

Cancer Stem Cell Literatures

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

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<http://www.sciencepub.net/stem>. 3

Key words: stem cell; life; gene; DNA; protein; cancer

Literatures:

Adams, G. and D. Scadden (2004). "Defining the hematopoietic stem cell niche." *Discov Med* 4(21): 118-9.

Extract: Stem cells modulate tissue formation and repair based on a complex interaction of cell autonomous and non-autonomous regulatory mechanisms. While reductionist approaches to understanding stem cell control continue to be extremely productive, understanding the physiological contexts in which stem cells function, will ultimately require definition of the microenvironments in which they live. The location of stem or precursor populations within numerous solid tissues has been described, but delineating specific associated cells and how they participate in regulating stem cell function has generally been lacking for mammalian tissues. However, the use of invertebrate-based models has created particularly productive systems in which to examine the niche context of stem cells. Gonadal tissue from *C. elegans* and *D. melanogaster* has permitted the definition and identification of ancillary niche cells, physical interactions and the molecular pathways such as Notch paralogues that govern the interplay between the stem cell and its local environment. We sought to determine a niche component for a mammalian tissue and focused on the hematopoietic system. We focused on hematopoiesis for multiple reasons, but in particular because of the potential for applying the information gained to a medical context.

Allen, J. E., L. S. Hart, et al. (2009). "Visualization and enrichment of live putative cancer stem cell populations following p53 inactivation or Bax deletion using non-toxic fluorescent dyes." *Cancer Biol Ther* 8(22): 2194-205.

Putative cancer stem cell (CSC) populations efflux dyes such as Hoechst 33342 giving rise to side populations (SP) that can be analyzed or isolated by flow cytometry. However, Hoechst 33342 is highly toxic, more so to non-SP cells, and thus presents difficulties in interpreting in vivo studies where non-SP cells appear less tumorigenic than SP cells in immunodeficient mice. We searched for non-toxic dyes to circumvent this problem as well as to image these putative CSCs. We found that the fluorescent dye calcein, a product of intracellular Calcein AM cleavage, is effluxed by a small subpopulation, calcein low population (C(lo)P). This population overlaps with SP and demonstrated long term cell viability, lack of cell stress and proliferation in several cancer cell lines when stained whereas Hoechst 33342 staining caused substantial apoptosis and ablated proliferation. We also found that the effluxed dye D-luciferin exhibits strong UV-fluorescence that can be imaged at cellular resolution and spatially overlaps with Calcein AM. In order to evaluate the hypothesis that p53 loss promotes enrichment of putative CSC populations we used Calcein AM, D-luciferin and Mitotracker Red FM as a counterstain to visualize dye-effluxing cells. Using fluorescence microscopy and flow cytometry we observed increased dye-effluxing populations in DLD-1 colon tumor cells with mutant p53 versus wild-type (WT) p53-expressing HCT116 cells. Deletion of the wild-type p53 or pro-apoptotic Bax genes induced the putative CSC populations in the HCT116 background to significant levels. Restoration of WT p53 in HCT116 p53(-/-) cells by an adenovirus vector eliminated the putative CSC populations whereas a control adenovirus vector, Ad-LacZ, maintained the putative CSC population. Our results suggest it is possible to image and quantitatively analyze putative CSC

populations within the tumor microenvironment and that loss of pro-apoptotic and tumor suppressing genes such as Bax or p53 enrich such tumor-prone populations.

Amano, T., Y. Kato, et al. (2001). "Full-term development of enucleated mouse oocytes fused with embryonic stem cells from different cell lines." *Reproduction* **121**(5): 729-33.

The developmental potential of enucleated mouse oocytes receiving embryonic stem cells from ten lines with either the same or different genetic backgrounds using the cell fusion method was examined in vitro and in vivo. The development of nuclear-transferred oocytes into blastocysts was high (34-88%). However, there was no clear correlation between development into blastocysts after nuclear transfer and the chimaera formation rate of embryonic stem cells. The development into live young was low (1-3%) in all cell lines and 14 of 19 young died shortly after birth. Most of the live young had morphological abnormalities. Of the five remaining mice, two died at days 23 and 30 after birth, but the other three mice are still active at days 359 (mouse 1) and 338 (mice 4 and 5) after birth, with normal fertility. However, the reasons for the abnormalities and postnatal death of embryonic stem cell-derived mice are unknown.

Anderson, G. S., K. Miyagi, et al. (2002). "Anti-tumor effect of Merocyanine 540-mediated photochemotherapy combined with Edelfosine: potential implications for the ex vivo purging of hematopoietic stem cell grafts from breast cancer patients." *J Photochem Photobiol B* **68**(2-3): 101-8.

High-dose chemotherapy combined with autologous stem cell support has improved response rates in high-risk and metastatic breast cancer, but has failed to improve long-term survival. Breast cancer has a tendency to metastasize to the bone marrow, and live tumor cells are known to circulate in the peripheral blood of breast cancer patients. Sensitive immunohistochemical, culture-based, and reverse transcriptase polymerase chain reaction (RT-PCR)-based methods have shown that about 50% of histologically normal stem cell grafts from breast cancer patients are contaminated with occult tumor cells, which may cause or contribute to tumor recurrences. Merocyanine 540 (MC540)-mediated photodynamic therapy (PDT) inactivates a wide range of leukemia and lymphoma cells and is well tolerated by normal hematopoietic stem and progenitor cells. Unfortunately, most solid tumor cells (including breast cancer cells) are only moderately sensitive or refractory to MC540-PDT. We report here that if MC540-PDT is followed by a 1-h incubation with the

alkyl-lysophospholipid, Edelfosine (ET-18-OCH₃), the depletion of murine and human breast cancer cells is greatly enhanced whereas the recovery of normal hematopoietic stem and progenitor cells is only minimally degraded. When used under conditions that reduce CD34-positive human bone marrow cells only 5.1-fold, and murine and human granulocyte/macrophage progenitors 6.8- and 3-fold, respectively, combination purging with MC540-PDT and Edelfosine depletes murine (Mm5MT) and human (MDA-MB-435S) breast cancer cells >17,000- and >125,000-fold, respectively. These data suggest that combination purging with MC540-PDT and Edelfosine may offer a simple, safe and effective method for the ex vivo purging of autologous stem cell grafts from breast cancer patients.

Anderson, G. S., I. Tsujino, et al. (2003). "Preferential inactivation of paediatric solid tumour cells by sequential exposure to Merocyanine 540-mediated photodynamic therapy and Edelfosine: implications for the ex vivo purging of autologous haematopoietic stem cell grafts." *J Photochem Photobiol B* **69**(2): 87-95.

Paediatric solid tumours exhibit steep dose-response curves to alkylating agents and are therefore considered candidates for high-dose chemotherapy and autologous stem cell support. There is growing evidence that autologous stem cell grafts from patients with solid tumours are frequently contaminated with live tumour cells. The objective of this study was to perform, in a preclinical purging model, an initial assessment of the safety and efficacy of a two-step purging procedure that combined Merocyanine 540-mediated photodynamic therapy (MC540-PDT) with a brief exposure to the alkyl-lysophospholipid, Edelfosine. Human and murine bone marrow cells and Neuro-2a murine neuroblastoma, SK-N-SH human neuroblastoma, SK-ES-1 and U-2 OS human osteosarcoma, G-401 and SK-NEP-1 human Wilms' tumour, and A-204 human rhabdomyosarcoma cells were exposed to a fixed dose of MC540-PDT followed by a brief incubation with graded concentrations of Edelfosine. Survival was subsequently assessed by in vitro clonal assay or, in the case of CD34-positive haematopoietic stem cells, by an immunohistochemical method. Combination purging with MC540-PDT and Edelfosine depleted all tumour cells by >4 log while preserving at least 15% of murine granulocyte/macrophage progenitors (CFU-GM), 34% of human CFU-GM, and 31% of human CD34-positive cells. The data suggest that combination purging with MC540-PDT and Edelfosine may be useful for the ex vivo purging of autologous stem cell grafts from patients with paediatric solid tumours.

Andrew, D. and R. Aspinall (2001). "IL-7 and not stem cell factor reverses both the increase in apoptosis and the decline in thymopoiesis seen in aged mice." *J Immunol* **166**(3): 1524-30.

Thymic atrophy is an age-associated decline in commitment to the T cell lineage considered to be associated with defective TCR beta-chain rearrangement. Both IL-7 and stem cell factor (SCF) have dominant roles at this stage of triple negative (TN) thymocyte development. Because there is no age-associated decrease in the number of CD44(+)CD25(-)CD3(-)CD4(-)CD8(-) cells, this study investigated whether alterations in apoptosis within the TN pathway accounted for diminishing thymocyte numbers with age. Here we show significant age-associated increases in apoptotic TN thymocytes, specifically within CD44(+)CD25(+) and CD44(-)CD25(+) subpopulations, known to be the location of TCR beta-chain rearrangement. IL-7 added to TN cultures established from old mice significantly both reduces apoptosis and increases the percentage of live cells within CD44(+)CD25(+) and CD44(-)CD25(+) subpopulations after 24 h, with prosurvival effects remaining after 5 days. SCF failed to demonstrate prosurvival effects in old or young cultures, and IL-7 and SCF together did not improve upon IL-7 alone. IL-7R expression did not decline with age, ruling out the possibility that the age-associated increase in apoptosis was attributed to reduced IL-7R expression. Compared with PBS, treatment of old mice with IL-7 produced significant increases in live TN cells. By comparison, treatment with SCF failed to increase live TN numbers, and IL-7 and SCF together failed to significantly improve thymopoiesis above that shown by IL-7 alone. Thus, treatment with IL-7 alone can reverse the age-associated defect in TN thymocyte development revealed by *in vitro* studies to be located at the stages of TCR beta-chain rearrangement.

