. Abbott, et al. (2008). "Potential for cripto-1 in defining stem cell-like characteristics in human malignant melanoma." Cell Cycle **7**(13): 1931-5.

The diagnosis of melanoma is becoming ever more frequent. Although surgical excision of early lesions is associated with relatively significant high cure rates, treatment modalities are largely unsuccessful for advanced disease. Characteristics such as cellular heterogeneity and plasticity, expression of certain molecules such as the multidrug resistance protein-1 (MDR1) or the aberrant expression of embryonic signaling molecules and morphogens like Nodal, important for self renewal and pluripotency, suggest that a stem cell-like population may reside in aggressive melanomas. This perspective focuses on preliminary findings obtained in our laboratory which indicate that the expression of the Nodal coreceptor, Cripto-1, in a subset of malignant melanoma cells may be exploited to identify possible melanoma stem cells (MSC). In fact, the use of anti-Cripto-1 antibodies to cell sort Cripto-1-positive cells in the metastatic melanoma cell line C8161 has identified a slow growing, sphere forming subpopulation that expresses increased levels of Oct4, Nanog and MDR1. If current in vivo studies confirm the self renewal and tumorigenic characteristics of these cells, the expression of Cripto-1 may represent a useful marker to identify cancer stem cells in melanoma, and possibly other aggressive tumors as well.

Sugaya, K. (2003). "Stem cell strategies, future and beyond." Seishin Shinkeigaku Zasshi **105**(1): 68-80.

The use of stem cells for neuroreplacement therapy is no longer science fiction--it is science fact. We have succeeded in the development of neural and mesenchymal stem cell transplantation to produce neural cells in the brain. We have seen the improvement of cognitive function in a memory-impaired aged animal model following stem cell transplantation. These results may promise a bright future for stem cell strategies. Before we begin to think about clinical applications beyond the present preclinical studies or even consider the pathophysiological environments of individual diseases, we must address and weigh the factors that may affect stem cell biology. Here, we not only show the potential for therapeutic applications for stem cell strategies in neuropathological conditions, but we also discuss the effects on the biology of stem cells of those factors that are altered under disease conditions.

Sugaya, K. and S. Merchant (2008). "How to approach Alzheimer's disease therapy using stem cell technologies." J Alzheimers Dis **15**(2): 241-54.

The use of stem cells for neuroreplacement therapy is no longer science fiction - it is science fact. We have succeeded in producing neural cells in the brain using both neural and mesenchymal stem cell transplantation and even systemic injection using a small molecular compound. We have seen the improvement of cognitive function in animal models following the application of these stem cell technologies. These results may promise a bright future for stem cell based neuroreplacement therapies for neurodegenerative diseases including Alzheimer's disease (AD). However, we have to consider the pathophysiological environments of individual diseases before clinical applications can be introduced. We must find the factors in the pathology that may affect stem cell biology and overcome the negative effects on neuroreplacement. Here, we discuss not only the potential for therapeutic applications of stem cell strategies in neuropathological conditions, but also how to overcome the adverse effects on the biology of stem cells due to the factors that are altered under AD pathology.

Sulmasy, D. P. (2009). "Deliberative Democracy and stem cell research in New York State: the good, the bad, and the ugly." Kennedy Inst Ethics J **19**(1): 63-78.

Many states in the U.S. have adopted policies regarding human embryonic stem cell (hESC) research in the last few years. Some have arrived at these policies through legislative debate, some by referendum, and some by executive order. New York has chosen a unique structure for addressing policy decisions regarding this morally controversial issue by creating the Empire State Stem Cell Board with two Committees--an Ethics Committee and a Funding Committee. This essay explores the pros and cons of various policy arrangements for making public policy decisions about morally controversial issues in bioethics (as well as other issues) through the lens of Deliberative Democracy, focusing on the principles of reciprocity, publicity, and accountability. Although New York's unique mechanism potentially offers an opportunity to make policy decisions regarding a morally controversial subject like hESC research in accord with the principles of Deliberative Democracy, this essay demonstrates its failure to do so in actual fact. A few relatively simple changes could make New York's program a real model for putting Deliberative Democracy into practice in making policy decisions regarding controversial bioethical issues.

Suratt, B. T., C. D. Cool, et al. (2003). "Human pulmonary chimerism after hematopoietic stem cell transplantation." Am J Respir Crit Care Med **168**(3): 318-22.

Many of the body's tissues once thought to be only locally regenerative may, in fact, be actively replaced by circulating stem cells after hematopoietic stem cell transplantation. Localization of donor-derived cells ("chimerism") has recently been shown to occur in the lungs of mice after either hematopoietic stem cell transplantation or infusion of cultured marrow. To determine whether tissues of the human lung might be similarly derived from extrapulmonary sources, we examined lung specimens from a retrospective cohort of female allogeneic hematopoietic stem cell transplant recipients who received stem cells from male donors. Tissue samples from three such patients who had undergone diagnostic lung biopsy or autopsy were examined. Slides were stained by immunohistochemistry for cytokeratin (epithelium) and platelet endothelial cell adhesion molecule, CD31 (PECAM) (endothelium) and were imaged and then examined by fluorescent in situ hybridization analysis to identify male cells. The resulting overlapping in situ hybridization and immunohistochemistry images were examined for the presence and, if present, cell type of donor cells in the lung. We found significant rates of epithelial (2.5-8.0%) and endothelial (37.5-42.3%) chimerism. These results suggest that significant chimerism of the human lung may follow hematopoietic stem cell transplantation and that adult human stem cells could potentially play a therapeutic role in treatment of the damaged lung.

Swijnenburg, R. J., S. Schrepfer, et al. (2008). "Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts." Proc Natl Acad Sci U S A **105**(35): 12991-6.

Given their self-renewing and pluripotent capabilities, human embryonic stem cells (hESCs) are well poised as a cellular source for tissue regeneration therapy. However, the host immune response against transplanted hESCs is not well characterized. In fact, controversy remains as to whether hESCs have immune-privileged properties. To address this issue, we used in vivo bioluminescent imaging to track the fate of transplanted hESCs stably transduced with a double-fusion reporter gene consisting of firefly luciferase and enhanced GFP. We show that survival after transplant is significantly limited in immunocompetent as opposed to immunodeficient mice. Repeated transplantation of hESCs into immunocompetent hosts results in accelerated hESC death, suggesting an adaptive donor-specific immune response. Our data demonstrate that transplanted hESCs trigger robust cellular and humoral immune responses, resulting in intragraft infiltration of inflammatory cells and subsequent hESC rejection. Moreover, we have found CD4(+) T cells to be an important modulator of hESC immune-mediated rejection. Finally, we show that immunosuppressive drug regimens can mitigate the anti-hESC immune response and that a regimen of combined tacrolimus and sirolimus therapies significantly prolongs survival of hESCs for up to 28 days. Taken together, these data suggest that hESCs are immunogenic, trigger both cellular and humoral-mediated pathways, and, as a result, are rapidly rejected in xenogeneic hosts. This process can be mitigated by a combined immunosuppressive regimen as assessed by molecular imaging approaches.

Tang, C., C. L. Chua, et al. (2007). "Insights into the cancer stem cell model of glioma tumorigenesis." Ann Acad Med Singapore **36**(5): 352-7.

Not all cancer cells are born equal. While the great majority of the cells that make up tumours are destined to differentiate, albeit aberrantly, and eventually stop dividing, a handful of cancer cells

appear to possess limitless replicative potential. This review presents compelling evidence to suggest that the bulk of malignant cells of most cancers are generated by a rare fraction of stem cell-like cancer cells. Cancer stem cells that are capable of recapitulating brain tumours as xenografts in mice are characterised by defined stem cell markers. These brain tumour stem cells demonstrate enhanced chemoresistance and radioresistance mechanisms compared to non-stem cells in the heterogeneous tumour, which suggest that they may be the likely candidates for tumour progression and recurrence. Indeed, recent work has shown that such aberrant signalling pathways may be targeted in novel anti-cancer therapeutic strategies. The stem cell concept of tumour progression prompts immediate attention to a new paradigm in cancer research with a focus on this minority subset of cells, and the design of novel therapeutic strategies to target these cells that are insignificant within the population of tumour cells, but that are in fact the relevant cells to be destroyed.