Angelucci, E., F. Pilo, et al. (2009). "Hematopoietic stem cell transplantation in thalassemia and related disorders." *Mediterr J Hematol Infect Dis* **1**(1): e2009015.

The basis of allogeneic hemopoietic stem cell (HSC) transplantation in thalassemia consists in substituting the ineffective thalassaemic erythropoiesis with an allogeneic effective one. This cellular replacement therapy is an efficient way to obtain a long lasting, probably permanent, clinical effective correction of the anaemia avoiding transfusion requirement and subsequent complications like iron overload. The first HSC transplant for thalassemia was performed in Seattle on Dec 2, 1981. In the early eighties transplantation procedure was limited to very

few centres worldwide. Between 17 December 1981 and 31 January 2003, over 1000 consecutive patients, aged from 1 to 35 years, underwent transplantation in Pesaro. After the pioneering work by the Seattle and Pesaro groups, this therapeutic approach is now widely applied worldwide. Medical therapy of thalassemia is one of the most spectacular successes of the medical practice in the last decades. In recent years advances in knowledge of iron overload pathophysiology, improvement and diffusion of diagnostic capability together with the development of new effective and safe oral chelators promise to further increase success of medical therapy. Nevertheless situation is dramatically different in non-industrialized countries where the very large majority of patients live today. Transplantation technologies have improved substantially during the last years and transplantation outcome is likely to be much better today than in the '80s. Recent data indicated a probability of overall survival and thalassemia free survival of 97% and 89% for patients with no advanced disease and of 87% and 80% for patients with advanced disease. Thus the central role of HSC in thalassemia has now been fully established. HSC remains the only definitive curative therapy for thalassemia and other hemoglobinopathies. The development of oral chelators has not changed this position. However this has not settled the controversy on how this curative but potentially lethal treatment stands in front of medical therapy for adults and advanced disease patients. In sickle cell disease HSC transplantation currently is reserved almost exclusively for patients with clinical features that indicate a poor outcome or significant sickle-related morbidity.

Benoit, D. S., A. R. Durney, et al. (2007). "The effect of heparin-functionalized PEG hydrogels on three-dimensional human mesenchymal stem cell osteogenic differentiation." *Biomaterials* **28**(1): 66-77.

Poly(ethylene glycol) (PEG) hydrogels functionalized with heparin were utilized as a three-dimensional culture system for human mesenchymal stem cells (hMSCs). Heparin-functionalized hydrogels supported hMSC viability, as quantified through live/dead imaging, and induced osteogenic differentiation, as measured by increased alkaline phosphatase (ALP) production and osteopontin (OPN) and collagen I (COL I) gene expression over the 5-week study. Further exploration of the potential mechanism of heparin-induced osteogenic differentiation was performed. Specifically, the availability of bone morphogenetic protein 2 (BMP2) and fibronectin (FN) in the culture system was controlled and hMSC osteogenic differentiation was evaluated as a function of the microenvironment.

BMP2 availability increased both ALP production and OPN gene expression, while FN increased ALP production, but not OPN gene expression. Furthermore, immunostaining of integrin expression revealed that viability and differentiation were differentially affected by integrin production, where both alpha5beta1 and alphavbeta3 integrin-ligand interactions supported viability, while only the alpha5beta1 integrin played a role in hMSC osteogenic differentiation.

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.

Cai, C., J. Thorne, et al. (2008). "Hedgehog serves as a mitogen and survival factor during embryonic stem cell neurogenesis." *Stem Cells* **26**(5): 1097-108.

Hedgehog (Hh) signaling is involved in a wide range of important biological activities. Within the vertebrate central nervous system, Sonic Hedgehog (Shh) can act as a morphogen or mitogen that regulates the patterning, proliferation, and survival of neural stem cells (NSCs). However, its role in embryonic stem cell (ESC) neurogenesis has not been explored in detail. We have previously shown that Hh signaling is required for ESC neurogenesis. In order to elucidate the underlying mechanism, we utilized the Sox1-GFP ESC line, which has a green fluorescent protein (GFP) reporter under the control of the Sox1 gene promoter, providing an easy means of detecting NSCs in live cell culture. We show here that ESC differentiation in adherent culture follows the ESC--> primitive ectoderm --> neurectoderm transitions observed in vivo. Selective death of the Sox1-GFP-negative cells contributes to the enrichment of Sox1-GFP-positive NSCs. Interestingly, Shh is expressed exclusively by the NSCs themselves and elicits distinct downstream gene expression in Sox1-GFP-positive and -negative cells. Suppression of Hh signaling by antagonist treatment leads to different responses from these two populations as well:

increased apoptosis in Sox1-GFP-positive NSCs and decreased proliferation in Sox1-GFP-negative primitive ectoderm cells. Hedgehog agonist treatment, in contrast, inhibits apoptosis and promotes proliferation of Sox1-GFP-positive NSCs. These results suggest that Hh acts as a mitogen and survival factor during early ESC neurogenesis, and evidence is presented to support a novel autocrine mechanism for Hh-mediated effects on NSC survival and proliferation.

Cantoni, N., M. Weisser, et al. (2009). "Infection prevention strategies in a stem cell transplant unit: impact of change of care in isolation practice and routine use of high dose intravenous immunoglobulins on infectious complications and transplant related mortality." *Eur J Haematol* **83**(2): 130-8.

OBJECTIVE: Nursing in 'live islands' and routine high dose intravenous immunoglobulins after allogeneic hematopoietic stem cell transplantation were abandoned by many teams in view of limited evidence and high costs. **METHODS:** This retrospective single-center study examines the impact of change from nursing in 'live islands' to care in single rooms (SR) and from high dose to targeted intravenous immunoglobulins (IVIG) on mortality and infection rate of adult patients receiving an allogeneic stem cell or bone marrow transplantation in two steps and three time cohorts (1993-1997, 1997-2000, 2000-2003). **RESULTS:** Two hundred forty-eight allogeneic hematopoietic stem cell transplantations were performed in 227 patients. Patient characteristics were comparable in the three cohorts for gender, median age, underlying disease, and disease stage, prophylaxis for graft versus host disease (GvHD) and cytomegalovirus constellation. The incidence of infections (78.4%) and infection rates remained stable (rates/1000 days of neutropenia for sepsis 17.61, for pneumonia 6.76). Cumulative incidence of GvHD and transplant-related mortality did not change over time. **CONCLUSIONS:** Change from nursing in 'live islands' to SR and reduction of high dose to targeted IVIG did not result in increased infection rates or mortality despite an increase in patient age. These results support the current practice.

Chapon, C., J. S. Jackson, et al. (2009). "An in vivo multimodal imaging study using MRI and PET of stem cell transplantation after myocardial infarction in rats." *Mol Imaging Biol* **11**(1): 31-8.

PURPOSE: The purpose of the study is to track iron-oxide nanoparticle-labelled adult rat bone marrow-derived stem cells (IO-rBMSCs) by magnetic resonance imaging (MRI) and determine their effect in host cardiac tissue using 2-deoxy-2-[F-18]fluoro-D: -glucose-positron emission tomography (FDG-PET).

PROCEDURES: Infarcted rats were randomised to receive (1) live IO-rBMSCs by direct local injection, or (2) dead IO-rBMSCs as controls; (3) sham-operated rats received live IO-rBMSCs. The rats were then imaged from 2 days to 6 weeks post-cell implantation using both MRI at 9.4T and FDG-PET. **RESULTS:** Implanted IO-rBMSCs were visible in the heart by MRI for the duration of the study. Histological analysis confirmed that the implanted IO-rBMSCs were present for up to 6 weeks post-implantation. At 1 week post-IO-rBMSC transplantation, PET studies demonstrated an increase in FDG uptake in infarcted regions implanted with live IO-rBMSC compared to controls. **CONCLUSIONS:** Noninvasive multimodality imaging allowed us to visualise IO-rBMSCs and establish their affect on cardiac function in a rat model of myocardial infarction (MI).

Chin, V. I., P. Taupin, et al. (2004). "Microfabricated platform for studying stem cell fates." *Biotechnol Bioeng* **88**(3): 399-415.

Platforms that allow parallel, quantitative analysis of single cells will be integral to realizing the potential of postgenomic biology. In stem cell biology, the study of clonal stem cells in multiwell formats is currently both inefficient and time-consuming. Thus, to investigate low-frequency events of interest, large sample sizes must be interrogated. We report a simple, versatile, and efficient micropatterned arraying system conducive to the culture and dynamic monitoring of stem cell proliferation. This platform enables: 1) parallel, automated, long-term (approximately days to weeks), live-cell microscopy of single cells in culture; 2) tracking of individual cell fates over time (proliferation, apoptosis); and 3) correlation of differentiated progeny with founder clones. To achieve these goals, we used microfabrication techniques to create an array of approximately 10,000 microwells on a glass coverslip. The dimensions of the wells are tunable, ranging from 20 to >500 microm in diameter and 10-500 microm in height. The microarray can be coated with adhesive proteins and is integrated into a culture chamber that permits rapid (approximately min), addressable monitoring of each well using a standard programmable microscope stage. All cells share the same media (including paracrine survival signals), as opposed to cells in multiwell formats. The incorporation of a coverslip as a substrate also renders the platform compatible with conventional, high-magnification light and fluorescent microscopy. We validated this approach by analyzing the proliferation dynamics of a heterogeneous adult rat neural stem cell population. Using this platform, one can further interrogate the response of distinct stem cell subpopulations to microenvironmental cues

(mitogens, cell-cell interactions, and cell-extracellular matrix interactions) that govern their behavior. In the future, the platform may also be adapted for the study of other cell types by tailoring the surface coatings, microwell dimensions, and culture environment, thereby enabling parallel investigation of many distinct cellular responses.