Tauchmanova, L., C. Alviggi, et al. (2007). "Cryptozoospermia with normal testicular function after allogeneic stem cell transplantation: a case report." Hum Reprod **22**(2): 495-9.

One of the most frequent consequences of allogeneic haemopoietic stem cell transplantation (allo-SCT) in both males and females is gonadal insufficiency. We report the case of a 27-year-old myelodysplastic male who developed azoospermia after allogeneic transplantation of haemopoietic stem cells from his HLA-identical sister. Post-transplant azoospermia was alternated with intermittent severe oligospermia. The patient had a normal endocrine pattern and evidence of mild chronic graft-versus-host disease (cGVHD). Normal intratesticular spermatogenesis was revealed by bilateral fine needle aspiration (FNA) cytology. Inflammation was evident at semen analysis, but no infection was detected by microbiological examination and sperm culture. These findings, together with the re-appearance of sperm cells at semen analysis after a low-dose immunosuppressive treatment, suggested the presence of cGVHD of the urogenital tract, causing a reversible obstruction of the spermatic tract and cryptozoospermia. This is the first case report documenting a severe impairment of sperm count because of a reversible obstruction of the seminal tract, likely caused by cGVHD, in a long-term survivor of allo-SCT with normal endocrine pattern. An important practical consequence of this case report is the fact that azoospermia was cured using low-dose immunosuppressive therapy, and this allowed us to avoid expensive stimulatory treatments with gonadotrophins, which remain, however, ineffective if the obstruction of spermatic tracts is not removed. A spontaneous uncomplicated pregnancy occurred in the partner of the patient 3 months after the corticosteroid treatment withdrawal.

Taylor, S. E., R. K. Smith, et al. (2007). "Mesenchymal stem cell therapy in equine musculoskeletal disease: scientific fact or clinical fiction?" Equine Vet J **39**(2): 172-80.

The goal in the therapeutic use of mesenchymal stem cells (MSCs) in musculoskeletal disease is to harness the regenerative nature of these cells focussing on their potential to grow new tissues and organs to replace damaged or diseased tissue. Laboratory isolation of MSCs is now well established and has recently been demonstrated for equine MSCs. Stem cell science has attracted considerable interest in both the scientific and clinical communities because of its potential to regenerate tissues. Research into the use of MSCs in tissue regeneration in general reflects human medical needs, however, the nature, prevalence and prognosis of superficial digital flexor tendonitis has put equine veterinary science at the forefront of tendon regeneration research. Much has been investigated and learnt but it must be appreciated that in spite of this, the field is still relatively young and both communities must prepare themselves for considerable time and effort to develop the technology into a highly efficient treatments. The promise of functional tissue engineering to replace old parts with new fully justifies the interest. At present, however, it is important to balance the understanding of our current limitations with a desire to progress the technology.

Tilly, J. L. and B. R. Rueda (2008). "Minireview: stem cell contribution to ovarian development, function, and disease." Endocrinology **149**(9): 4307-11.

By virtue of the fact that oocytes not only serve to produce embryos after fertilization but also can effectively reprogram adult somatic cell nuclei to a pluripotent state, much of the interest in the role of stem cells in ovarian biology has been focused on the germline. However, very recent studies have revealed that somatic stem cells may also be of considerable relevance to the study of normal ovarian function. Furthermore, stem cell dysfunction may underlie or contribute to disease states such as ovarian cancer and polycystic ovary syndrome. Our objective is to explore these concepts in greater detail, with the hope of stimulating further research efforts into understanding what role stem cells may play in the physiology and pathology of the mammalian female gonads.

Torella, D., G. M. Ellison, et al. (2005). "Cardiac stem and progenitor cell biology for regenerative medicine." Trends Cardiovasc Med **15**(6): 229-36.

Stem cell therapy is a new and promising treatment of heart disease. However, the race is still on to find the "best" cell to reconstitute the myocardium and improve function after myocardial damage. The recent discovery in the adult mammalian myocardium of a small cell population with the phenotype, behavior, and regenerative potential of cardiac stem and progenitor cells has proposed these cells as the most appropriate for cell therapy. The existence of these cells has provided an explanation for the hitherto unexplained existence of a subpopulation of immature cycling myocytes in the adult myocardium. Although CSCs (cardiac cells proven to have stem and/or progenitor characteristics) can be isolated and amplified in vitro or stimulated to differentiate in situ, it has become reasonable to exploit this endogenous regenerative potential to replace the lost muscle with autologous functional myocardium. Therefore, it is imperative to obtain a better understanding of the biology and regenerative potential of the endogenous CSCs. This will enable us to design better protocols for the regeneration of functional contractile mass after myocardial injury.

Tran, H. T., T. Madden, et al. (2000). "Individualizing high-dose oral busulfan: prospective dose adjustment in a pediatric population undergoing allogeneic stem cell transplantation for advanced hematologic malignancies." Bone Marrow Transplant **26**(5): 463-70.

We investigated whether adjusting the oral busulfan (BU) dosage on the basis of early pharmacokinetic data to achieve a targeted drug exposure could reduce transplant-related complications in children with advanced hematologic malignancies. Twenty-five children received a preparative regimen consisting of thiotepa (250 mg/m2 i.v. daily for 3 days), BU (40 mg/m2 per dose p.o. every 6 h for 12 doses), and cyclophosphamide (60 mg/kg i.v. daily for 2 days) and then underwent allogeneic stem cell transplantation. Busulfan clearance and area under concentration time-curve (AUC) were determined after the first dose using a one-compartment pharmacokinetic (PK) model with first-order absorption. The initial PK analysis was successfully completed after the first BU dose in 21 patients (84%). A final AUC of 1000-1500 microM x min/dose was targeted and subsequent doses were modified as necessary to achieve this value. Fourteen of the 25 patients (56%) required dose adjustment. Follow-up PK analysis was completed in 21 patients and 16 of these achieved the targeted BU exposure for the course of therapy. Interpatient variability in BU clearance was high (up to five-fold). The most frequent regimen-related toxicities were cutaneous and gastrointestinal (stomatitis and diarrhea). Only one patient developed hepatic veno-occlusive disease. Our study demonstrates the feasibility of adjusting the oral BU dose in individual pediatric patients. Although toxicity associated with BU seemed to be reduced, this conclusion is tempered by the fact that the overall regimen-related toxicity (RRT) remains substantial and reflected the effects of all agents used in the preparative regimen.

Trosko, J. E. (2005). "The role of stem cells and cell-cell communication in radiation carcinogenesis: ignored concepts." BJR Suppl **27**: 132-8.

Given the complexity of the carcinogenic process and the relative lack of mechanistic understanding about how ionising radiation at low level exposures affects the multistage, multimechanism processes of carcinogenesis, it is imperative that major concepts and paradigms be re-examined when extrapolating from high level to low level results. Clearly, any health effect directly linked to low level radiation exposure must have molecular/biochemical and biological bases. On the other hand, demonstrating some molecular/biochemical or cellular effect, using surrogate systems for the whole human being, may not have a corresponding health effect. Given the general acceptance of an extrapolated linear no-threshold (LNT) model, our current understanding of the multistage, multimechanism process of carcinogenesis cries out for a resolution of a real problem. How can a low level acute, or even chronic, exposure of ionising radiation bring about all the different mechanisms (mutagenic, cytotoxic and epigenetic) and genotypic/phenotypic changes needed to convert a normal cell in a body to an invasive, malignant cell, given all the protective, repair and suppressive systems known to exist in the human body? Until recently, the prevailing paradigm that ionising radiation brings about cancer via DNA damage and its conversion to gene and chromosomal mutations drove our interpretation of radiation carcinogenesis. Today, our knowledge includes both the fact that epigenetic events play a major role in carcinogenesis and that low level radiation can also induce epigenetic events in and between cells in tissues, and this challenges any simple extrapolation of the LNT model. Although a recent description of the "hallmarks" of the cancer process has helped to focus on how ionising radiation might contribute to the induction of cancers, several other previously ignored hallmarks, namely the stem cells in tissues as targets for carcinogenesis and the role of cell-cell