Cho, M., M. Jang, et al. (2006). "An alternative method of deriving embryonic stem cell-like clones by aggregation of diploid cells with tetraploid embryos." *Fertil Steril* **85 Suppl 1**: 1103-10.

OBJECTIVE: To assess whether embryonic stem (ES) cells could be derived from the aggregation of diploid cells with tetraploid embryos. **DESIGN:** Randomized, prospective study. **SETTING:** University embryology and gamete biotechnology laboratory. **ANIMAL(S):** F1 (C57BL6/DBA2) mice. **INTERVENTION(S):** Four- to eight-cell F1 tetraploid embryos were aggregated with 10 to 15 donor E14 ES cells. **MAIN OUTCOME MEASURE(S):** Embryogenesis and ES cell establishment. **RESULT(S):** No difference (78% to 89%) in blastocyst formation was detected between the aggregated tetraploid and the control diploid embryos. In a total of 27 transfers, pregnancy was detected in three tetraploid (23.1%) and five diploid (35.7%) cases, and three live births developed from the aggregated tetraploid embryos. The tetraploid blastocysts without aggregation were plated, but no ES cell-like colony was formed. Six of eight aggregated blastocysts derived well-proliferated colonies, which were positive for anti-stage-specific embryonic antigen (SSEA)-1 antibody, Oct-4, and alkaline phosphatase. The microsatellite assay confirmed the homogenous makeup among the donor E14 cells and live-birth and ES-like cells derived from the E14-aggregated, tetraploid embryo. **CONCLUSION(S):** The aggregation of pluripotent diploid cells with tetraploid embryos yielded live births and ES-like cells that were homogenous to the donor diploid cells.

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.

Cogle, C. R., S. M. Guthrie, et al. (2003). "An overview of stem cell research and regulatory issues." *Mayo Clin Proc* 78(8): 993-1003.

Stem cells are noted for their ability to self-renew and differentiate into a variety of cell types. Some stem cells, described as totipotent cells, have tremendous capacity to self-renew and differentiate. Embryonic stem cells have pluripotent capacity, able to form tissues of all 3 germ layers but unable to form an entire live being. Research with embryonic stem cells has enabled investigators to make substantial gains in developmental biology, therapeutic tissue engineering, and reproductive cloning. However, with these remarkable opportunities many ethical challenges arise, which are largely based on concerns for safety, efficacy, resource allocation, and methods of harvesting stem cells. Discussing the moral and legal status of the human embryo is critical to the debate on stem cell ethics. Religious perspectives and political events leading to regulation of stem cell research are presented and discussed, with special attention directed toward the use of embryonic stem cells for therapeutic and reproductive cloning. Adult stem cells were previously thought to have a restricted capacity to differentiate; however, several reports have described their plasticity potential. Furthermore, there have been close ties between the behavior of stem cells and cancer cells. True eradication of cancer will require a deeper understanding of stem cell biology. This article was written to inform medical scientists and practicing clinicians across the spectrum of medical education about the research and regulatory issues affecting the future of stem cell therapy.

Dahan, J. F. and C. F. Auerbach (2006). "A qualitative study of the trauma and posttraumatic growth of multiple myeloma patients treated with peripheral blood stem cell transplant." *Palliat Support Care* 4(4): 365-87.

OBJECTIVE: The study was conducted to understand the emotional impact of multiple myeloma, as well as the impact of its principle treatment, peripheral blood stem cell transplant (PBSCT). The absence of psycho-oncology research literature on this population prompted the need for a

hypothesis-generating investigation. Thus, a qualitative design was used to construct a theoretical model of the trauma relating to diagnosis and treatment of myeloma. The study also incorporates the important period of reflection and growth following treatment. **METHODS:** The sample consisted of 3 women and 3 men treated for myeloma at a New York City-based cancer treatment center. Data from individual interviews were audiotaped and transcribed. After extensive review, the data were categorized into groups of repeating ideas, themes and broad theoretical constructs. **RESULTS:** A five-construct model emerged from the data analysis that integrated a model of trauma and growth presented in earlier work (Auerbach et al., 2006). These constructs roughly correspond with stages of illness, but do not necessarily imply a linear process, as suggested by stage models. The first construct is diagnosis. Patients receive the news that they have multiple myeloma. Initial reactions are discussed and a treatment plan takes form. In the second construct, treatment, patients highlight the physical and emotional hurdles confronted throughout treatment. The third construct, network of safety, presents social factors that play a role in comforting patients throughout illness. Patients recognize the importance of a strong support system during their experiences. In the fourth construct, recuperation, physical energy is regained after an arduous recovery period. This contributes to higher spirits and a motivation to reengage with life. The fifth construct is reflection and new existence. Patients strive to balance a new reality that relapse and death are inevitable, along with their need to live a meaningful life. Many do not yet appreciate how their disease has impacted them, but describe how their interpersonal lives and perceptions have changed, both positively and negatively. **SIGNIFICANCE OF RESULTS:** Limitations of the study, future directions for research and clinical implications are discussed.

Drapeau, C., H. Ma, et al. (2009). "The stem cell mobilizer StemEnhance does not promote tumor growth in an orthotopic model of human breast cancer." *Anticancer Res* 29(1): 443-7.

Bone marrow-derived stem cells (BMDSC) have been implicated in tumor formation, though it is not clear whether they contribute to tumor growth. A novel mobilizer of BMDSC (StemEnhance; SE) was used to investigate whether its daily administration promotes tumor growth. Forty mice were surgically transplanted with human MDA-MB-435-GFP breast cancer into the mammary fat pad of nude mice, The mice were gavaged for six weeks with 300 mg/kg of SE. Tumor growth was monitored using live whole-body fluorescence imaging. At the end of the study, tumors were excised and weighed. At the start of the

feeding trial, tumor areas for both control and experimental group were statistically identical. Tumor growth rate was slower in the SE group ($p = 0.014$) when compared to the control group. After 6 weeks, tumor areas were 40% larger in the control ($p < 0.01$) and mean tumor weight was 35% smaller in the SE-treated group (0.44 g vs. 0.68 g; $p = 0.031$). Feeding of SE did not promote tumor growth but rather reduced the growth of human MDA-MB-435 breast cancer.

Egger, B., J. Q. Boone, et al. (2007). "Regulation of spindle orientation and neural stem cell fate in the *Drosophila* optic lobe." *Neural Dev* 2: 1.

BACKGROUND: The choice of a stem cell to divide symmetrically or asymmetrically has profound consequences for development and disease. Unregulated symmetric division promotes tumor formation, whereas inappropriate asymmetric division affects organ morphogenesis. Despite its importance, little is known about how spindle positioning is regulated. In some tissues cell fate appears to dictate the type of cell division, whereas in other tissues it is thought that stochastic variation in spindle position dictates subsequent sibling cell fate. **RESULTS:** Here we investigate the relationship between neural progenitor identity and spindle positioning in the *Drosophila* optic lobe. We use molecular markers and live imaging to show that there are two populations of progenitors in the optic lobe: symmetrically dividing neuroepithelial cells and asymmetrically dividing neuroblasts. We use genetically marked single cell clones to show that neuroepithelial cells give rise to neuroblasts. To determine if a change in spindle orientation can trigger a neuroepithelial to neuroblast transition, we force neuroepithelial cells to divide along their apical/basal axis by misexpressing *Inscuteable*. We find that this does not induce neuroblasts, nor does it promote premature neuronal differentiation. **CONCLUSION:** We show that symmetrically dividing neuroepithelial cells give rise to asymmetrically dividing neuroblasts in the optic lobe, and that regulation of spindle orientation and division symmetry is a consequence of cell type specification, rather than a mechanism for generating cell type diversity.

Eve, D. J., P. J. Marty, et al. (2008). "Stem Cell Research and Health Education." *Am J Health Educ* 39(3): 167-179.

Stem cells are being touted as the greatest discovery for the potential treatment of a myriad of diseases in the new millennium, but there is still much research to be done before it will be known whether they can live up to this description. There is also an ethical debate over the production of one of the most

valuable types of stem cell: the embryonic form. Consequently, there is public confusion over the benefits currently being derived from the use of stem cells and what can potentially be expected from their use in the future. The health educator's role is to give an unbiased account of the current state of stem cell research. This paper provides the groundwork by discussing the types of cells currently identified, their potential use, and some of the political and ethical pitfalls resulting from such use.

Faast, R., S. J. Harrison, et al. (2006). "Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs." *Cloning Stem Cells* 8(3): 166-73.

Mesenchymal stem cells (MSCs) isolated from bone marrow were used to examine the hypothesis that a less differentiated cell type could increase adult somatic cell nuclear transfer (SCNT) efficiencies in the pig. SCNT embryos were produced using a fusion before activation protocol described previously and the rate at which these developed to the blastocyst stage compared with that using fibroblasts obtained from ear tissue from the same animal. The use of bone marrow MSCs did not increase cleavage rates compared with adult fibroblasts. However, the percentage of embryos that developed to the blastocyst stage was almost doubled, providing support for the hypothesis that a less differentiated cell can increase cloning efficiencies. As MSCs are relatively difficult to isolate from the bone marrow of live animals, a second experiment was undertaken to determine whether MSCs could be isolated from the peripheral circulation and used for SCNT. Blood MSCs were successfully isolated from four of the five pigs sampled. These cells had a similar differentiation capacity and marker profile to those isolated from bone marrow but did not result in increased rates of development. This is the first study to our knowledge, to report that MSCs can be derived from peripheral blood and used for SCNT for any species. These cells can be readily obtained under relatively sterile conditions compared with adult fibroblasts and as such, may provide an alternative cell type for cloning live animals.

Fernandes, M., V. S. Sangwan, et al. (2004). "Limbal stem cell transplantation." *Indian J Ophthalmol* 52(1): 5-22.

The past two decades have witnessed remarkable progress in limbal stem cell transplantation. In addition to harvesting stem cells from a cadaver or a live related donor, it is now possible to cultivate limbal stem cells in vitro and then transplant them onto the recipient bed. A clear understanding of the basic disease pathology and a

correct assessment of the extent of stem cell deficiency are essential. A holistic approach towards management of limbal stem cell deficiency is needed. This also includes management of the underlying systemic disease, ocular adnexal pathology and dry eye. Conjunctival limbal autografts from the healthy contralateral eye are performed for unilateral cases. In bilateral cases, tissue may be harvested from a cadaver or a living related donor; prolonged immunosuppression is needed to avoid allograft rejection in such cases. This review describes the surgical techniques, postoperative treatment regimes (including immunosuppression for allografts), the complications and their management. The short and long-term outcomes of the various modalities reported in the literature are also described.

Gao, S., M. McGarry, et al. (2003). "Effect of cell confluence on production of cloned mice using an inbred embryonic stem cell line." *Biol Reprod* **68**(2): 595-603.

Mice have been successfully cloned from both somatic cells and hybrid embryonic stem (ES) cells. Heterozygosity of the donor ES cell genome has been suggested as a crucial factor for long-term survival of cloned mice. In the present study, an inbred ES cell line, HM-1 (129/Ola), and a well-tested ES cell line, R1 (129/Sv x 129/Sv-CP), were used as donor cells to evaluate the developmental potential of nuclear transfer embryos. We found that ES cell confluence dramatically affects the developmental potential of reconstructed embryos. With the ES cell line HM-1 and 80-90% confluence, 49% of reconstructed embryos developed to the morula/blastocyst stage, 9% of these embryos developed to live pups when transferred to the surrogate mothers, and 5 of 18 live pups survived to adulthood. By contrast, at 60-70% confluence, only 22% of embryos developed to the morula/blastocyst stage, and after transfer, only a single fetus reached term. Consistent with previous reports, the nuclei of R1 ES cells were also shown to direct development to term, but no live pups were derived from cells at later passages (>20). Our results show that the developmental potential of reconstructed embryos is determined by both cell confluence and cell passage. These results also demonstrate that the inbred ES cell line, HM-1, can be used to produce viable cloned mice, although less efficiently than most heterozygous ES cell lines.

Gasparrini, B., S. Gao, et al. (2003). "Cloned mice derived from embryonic stem cell karyoplasts and activated cytoplasts prepared by induced enucleation." *Biol Reprod* **68**(4): 1259-66.

Our objective was to induce enucleation (IE) of activated mouse oocytes to yield cytoplasts capable of supporting development following nuclear transfer. Fluorescence microscopy for microtubules, microfilaments, and DNA was used to evaluate meiotic resumption after ethanol activation and the effect of subsequent transient treatments with 0.4 micro g/ml of demecolcine. Using oocytes from B6D2F1 (C57BL/6 x DBA/2) donors, the success of IE of chromatin into polar bodies (PBs) was dependent on the duration of demecolcine treatment and the time that such treatment was initiated after activation. Similarly, variations in demecolcine treatment altered the proportions of oocytes exhibiting a reversible compartmentalization of chromatin into PBs. Treatment for 15 min begun immediately after activation yielded an optimized IE rate of 21% (n = 80) when oocytes were evaluated after overnight recovery in culture. With this protocol, 30-50% of oocytes were routinely scored as compartmentalized when assessed 90 min postactivation. No oocytes could be scored as such following overnight recovery, with 66% of treated oocytes cleaving to the 2-cell stage (n = 80). Activated cytoplasts were prepared by mechanical removal of PBs from oocytes whose chromatin had undergone IE or compartmentalization. These cytoplasts were compared with mechanically enucleated, metaphase (M) II cytoplasts whose activation was delayed in nuclear transfer experiments using HM-1 embryonic stem cells. Using oocytes from either B6D2F1 or B6CBAF1 (C57BL/6 x CBA) donors, the in vitro development of cloned embryos using activated cytoplasts was consistently inferior to that observed using MII cytoplasts. Live offspring were derived from both oocyte strains using the latter, whereas a single living mouse was cloned from activated B6CBAF1 cytoplasts.

Good, R. A., B. Y. Wang, et al. (2002). "Mixed bone marrow or mixed stem cell transplantation for prevention or treatment of lupus-like diseases in mice." *Exp Nephrol* **10**(5-6): 408-20.

Scientific analyses fortified by interpretations of immunodeficiency diseases as 'experiments of nature' have revealed the specific immune systems to be comprised of T cells subserving cell-mediated immunities plus B cells and plasma cells which produce and secrete antibodies. These two separate cellular systems regularly interact with each other to produce a coordinated defense which permits mammals to live within a sea of microorganisms that threaten the integrity and the survival of individuals. We have shown that bone marrow transplantation (BMT) can be used as a form of cellular engineering to construct or reconstruct the immune systems and cure otherwise fatal severe combined

immunodeficiency. When severe aplastic anemia complicated the first BMT which was performed to cure a fatal severe combined immunodeficiency, a second BMT cured for the first time a complicating severe aplastic anemia. Subsequently, BMT has been used effectively to treat some 75 otherwise fatal diseases such as resistant leukemias, lymphomas, inborn errors of metabolism, and genetic anomalies of the hematopoietic development such as sickle cell anemia, thalassemia, congenital neutropenias, and many other diseases. More recently, we have employed BMT in mice both to cure and cause autoimmunities, and, together, these experiments showed that autoimmunities actually reside in the hematopoietic stem cells. We have also found that mixed BMT or mixed hematopoietic stem cell transplantation (HSCT) can be used to prevent and cure the most complex autoimmunities such as those occurring in BXSB mice and in (NZW x BXSB)F1 W/BF1 mice. Untreated, the former develop fulminating lethal glomerulonephritis plus numerous humoral autoimmunities. Mice of the (W/B)F1 strain develop autoimmune thrombocytopenic purpura, coronary vascular disease with myocardial infarction, glomerulonephritis, and numerous autoantibodies. All of these abnormalities are prevented or cured by mixed syngeneic (autoimmune) plus allogeneic (normal healthy) BMT or mixed peripheral blood HSCT. Thus, the most complex autoimmune diseases can be prevented or cured in experimental animals by mixed syngeneic plus allogeneic BMT or HSCT which produce stable mixed chimerism as a form of cellular engineering.

Goossens, E., M. De Rycke, et al. (2009). "DNA methylation patterns of spermatozoa and two generations of offspring obtained after murine spermatogonial stem cell transplantation." Hum Reprod **24**(9): 2255-63.

BACKGROUND: Apart from its use in research, spermatogonial stem cell transplantation (SSCT) may have important clinical applications. This controlled study aimed at evaluating the safety of SSCT by analyzing the DNA methylation pattern of Igf2, Peg1 and alpha-Actin both in spermatozoa and live born offspring obtained after SSCT in mice. **METHODS:** Testicular cell suspensions were transplanted to the testes of genetically sterile WW recipients. Transplanted males were mated with fertile females and their first and second generation offspring were examined and compared with controls with respect to weight, length and DNA methylation patterns. Sodium-bisulfite treated genomic DNA extracted from post-transplantation spermatozoa, liver, kidney and placenta of first and second generation offspring was PCR-amplified to obtain Igf2, Peg1 and

alpha-Actin gene fragments. Pyrosequencing was used to individually quantify the resulting artificial C/T sequence variation at CpG sites. **RESULTS:** First and second generation offspring developed normally with their length and weight not being different from controls. Also the DNA methylation patterns of Igf2, Peg1 and alpha-Actin were not different among controls and first and second generation offspring after SSCT. **CONCLUSIONS:** SSCT between syngenic individuals was not associated with changes in fetal development nor with differences in the DNA methylation patterns of Igf2, Peg1 and alpha-Actin in spermatozoa or other tissues from two subsequent generations of offspring obtained after SSCT.

Goossens, E., V. Frederickx, et al. (2006). "Evaluation of in vivo conception after testicular stem cell transplantation in a mouse model shows altered post-implantation development." Hum Reprod **21**(8): 2057-60.

BACKGROUND: Apart from research applications, testicular stem cell transplantation (TSCT) may one day also have valuable clinical applications. Therefore, it is important to investigate whether this technique is a safe method to have progeny. This controlled study aims at evaluating the fetuses and the live born offspring obtained after TSCT in male mice. **METHODS:** Male mice were mated with wild-type (WT) females after TSCT to produce offspring. First, fetuses were evaluated on the 17th gestational day. The length, weight and morphological age were compared to those of control mouse fetuses. The live born offspring were then investigated for their reproductive potential over three generations. **RESULTS:** The litter sizes after TSCT were decreased compared to controls. Fetuses showed developmental retardation of a quarter of a day, but no major external abnormalities were observed. The live born pups were able to produce normal litter sizes, at least until the third generation. **CONCLUSIONS:** Transplanted animals are able to reproduce naturally. Although litter sizes are lower and development is retarded, no major morphological or procreative abnormalities were observed.