communication processes in modulating the radiation effects on the target cell, must be considered.

Tsirigotis, P. D., I. B. Resnick, et al. (2009). "Post-hematopoietic stem cell transplantion immune-mediated cytopenias." Immunotherapy **1**(1): 39-47.

Immune-mediated cytopenias after allogeneic stem cell transplantation can be categorized as either alloimmune when host or donor immunity reacts against donor or host elements, respectively, or autoimmune when donor immunity reacts against donor hematopoietic tissue, owing to poorly understood mechanisms that result in severe impairment of central and peripheral tolerance. Immune cytopenias are manifested as monolineage or more rarely as bilineage cytopenias, and are usually mediated through humoral immune mechanisms. On the contrary, immune-mediated pancytopenia is a rare event with only few cases reported in the literature. The exact pathogenesis of immune pancytopenia is not well known although it is possible that cellular immunity may play a significant role. The importance of these syndromes lies in the fact that they can cause severe morbidity and mortality. Differential diagnosis from other causes of post-transplant pancytopenia is of extreme value because these disorders can respond to various treatment modalities.

Turpeinen, H., L. Volin, et al. (2009). "Genetic similarity of chromosome 6 between patients receiving hematopoietic stem cell transplantation and HLA matched sibling donors." Haematologica **94**(4): 528-35.

BACKGROUND: Matching for HLA genes located on chromosome 6 is required in hematopoietic stem cell transplantation to reduce the incidence of graft-versus-host disease. However, a considerable proportion of patients still suffer from it, obviously due to genetic differences outside the HLA gene region. DESIGN AND METHODS: We studied the similarity of almost 4,000 single nucleotide polymorphisms on chromosome 6 between patients receiving hematopoietic stem cell transplantation and their HLA-matched sibling donors. RESULTS: We observed that as a result of routine HLA matching the siblings in fact shared surprisingly long chromosomal fragments with similar single nucleotide polymorphism genotypes--from 11.65 Mb to 134.66 Mb. The number of genes mapped on these shared fragments varied from 402 to 1,302. Considering the whole chromosome 6, the HLA-matched siblings were apparently identical for 65.2-97.8% of the single nucleotide polymorphisms. CONCLUSIONS: Potentially, genes similar in some transplantation pairs while different in others might have a significant role in determining the outcome after hematopoietic stem cell transplantation.

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.

Voralia, M., A. Semeluk, et al. (1987). "Facilitation of syngeneic stem cell engraftment by anti-class I monoclonal antibody pretreatment of unirradiated recipients." Transplantation **44**(4): 487-94.

We have established a murine model of syngeneic bone marrow transplantation based on the use of monoclonal antibody as the sole conditioning regimen in unirradiated recipients. Administration of a single injection of monoclonal antibody directed against major histocompatibility complex-encoded class I determinants facilitated permanent hemopoietic stem cell engraftment without any apparent side-effects. Whereas untreated hosts exhibited a maximal chimerism of 15% at donor cell doses of up to 12 X 10(7) bone marrow cells, pretreatment by 2 mg of anti-class I antibody one week prior to transplantation