Haimi, S., G. Gorianc, et al. (2009). "Characterization of zinc-releasing three-dimensional bioactive glass scaffolds and their effect on human adipose stem cell proliferation and osteogenic differentiation." Acta Biomater **5**(8): 3122-31.

While the addition of zinc ions to bioactive ceramics has been shown to enhance the proliferation and osteogenic differentiation of osteoblast-like cells, contradictory results have been found. Therefore, the effect of zinc-releasing ceramics on cell proliferation and differentiation into osteogenic lineages requires

further clarification. The aim of this study was to evaluate the effects of zinc addition on the degradation profile of three-dimensional bioactive glass scaffold, and on the proliferation and osteogenesis of human adipose stem cells (hASCs) in these scaffolds. Bioactive glass scaffolds containing Na₂O, K₂O, MgO, CaO, B₂O₃, TiO₂, P₂O₅ and SiO₂ were prepared. The degradation was evaluated by weight loss measurement, scanning electron microscopy and elemental analysis. The degradation profile of bioactive glass was shown to slow down with the addition of zinc. Qualitative live/dead staining showed that zinc addition to bioactive glass inhibits cell spreading and proliferation of hASCs. However, zinc addition had no significant effect on DNA content, alkaline phosphatase activity and osteopontin concentration of hASCs when measured quantitatively. Our results suggest that the possible stimulatory effect of addition of zinc on hASC proliferation and osteogenesis was not detected because addition of zinc slowed down the degradation rate of the studied bioactive glass scaffolds.

Hanson, A. D., M. E. Wall, et al. (2007). "Effects of oxygen plasma treatment on adipose-derived human mesenchymal stem cell adherence to poly(L-lactic acid) scaffolds." *J Biomater Sci Polym Ed* **18**(11): 1387-400.

Plasma treatment of substrate surfaces can be utilized to improve adhesion of cells to tissue-engineered scaffolds. The purpose of this study was to enhance cell adhesion to non-woven poly(L-lactic acid) (PLLA) scaffolds using oxygen plasma treatment to increase surface hydroxyl groups and thereby enhance substrate hydrophilicity. It was hypothesized that oxygen plasma treatment would increase the number of adipose-derived human mesenchymal stem cells (hMSCs) that adhered to melt-blown, non-woven PLLA scaffolds without affecting cell viability. The number of cells that adhered to the oxygen plasma-treated (10 min at 100 W) or untreated PLLA scaffolds was assessed at 2, 4, 8, 12, 24 and 48 h post-seeding via DNA analysis. Cell viability and morphology were also assessed at 2, 4, 8, 12 and 24 h post-seeding via a live/dead assay and hematoxylin staining, respectively. Oxygen plasma treatment decreased the contact angle of water from 75.6 degrees to 58.2 degrees, indicating an increase in the surface hydrophilicity of PLLA. The results of the DNA analysis indicated that there was an increased number of hMSCs on oxygen plasma treated scaffolds for two of the three donors. In addition, oxygen plasma treatment promoted a more even distribution of hMSCs throughout the scaffold and enhanced cell spreading at earlier time points without altering cell viability. This early induction of

cell spreading and the uniform distribution of cells, in turn, may increase future proliferation and differentiation of hMSCs under conditions that simulate the microenvironment in vivo.

Hedlund, E., J. Pruszek, et al. (2008). "Embryonic stem cell-derived Pitx3-enhanced green fluorescent protein midbrain dopamine neurons survive enrichment by fluorescence-activated cell sorting and function in an animal model of Parkinson's disease." *Stem Cells* **26**(6): 1526-36.

Both fetal ventral mesencephalic (VM) and embryonic stem (ES) cell-derived dopamine neurons have been used successfully to correct behavioral responses in animal models of Parkinson's disease. However, grafts derived from fetal VM cells or from ES cells contain multiple cell types, and the majority of these cells are not dopamine neurons. Isolation of ES cell-derived dopamine neurons and subsequent transplantation would both elucidate the capacity of these neurons to provide functional input and also further explore an efficient and safer use of ES cells for the treatment of Parkinson's disease. Toward this goal, we used a Pitx3-enhanced green fluorescent protein (Pitx3-eGFP) knock-in mouse blastocyst-derived embryonic stem (mES) cell line and fluorescence-activated cell sorting (FACS) to select and purify midbrain dopamine neurons. Initially, the dopaminergic marker profile of intact Pitx3-eGFP mES cultures was evaluated after differentiation in vitro. eGFP expression overlapped closely with that of Pitx3, Nurr1, Engrailed-1, Lmx1a, tyrosine hydroxylase (TH), l-aromatic amino acid decarboxylase (AADC), and vesicular monoamine transporter 2 (VMAT2), demonstrating that these cells were of a midbrain dopamine neuron character. Furthermore, postmitotic Pitx3-eGFP(+) dopamine neurons, which constituted 2%-5% of all live cells in the culture after dissociation, could be highly enriched to >90% purity by FACS, and these isolated neurons were viable, extended neurites, and maintained a dopaminergic profile in vitro. Transplantation to 6-hydroxydopamine-lesioned rats showed that an enriched dopaminergic population could survive and restore both amphetamine- and apomorphine-induced functions, and the grafts contained large numbers of midbrain dopamine neurons, which innervated the host striatum. Disclosure of potential conflicts of interest is found at the end of this article.

Heng, B. C., T. Cao, et al. (2004). "An overview and synopsis of techniques for directing stem cell differentiation in vitro." *Cell Tissue Res* **315**(3): 291-303.

The majority of studies on stem cell differentiation have so far been based in vivo, on live

animal models. The usefulness of such models is limited, since it is much more technically challenging to conduct molecular studies and genetic manipulation on live animal models compared to in vitro cell culture. Hence, it is imperative that efficient protocols for directing stem cell differentiation into well-defined lineages in vitro are developed. The development of such protocols would also be useful for clinical therapy, since it is likely that the transplantation of differentiated stem cells would result in higher engraftment efficiency and enhanced clinical efficacy, compared to the transplantation of undifferentiated stem cells. The in vitro differentiation of stem cells, prior to transplantation in vivo, would also avoid spontaneous differentiation into undesired lineages at the transplantation site, as well as reduce the risk of teratoma formation, in the case of embryonic stem cells. Hence, this review critically examines the various strategies that could be employed to direct and control stem cell differentiation in vitro.

Hernandez, D., P. J. Mee, et al. (1999). "Transchromosomal mouse embryonic stem cell lines and chimeric mice that contain freely segregating segments of human chromosome 21." *Hum Mol Genet* **8**(5): 923-33.

At least 8% of all human conceptions have major chromosome abnormalities and the frequency of chromosomal syndromes in newborns is >0.5%. Despite these disorders making a large contribution to human morbidity and mortality, we have little understanding of their aetiology and little molecular data on the importance of gene dosage to mammalian cells. Trisomy 21, which results in Down syndrome (DS), is the most frequent aneuploidy in humans (1 in 600 live births, up to 1 in 150 pregnancies worldwide) and is the most common known genetic cause of mental retardation. To investigate the molecular genetics of DS, we report here the creation of mice that carry different human chromosome 21 (Hsa21) fragments as a freely segregating extra chromosome. To produce these 'transchromosomal' animals, we placed a selectable marker into Hsa21 and transferred the chromosome from a human somatic cell line into mouse embryonic stem (ES) cells using irradiation microcell-mediated chromosome transfer (XMMCT). 'Transchromosomal' ES cells containing different Hsa21 regions ranging in size from approximately 50 to approximately 0.2 Mb have been used to create chimeric mice. These mice maintain Hsa21 sequences and express Hsa21 genes in multiple tissues. This novel use of the XMMCT protocol is applicable to investigations requiring the transfer of large chromosomal regions into ES or other cells and, in particular, the modelling of DS and other human aneuploidy syndromes.

Hogan, B. L. (2008). "Summary: present and future challenges for stem cell research." *Cold Spring Harb Symp Quant Biol* **73**: 593-600.

Stem cell research is being driven forward at an intense pace by creative interactions among scientists working in different fields. These include developmental and reproductive biology, regeneration, genomics, live cell imaging, RNA biology, and cancer biology, to name a few. Numerous model systems and techniques are being exploited, and lab scientists are teaming up with bioengineers and clinicians. The ferment of ideas that makes the field so exciting was in full evidence throughout the Symposium. However, many challenges still need to be overcome to translate basic discoveries into therapeutic outcomes that will save lives and fulfill the promises that have been made. This chapter summarizes some of the highlights of the Symposium and indicates future directions that are being taken by leaders in the field.

Huang, J., K. Deng, et al. (2008). "Efficient production of mice from embryonic stem cells injected into four- or eight-cell embryos by piezo micromanipulation." *Stem Cells* **26**(7): 1883-90.

The conventional method for producing embryonic stem (ES) cell-derived knockout or transgenic mice involves injection of ES cells into normal, diploid blastocysts followed by several rounds of breeding of resultant chimeras and thus is a time-consuming and inefficient procedure. F0 ES cell pups can also be derived directly from tetraploid embryo complementation, which requires fusion of two-cell embryos. Recently, F0 ES cell pups have been produced by injection of ES cells into eight-cell embryos using a laser-assisted micromanipulation system. We report a simple method for producing F0 ES cell germline-competent mice by piezo injection of ES cells into four- or eight-cell embryos. The efficiency of producing live, transgenic mice by this method is higher than that with the tetraploid blastocyst complementation method. This efficient and economical technique for directly producing F0 ES cell offspring can be applicable in many laboratories for creating genetically manipulated mice using ES cell technology and also for stringent testing of the developmental potency of new ES cell or other types of pluripotent stem cell lines.

Issaragrisil, S. (2002). "Stem cell transplantation for thalassemia." *Int J Hematol* **76 Suppl 1**: 307-9.

Thalassemia, one of the most common genetic disorders, is considered to be a global problem. Several millions of the patients suffer from severe thalassaemic diseases. Stem cell transplantation is currently the only curative therapy. Bone marrow

transplantation offers a high probability of cure when performed in young children. There is a higher risk as the patient becomes older, especially the high incidence of graft rejection. Modified conditioning regimens have been developed to overcome graft rejection in patients with class III or full blown manifestations. The alternative use of stem cell from cord blood makes possible earlier transplant with better chance of cure, although the engraftment is slower compared to bone marrow transplantation. More experiences with regard to stem cell transplantation in adult patients, the use of stem cell transplantation from related donors as well as matched unrelated donors are necessary.

Iwasaki, S. and H. Kimura (2003). "Further studies on thermal treatment of two-cell stage embryos to produce complete embryonic stem-cell-derived mice by cell-aggregation methods." *Dev Growth Differ* **45**(5-6): 427-34.

Employing aggregation techniques with two embryonic sources, one from two-cell stage embryos treated by thermal stimulation and the other from mouse embryonic stem (ES) cells that had been obtained from a feeder layer, simple and most effective methods of producing a complete generation of mice from ES cells were explored. Although thermal treatment affected embryos at various developmental stages, the embryos at the two-cell stage of development were selected because of the remarkably reduced number of cells present in the inner cell mass (ICM) at blastocyst stage after thermal conditioning. Under these conditions, a combination of thermally treated host embryos and an aggregated ES cell-clump was found to produce a high rate of live newborns by natural delivery. That the newborns were completely derived from ES cells was checked by two criteria: microsatellite analysis and coat color analysis. Importantly, all of these mice were healthy and fertile. The aggregation techniques reported here might well be applied to other animal species whose ES cells form stable colonies on a feeder layer.

Kim, K. M. and D. Shibata (2002). "Methylation reveals a niche: stem cell succession in human colon crypts." *Oncogene* **21**(35): 5441-9.

Little is known about human stem cells although they are likely to be the earliest progenitors of carcinomas. Just as methylation can substitute for mutations to inactivate tumor suppressor genes, methylation can also substitute for mutations in a phylogenetic analysis. This review explains why stem cell dynamics may be important to tumor progression and how methylation patterns found in a normal human colon can be used to reconstruct the behavior of crypt stem cells. Histories are recorded in

sequences and strategies used to reconstruct phylogenies from sequences likely apply to methylation patterns because both exhibit somatic inheritance. Such a quantitative analysis of colon methylation patterns infers stem cells live in niches containing multiple 'stem' cells. Although niche stem cell numbers remain constant, clonal succession is inherent to niches because periodically progeny from a single stem cell become dominant. These niche succession cycles may potentially accumulate multiple alterations because they resemble superficially the clonal succession of tumor progression except that they occur invisibly in the absence of selection or phenotypic change. Alterations without immediate selective value may hitchhike passively in the stem cells that become dominant during niche succession cycles. The inherent ability of a niche to fix alterations (Muller's ratchet) is another potential mechanism besides instability and selection to sequentially accumulate multiple alterations. Many alterations found in colorectal tumors may reflect such occult clonal progression in normal colon.

Kitaori, T., H. Ito, et al. (2009). "Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model." *Arthritis Rheum* **60**(3): 813-23.

OBJECTIVE: Stromal cell-derived factor 1 (SDF-1; CXCL12/pre-B cell growth-stimulating factor) is a dominant chemokine in bone marrow and is known to be involved in inflammatory diseases, including rheumatoid arthritis. However, its role in bone repair remains unknown. The purpose of this study was to investigate the role of SDF-1 and its receptor, CXCR4, in bone healing. **METHODS:** The expression of SDF-1 during the repair of a murine structural femoral bone graft was examined by real-time polymerase chain reaction and immunohistochemical analysis. The bone graft model was treated with anti-SDF-1 neutralizing antibody or TF14016, an antagonist for CXCR4, and evaluated by histomorphometry. The functional effect of SDF-1 on primary mesenchymal stem cells was determined by *in vitro* and *in vivo* migration assays. New bone formation in an exchanging-graft model was compared with that in the autograft models, using mice partially lacking SDF-1 (SDF-1(+/-)) or CXCR4 (CXCR4(+/-)). **RESULTS:** The expression of SDF1 messenger RNA was increased during the healing of live bone grafts but was not increased in dead grafts. High expression of SDF-1 protein was observed in the periosteum of the live graft. New bone formation was inhibited by the administration of anti-SDF-1 antibody or TF14016. SDF-1 increased mesenchymal stem cell chemotaxis *in vitro* in a dose-dependent manner. The

in vivo migration study demonstrated that mesenchymal stem cells recruited by SDF-1 participate in endochondral bone repair. Bone formation was decreased in SDF-1(+/-) and CXCR4(+/-) mice and was restored by the graft bones from CXCR4(+/-) mice transplanted into the SDF-1(+/-) femur, but not vice versa. CONCLUSION: SDF-1 is induced in the periosteum of injured bone and promotes endochondral bone repair by recruiting mesenchymal stem cells to the site of injury.

Lachmann, P. J. (2002). "Stem cell therapy: medical advance or moral challenge?" C R Biol **325**(10): 1049-51.

My professional lifetime has seen progress in the biomedical sciences that beggars belief. This has led to astonishing advances in the ability to prevent and treat disease and, in the developed world at least, people live longer and healthier lives than ever before. Paradoxically, this has gone hand in hand with the growth of a vocal and influential anti-science lobby that not only rejects much modern science but is also deeply suspicious of new medical interventions. The prospect of cell therapy in the near or middle future is their current target especially where the use of embryonic stem cells or of cell nuclear transfer techniques is concerned. The prospect of cell therapy is welcomed with enthusiasm by patients with genetic and degenerative diseases who hope to benefit from them. On the other hand the whole idea is regarded as repugnant by the anti-science lobby. While some of this opposition is essentially luddite in nature, there are some more persuasive arguments raised particularly to any research than uses embryonic or foetal materials. These arguments will be examined critically. The moral problems of denying the sick the hope of effective treatments have to be weighed against those seen in the development of such treatments. (This article is closely based on an already published paper. P. Lachmann, Stem cell research: why is it regarded as a threat? An investigation of the economic and ethical arguments made against research with human embryonic stem cells.

Leung, A. Y., H. C. Chow, et al. (2007). "Safety of vaccinating sibling donors with live-attenuated varicella zoster vaccine before hematopoietic stem cell transplantation." Bone Marrow Transplant **39**(11): 661-5.

Reactivation of varicella zoster virus (VZV), clinically manifested as herpes zoster (HZ) is a common complication after hematopoietic stem cell transplantation (HSCT). The optimum prophylaxis for this disease has not been defined. In this study, we examined the effects of vaccinating donors with a live-attenuated vaccine with particular reference to

their immune responses and the outcome of HSCT patients. Forty prospective HLA-matched sibling donors were vaccinated before HSCT. There were humoral immune responses in both sero-positive ($P < 0.01$) and sero-negative ($P = 0.058$) donors. Cellular immune response was assayed in 26 donors. Significant correlation was observed between cellular immune responses as enumerated by thymidine incorporation and interferon gamma secretion ($P < 0.001$) and the latter was used in subsequent analyses. Significant response was observed in sero-negative (6/26) and a group of sero-positive (13/26) donors while 7/26 sero-positive donors showed no response. Thirty-four HSCT were performed. These patients have a lower, albeit insignificant, risk of HZ compared with historical controls and only 3/34 patients developed single dermatomal HZ at 6, 9 and 28 months after HSCT. No patients developed VZV-related mortality. Vaccinating donors with live-attenuated VZV vaccine was safe, but whether it confers a significant protection to the patients would require further study.

Ljungman, P., F. Z. Wang, et al. (2003). "Vaccination of autologous stem cell transplant recipients with live varicella vaccine: a pilot study." Support Care Cancer **11**(11): 739-41.

A pilot study of vaccination with live, attenuated varicella vaccine for prevention of zoster was performed in autologous stem cell transplant (SCT) recipients. Nine patients were vaccinated between 3-4 months after transplantation. The antigen-specific immune response was studied by lymphocyte proliferation. No systemic side effects were seen. One of nine patients developed herpes zoster HZ during follow-up. There was a tendency for a strengthened specific immune response after SCT ($p = 0.12$). This pilot study shows that vaccination with live, attenuated varicella vaccine can be performed safely 3-4 months after autologous stem cell transplantation. Additional studies are needed to assess efficacy of this approach in the prevention of HZ.

Machado, C. M. (2005). "Reimmunization after hematopoietic stem cell transplantation." Expert Rev Vaccines **4**(2): 219-28.

Hematopoietic stem cell transplant recipients lose immune memory of exposure to infectious agents and vaccines accumulated through a lifetime, and therefore need to be revaccinated. Reimmunization protocols vary greatly among hematopoietic stem cell transplant centers. Diphtheria and tetanus toxoids, pertussis vaccine, Haemophilus influenza type B conjugate, 23-valent pneumococcal polysaccharide, inactivated influenza and polio vaccine and live

attenuated measles-mumps-rubella vaccine are the currently recommended vaccines to be included in a vaccination program after hematopoietic stem cell transplant. Other variables, such as stem cell source, new adjuvants, T-cell depleted transplants, nonmyeloablative conditioning and donor immunization have recently been introduced and a constant update of current recommendations are needed. Studies recently published, the use of other vaccines and the perspectives for different vaccination protocols are discussed in this review.