of 3 X 10(7) syngeneic bone marrow cells resulted in a mean donor representation of about 80%. The antibody can be given up to four weeks prior to transplantation, and the degree of donor engraftment observed is a function of the dose of antibody administered. The fact that specific antibody enhanced engraftment in two strain combinations indicates that antibody is the active agent in facilitating engraftment and that facilitation is not strain-restricted. Anti-class I antibodies of the IgG2a, but not IgG1, isotype are effective in promoting engraftment. Although the isotype requirement suggests a role for antibody-mediated cytotoxicity in promoting stem cell engraftment, the extensive time-frame of facilitation suggests that other effects of the antibody may also be involved. The model of syngeneic bone marrow transplantation we describe here will be useful in studying the mechanisms regulating stem cell engraftment and may have potential clinical application as an approach to autologous marrow transplantation.

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., J. Imitola, et al. (2008). "Paradoxical dysregulation of the neural stem cell pathway sonic hedgehog-Gli1 in autoimmune encephalomyelitis and multiple sclerosis." Ann Neurol **64**(4): 417-27.

OBJECTIVE: Neurovascular niches have been proposed as critical components of the neural stem cell (NSC) response to acute central nervous system injury; however, it is unclear whether these potential reparative niches remain functional during chronic injury. Here, we asked how central nervous system inflammatory injury regulates the intrinsic properties of NSCs and their niches. METHODS: We investigated the sonic hedgehog (Shh)-Gli1 pathway, an important signaling pathway for NSCs, in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS), and its regulation by inflammatory cytokines. RESULTS: We show that Shh is markedly upregulated by reactive and perivascular astroglia in areas of injury in MS lesions and during EAE. Astroglia outside the subventricular zone niche can support NSC differentiation toward neurons and oligodendrocytes, and Shh is a critical mediator of this effect. Shh induces differential upregulation of the transcription factor Gli1, which mediates Shh-induced NSC differentiation. However, despite the increase in Shh and the fact that Gli1 was initially increased during early inflammation of EAE and active lesions of MS, Gli1 was significantly decreased in spinal cord oligodendrocyte precursor cells after onset of EAE, and in chronic active and inactive lesions from MS brain. The Th1 cytokine interferon-gamma was unique in inducing Shh expression in astroglia and NSCs, while paradoxically suppressing Gli1 expression in NSCs and inhibiting Shh-mediated NSC differentiation. INTERPRETATION: Our data suggest that endogenous repair potential during chronic injury appears to be limited by inflammation-induced alterations in intrinsic NSC molecular pathways such as Gli1.

Wanner, M., S. Bochert, et al. (2009). "Losing the genetic twin: donor grief after unsuccessful unrelated stem cell transplantation." BMC Health Serv Res **9**: 2.

BACKGROUND: Stem cell transplantations from related or unrelated donors are used to cure leukaemia and other blood diseases. When a patient dies after an unsuccessful transplantation, interested unrelated donors are informed about the failure by their donor centre. Studies focussing on failed related

donations show that donors undergo an intense grieving process. Questionnaires were sent to 395 unrelated donors who received the news of their recipients' deaths between November 2005 and August 2006. In addition, twelve in-depth interviews with selected donors were carried out. RESULTS: Unrelated donors were emotionally affected by the recipients' deaths, and it is appropriate to speak about a "Donor Grief" phenomenon, as the results of 325 returned questionnaires (return rate 82.3%) and in-depth interviews show. Donors demonstrated a range of feelings such as sadness, disappointment, grief, and helplessness. These feelings were often unexpectedly intense given the fact that the recipient was a stranger. Although the news caused grief, donors underlined that they nevertheless wanted to be informed. They preferred knowledge of the failure to uncertainty. The method of providing the information is only of secondary importance. Most donors favoured the way of communication they had experienced. CONCLUSION: This result indicates that both phone and letter communication can be justified. However, phone communication seems to be superior with respect to aspects of sensitivity. In spite of transplantation failure and the associated negative feelings, most donors were happy to have donated and would be willing to do so again. Our results underline the special responsibility of donor centres for informing and supporting unrelated volunteer donors in case their recipients have died.