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

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

Melville, A. J., J. Harrison, et al. (2006). "Mouse embryonic stem cell colonisation of carbonated apatite surfaces." *Biomaterials* **27**(4): 615-22.

Apatites play a crucial role in the body and have been used extensively in biomedical implants. The influence on stem cell behaviour is not known and so this study will explore whether sintered carbonated apatites are favourable for propagation of stem cells. Different weight substitutions of carbonated apatite, specifically 2.5 wt% (2.5 wt%CAP) and 5 wt% (5 wt%CAP), were sintered and characterised prior to the investigation of their potential as a matrix for the support of mouse embryonic stem (ES) cells. Characterisation of the apatites included elemental analysis, X-ray diffraction, surface roughness, specific surface area, density, and solubility. The ability of carbonated apatite to support mouse ES cell colonisation and maintenance in the presence of leukaemia inhibitory factor was determined by an enumeration of live versus dead cells within a population, and immunoreactivity to Oct4, a transcription factor and stem cell marker, following growth on each matrix. It was found that while both compositions allowed for the colonisation of mouse ES cells, the cells were not maintained in an undifferentiated state, as evidenced by a reduction in the number of cells staining positive for Oct4 expression. This study shows that an increase in carbonate content within sintered apatites leads to a higher cell number, a desired aspect for stem cells to populate scaffolds intended for tissue engineering. This study presents carbonated apatites as a suitable matrix for the initial colonisation and differentiation of ES cells for tissue engineering applications.

Miki, T., T. Lehmann, et al. (2005). "Stem cell characteristics of amniotic epithelial cells." *Stem Cells* **23**(10): 1549-59.

Amniotic epithelial cells develop from the epiblast by 8 days after fertilization and before gastrulation, opening the possibility that they might maintain the plasticity of pregastrulation embryo cells. Here we show that amniotic epithelial cells isolated from human term placenta express surface markers normally present on embryonic stem and germ cells. In addition, amniotic epithelial cells express the pluripotent stem cell-specific transcription factors octamer-binding protein 4 (Oct-4) and nanog. Under certain culture conditions, amniotic epithelial cells form spheroid structures that retain stem cell characteristics. Amniotic epithelial cells do not require other cell-derived feeder layers to maintain Oct-4 expression, do not express telomerase, and are nontumorigenic upon transplantation. Based on immunohistochemical and genetic analysis, amniotic epithelial cells have the potential to differentiate to all three germ layers--endoderm (liver, pancreas), mesoderm (cardiomyocyte), and ectoderm (neural cells) in vitro. Amnion derived from term placenta

after live birth may be a useful and noncontroversial source of stem cells for cell transplantation and regenerative medicine.

Mizrak, S. C., B. M. Gadella, et al. (2008). "Spermatogonial stem cell sensitivity to capsaicin: an in vitro study." *Reprod Biol Endocrinol* **6**: 52.

BACKGROUND: Conflicting reports have been published on the sensitivity of spermatogenesis to capsaicin (CAP), the pungent ingredient of hot chili peppers. Here, the effect of CAP on germ cell survival was investigated by using two testis germ cell lines as a model. As CAP is a potent agonist of the transient receptor potential vanilloid receptor 1 (TRPV1) and no information was available of its expression in germ cells, we also studied the presence of TRPV1 in the cultured cells and in germ cells in situ. **METHODS:** The rat spermatogonial stem cell lines Gc-5spg and Gc-6spg were used to study the effects of different concentrations of CAP during 24 and 48 h. The response to CAP was first monitored by phase-contrast microscopy. As germ cells appear to undergo apoptosis in the presence of CAP, the activation of caspase 3 was studied using an anti activated caspase 3 antibody or by quantifying the amount of cells with DNA fragmentation using flow cytometry. Immunolocalization was done with an anti-TRPV1 antibody either with the use of confocal microscopy to follow live cell labeling (germ cells) or on Bouin fixed paraffin embedded testicular tissues. The expression of TRPV1 by the cell lines and germ cells was confirmed by Western blots. **RESULTS:** Initial morphological observations indicated that CAP at concentrations ranging from 150 μ M to 250 μ M and after 24 and 48 h of exposure, had deleterious apoptotic-like effects on both cell lines: A large population of the CAP treated cell cultures showed signs of DNA fragmentation and caspase 3 activation. Quantification of the effect demonstrated a significant effect of CAP with doses of 150 μ M in the Gc-5spg cell line and 200 μ M in the Gc-6spg cell line, after 24 h of exposure. The effect was dose and time dependent in both cell lines. TRPV1, the receptor for CAP, was found to be expressed by the spermatogonial stem cells in vitro and also by premeiotic germ cells in situ. **CONCLUSION:** CAP adversely affects spermatogonial survival in vitro by inducing apoptosis to those cells and TRPV-1, a CAP receptor, may be involved in this effect as this receptor is expressed by mitotic germ cells.

Moioli, E. K., P. A. Clark, et al. (2008). "Autologous stem cell regeneration in craniosynostosis." *Bone* **42**(2): 332-40.

Craniosynostosis occurs in one of 2500 live human births and may manifest as craniofacial

disfiguration, seizure, and blindness. Craniotomy is performed to reshape skull bones and resect synostosed cranial sutures. We demonstrate for the first time that autologous mesenchymal stem cells (MSCs) and controlled-released TGFbeta3 reduced surgical trauma to localized osteotomy and minimized osteogenesis in a rat craniosynostosis model. Approximately 0.5 mL tibial marrow content was aspirated to isolate mononucleated and adherent cells that were characterized as MSCs. Upon resecting the synostosed suture, autologous MSCs in collagen carriers with microencapsulated TGFbeta3 (1 ng/mL) generated cranial suture analogs characterized as bone-soft tissue-bone interface by quantitative histomorphometric and microCT analyses. Thus, surgical trauma in craniosynostosis can be minimized by a biologically viable implant. We speculate that proportionally larger amounts of human marrow aspirates participate in the healing of craniosynostosis defects in patients. The engineered soft tissue-bone interface may have implications in the repair of tendons, ligaments, periosteum and periodontal ligament.

Moore, J., I. Nivison-Smith, et al. (2007). "Equivalent survival for sibling and unrelated donor allogeneic stem cell transplantation for acute myelogenous leukemia." *Biol Blood Marrow Transplant* **13**(5): 601-7.

Recent studies have shown comparable survival outcomes for unrelated donor (URD) stem cell transplantation in chronic myelogenous leukemia compared to sibling donors. We compared outcomes for 105 patients aged 16 to 59 years undergoing URD transplants for acute myelogenous leukemia (AML) who were reported to the Australasian Bone Marrow Transplant Recipient Registry between 1992 and 2002, and a strictly selected matching set of 105 HLA-matched sibling donor (MSD) transplants. There was no significant difference between URD and MSD controls in the distributions of time from diagnosis to transplant, donor-recipient sex match, prior therapies, donor age, or performance status. The median follow-up of live URD patients was 4.4 years and for live MSD controls was 6.3 years. There were 18 good risk (complete remission [CR]1) and 87 poor risk (>CR1) recipients in both URD and sibling groups. Five-year disease-free survival (DFS) was not significantly different for good-risk URD and sibling donor recipients (62% versus 40%, $P = .2$), or poor-risk URD and sibling recipients (21% versus 25%, $P = .2$). In a stratified multivariate Cox regression model, the independent adverse risk factors for DFS were recipient cytomegalovirus positivity ($P = .01$) and the interaction of URD and earlier year of transplant ($P = .006$). Both neutrophil and platelet engraftment were

significantly more rapid in the sibling group, but transplant-related mortality at 100 days was not significantly different. There was no difference in the cumulative incidence of acute graft-versus-host disease grade II or above at 100 days. Relapse occurred in 28% of good risk URD subjects and 16% of siblings ($P = .3$), and in poor risk subjects 39% and 29%, respectively ($P = .2$). Based on this data, URD allografts should be considered in AML patients without a matched sibling donor. This study provides a rationale for a larger prospective study of risk factors in allogeneic transplantation for AML and a guide on the subset of patients who may most benefit from an unrelated donor allograft in AML.

Moreau, J. L. and H. H. Xu (2009). "Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate-chitosan composite scaffold." *Biomaterials* **30**(14): 2675-82.

Calcium phosphate cement (CPC) can be molded or injected to form a scaffold in situ, has excellent osteoconductivity, and can be resorbed and replaced by new bone. However, its low strength limits CPC to non-stress-bearing repairs. Chitosan could be used to reinforce CPC, but mesenchymal stem cell (MSC) interactions with CPC-chitosan scaffold have not been examined. The objective of this study was to investigate MSC proliferation and osteogenic differentiation on high-strength CPC-chitosan scaffold. MSCs were harvested from rat bone marrow. At CPC powder/liquid (P/L) mass ratio of 2, flexural strength (mean \pm sd; n=5) was (10.0 \pm 1.1) MPa for CPC-chitosan, higher than (3.7 \pm 0.6) MPa for CPC ($p < 0.05$). At P/L of 3, strength was (15.7 \pm 1.7) MPa for CPC-chitosan, higher than (10.2 \pm 1.8) MPa for CPC ($p < 0.05$). Percentage of live MSCs attaching to scaffolds increased from 85% at 1 day to 99% at 14 days. There were (180 \pm 37) cells/mm² on scaffold at 1 day; cells proliferated to (1808 \pm 317) cells/mm² at 14 days. SEM showed MSCs with healthy spreading and anchored on nano-apatite crystals via cytoplasmic processes. Alkaline phosphatase activity (ALP) was (557 \pm 171) (pNPP mM/min)/(microg DNA) for MSCs on CPC-chitosan, higher than (159 \pm 47) on CPC ($p < 0.05$). Both were higher than (35 \pm 32) of baseline ALP for undifferentiated MSCs on tissue-culture plastic ($p < 0.05$). In summary, CPC-chitosan scaffold had higher strength than CPC. MSC proliferation on CPC-chitosan matched that of the FDA-approved CPC control. MSCs on the scaffolds differentiated down the osteogenic lineage and expressed high levels of bone marker ALP. Hence, the stronger CPC-chitosan scaffold may be useful for stem cell-based bone regeneration in moderate load-bearing maxillofacial and orthopedic applications.