Yanada, S., M. Ochi, et al. (2006). "Effects of CD44 antibody-- or RGDS peptide--immobilized magnetic beads on cell proliferation and chondrogenesis of mesenchymal stem cells." J Biomed Mater Res A **77**(4): 773-84.

We evaluated the efficacy of a novel mesenchymal stem cell (MSC) delivery system using an external magnetic field for cartilage repair in vitro. MSCs were isolated from the bone marrow of Sprague Drawley rats and expanded in a monolayer. To use the MSC delivery system, two types of MSC-magnetic bead complexes were designed and compared. Expanded MSCs were combined with small-sized (diameter: 310 nm) carboxyl group-combined (0.01-0.04 micromol/mg) magnetic beads, Ferri Sphere 100C, through either anti-rat CD44 mouse monoclonal antibodies or a synthetic cell adhesion factor, arginine (R)-glycine (G)-aspartic acid (D)-serine (S) (RGDS) peptide. Both cell complexes were successfully created, and were able to proliferate in monolayer culture up to at least day 7 after separation of magnetic beads from the cell surface, although the proliferation of the complexes was slower in the early period of culture than that of non-labeled rat MSCs (after 7 days of culture: proliferation of CD44 antibody-bead complexes, approximately 50%; RGDS peptide-bead complexes, 70% versus non-labeled rat MSCs, respectively). These complexes were seeded onto culture plates with or without an external magnetic force (magnetic flux density was 0.20 Tesla at a distance of 2 mm from plate base) generated by a neodymium magnet, and supplemented with chondrogenic differentiation medium. Both complexes could be attached and gathered effectively under the influence of the external magnet, and CD44-bead complexes could effectively generate chondrogenic matrix in monolayer culture. In a three-dimensional culture system, the production of a dense chondrogenic matrix and the expression of type II collagen and aggrecan mRNA were detected in both complexes, and the chondrogenic potential of these complexes was only a little less than that of rat MSCs alone. Thus, we conclude that due to the fact that MSC-RGDS peptide-bead complexes are composed using a biodegradable material, RGDS peptide, as a mediator, the RGDS peptide-bead complex is more useful for minimally invasive clinical applications using our design of magnetic MSC delivery system than CD44 antibody-beads.

Zangiacomi, V., N. Balon, et al. (2008). "Cord blood-derived neurons are originated from CD133+/CD34 stem/progenitor cells in a cell-to-cell contact dependent manner." Stem Cells Dev **17**(5): 1005-16.

Previous studies described that neurons could be generated in vitro from human umbilical cord blood cells. However, there are few data concerning their origin. Notably, cells generating neurons are not well characterized. The present study deals with the origin of cord blood cells generating neurons and mechanisms allowing the neuronal differentiation. We studied neuronal markers of both total fractions of cord blood and stem/progenitor cord blood cells before and after selections and cultures. We also compared neuronal commitment of cord blood cells to that observed for the neuronal cell line SK-N-BE(2). Before cultures, neuronal markers are found within the total fraction of cord blood cells. In CD133+ stem/progenitor cell fraction only immature neuronal markers are detected. However, CD133+ cells are unable to give rise to neurons in cultures, whereas this is achieved when total fraction of cord blood cells is used. In fact, mature functional neurons can be generated from CD133+ cells only in cell-to-cell close contact with either CD133- fraction or a neurogenic epithelium. Furthermore, since CD133+ fraction is heterogenous, we used several selections to precisely identify the phenotype of cord blood-derived neuronal stem/progenitor cells. Results reveal that only CD34- cells from CD133+ fraction possess neuronal potential. These data show the phenotype of cord

blood neuronal stem/progenitor cells and the crucial role of direct cell-to-cell contact to achieve their commitment. Identifying the neuron supporting factors may be beneficial to the use of cord blood neuronal stem/progenitor cells for regenerative medicine.

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