Muotri, A. R., K. Nakashima, et al. (2005). "Development of functional human embryonic stem cell-derived neurons in mouse brain." *Proc Natl Acad Sci U S A* **102**(51): 18644-8.

Human embryonic stem cells are pluripotent entities, theoretically capable of generating a whole-body spectrum of distinct cell types. However, differentiation of these cells has been observed only in culture or during teratoma formation. Our results show that human embryonic stem cells implanted in the brain ventricles of embryonic mice can differentiate into functional neural lineages and generate mature, active human neurons that successfully integrate into the adult mouse forebrain. Moreover, this study reveals the conservation and recognition of common signals for neural differentiation throughout mammalian evolution. The chimeric model will permit the study of human neural development in a live environment, paving the way for the generation of new models of human neurodegenerative and psychiatric diseases. The model also has the potential to speed up the screening process for therapeutic drugs.

Nagy, A., J. Rossant, et al. (1993). "Derivation of completely cell culture-derived mice from early-passage embryonic stem cells." *Proc Natl Acad Sci U S A* **90**(18): 8424-8.

Several newly generated mouse embryonic stem (ES) cell lines were tested for their ability to produce completely ES cell-derived mice at early passage numbers by ES cell \leftrightarrow tetraploid embryo aggregation. One line, designated R1, produced live offspring which were completely ES cell-derived as judged by isoenzyme analysis and coat color. These cell culture-derived animals were normal, viable, and fertile. However, prolonged in vitro culture negatively affected this initial totipotency of R1, and after passage 14, ES cell-derived newborns died at birth. However, one of the five subclones (R1-S3) derived from single cells at passage 12 retained the original totipotency and gave rise to viable, completely ES cell-derived animals. The total in vitro culture time of the sublines at the time of testing was equivalent to passage 24 of the original line. Fully potent early passage R1 cells and the R1-S3 subclone should be very useful not only for ES cell-based genetic manipulations but also in defining optimal in vitro culture conditions for retaining the initial totipotency of ES cells.

Nelson, T. J., A. Martinez-Fernandez, et al. (2009). "KCNJ11 knockout morula re-engineered by stem cell diploid aggregation." *Philos Trans R Soc Lond B Biol Sci* **364**(1514): 269-76.

KCNJ11-encoded Kir6.2 assembles with ATP-binding cassette sulphonylurea receptors to generate ATP-sensitive K⁺ (KATP) channel complexes. Expressed in tissues with dynamic metabolic flux, these evolutionarily conserved yet structurally and functionally unique heteromultimers serve as high-fidelity rheostats that adjust membrane potential-dependent cell functions to match energetic demand. Genetic defects in channel subunits disrupt the cellular homeostatic response to environmental stress, compromising organ tolerance in the adult. As maladaptation characterizes malignant KATP channelopathies, establishment of platforms to examine progression of KATP channel-dependent adaptive behaviour is warranted. Chimeras provide a powerful tool to assay the contribution of genetic variance to stress intolerance during prenatal or post-natal development. Here, KCNJ11 KATP channel gene knockout<-->wild-type chimeras were engineered through diploid aggregation. Integration of wild-type embryonic stem cells into zona pellucida-denuded morula derived from knockout embryos achieved varying degrees of incorporation of stress-tolerant tissue within the KATP channel-deficient background. Despite the stress-vulnerable phenotype of the knockout, ex vivo derived mosaic blastocysts tolerated intrauterine transfer and implantation, followed by full-term embryonic development in pseudopregnant surrogates to produce live chimeric offspring. The development of adult chimerism from the knockout<-->wild-type mosaic embryo offers thereby a new paradigm to probe the ecogenetic control of the KATP channel-dependent stress response.

Oktay, K. (2006). "Spontaneous conceptions and live birth after heterotopic ovarian transplantation: is there a germline stem cell connection?" *Hum Reprod* **21**(6): 1345-8.

Ovarian cryopreservation and transplantation is an emerging technology to preserve fertility in women and children undergoing cancer treatment. Recent reports of live births after orthotopic transplantation raised hopes for the future success of this procedure. However, doubts remained whether the reported pregnancies were as a result of resumed function in the remaining ovary. We recently performed an autologous heterotopic ovarian transplantation in a 32-year-old Hodgkin lymphoma survivor who was menopausal for 2.5 years as a result of a preconditioning chemotherapy given before a hematopoietic stem cell transplant. Subsequent to the transplantation, the patient conceived twice within 3 months and delivered a healthy female child at 40 weeks of gestation. The occurrence of spontaneous pregnancies after heterotopic ovarian transplantation

highlights the need for caution when interpreting the source of pregnancies in recipients with intact ovaries. On the other hand, the temporal relationship between the ovarian transplant and the spontaneous resumption of ovarian function and pregnancies in previously menopausal women is intriguing, especially in the light of recent reports of germ cell renewal and migration from the bone marrow to the ovary in rodents.

Piao, C. S., M. E. Gonzalez-Toledo, et al. (2009). "The role of stem cell factor and granulocyte-colony stimulating factor in brain repair during chronic stroke." *J Cereb Blood Flow Metab* **29**(4): 759-70.

Chronic stroke is a highly important but under-investigated scientific problem in neurologic research. We have reported earlier that stem cell factor (SCF) in combination with granulocyte-colony stimulating factor (G-CSF) treatment during chronic stroke improves functional outcomes. Here we have determined the contribution of bone marrow-derived cells in angiogenesis and neurogenesis, which are enhanced by SCF+G-CSF treatment during chronic stroke. Using bone marrow tracking, flow cytometry, 2-photon live brain imaging, and immunohistochemistry, we observed that the levels of circulating bone marrow stem cells (BMSCs) (CD34⁺/c-kit⁺) were significantly increased by SCF+G-CSF treatment. In addition, live brain imaging revealed that numerous bone marrow-derived cells migrate into the brain parenchyma in the treated mice. We also found that bone marrow-derived cells, bone marrow-derived endothelial cells, vascular density, and bone marrow-derived neurons were significantly augmented by SCF+G-CSF. It is interesting that, in addition to the increase in bone marrow-derived endothelial cells, the number of bone marrow-derived pericytes was reduced after SCF+G-CSF treatment during chronic stroke. These data suggest that SCF+G-CSF treatment can enhance repair of brain damage during chronic stroke by mobilizing BMSCs, and promoting the contribution of bone marrow-derived cells to angiogenesis and neurogenesis.

Pollard, S. M., K. Yoshikawa, et al. (2009). "Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens." *Cell Stem Cell* **4**(6): 568-80.

Human brain tumors appear to have a hierarchical cellular organization suggestive of a stem cell foundation. In vitro expansion of the putative cancer stem cells as stable cell lines would provide a powerful model system to study their biology. Here, we demonstrate routine and efficient derivation of adherent cell lines from malignant glioma that display

stem cell properties and initiate high-grade gliomas following xenotransplantation. Significantly, glioma neural stem (GNS) cell lines from different tumors exhibit divergent gene expression signatures and differentiation behavior that correlate with specific neural progenitor subtypes. The diversity of gliomas may, therefore, reflect distinct cancer stem cell phenotypes. The purity and stability of adherent GNS cell lines offer significant advantages compared to "sphere" cultures, enabling refined studies of cancer stem cell behavior. A proof-of-principle live cell imaging-based chemical screen (450 FDA-approved drugs) identifies both differential sensitivities of GNS cells and a common susceptibility to perturbation of serotonin signaling.

Pomper, M. G., H. Hammond, et al. (2009). "Serial imaging of human embryonic stem-cell engraftment and teratoma formation in live mouse models." *Cell Res* **19**(3): 370-9.

Two new types of lentiviral vectors expressing a reporter transgene encoding either firefly luciferase (fLuc) for bioluminescence imaging or the HSV1 thymidine kinase (HSV1-TK) for radiopharmaceutical-based imaging were constructed to monitor human embryonic stem cell (hESC) engraftment and proliferation in live mice after transplantation. The constitutive expression of either transgene did not alter the properties of hESCs in the culture. We next monitored the formation of teratomas in SCID mice to test (1) whether the gene-modified hESCs maintain their developmental pluripotency, and (2) whether sustained reporter gene expression allows noninvasive, whole-body imaging of hESC derivatives in a live mouse model. We observed teratoma formation from both types of gene-modified cells as well as wild-type hESCs 2-4 months after inoculation. Using an optical imaging system, bioluminescence from the fLuc-transduced hESCs was easily detected in mice bearing teratomas long before palpable tumors could be detected. To develop a noninvasive imaging method more readily translatable to the clinic, we also utilized HSV1-TK and its specific substrate, 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-[(125)I]iodouracil [(125)I]FIAU, as a reporter/probe pair. After systemic administration, [(125)I]FIAU is phosphorylated only by the transgene-encoded HSV1-TK enzyme and retained within transduced (and transplanted) cells, allowing sensitive and quantitative imaging by single-photon emission computed tomography. Noninvasive imaging methods such as these may enable us to monitor the presence and distribution of transplanted human stem cells repetitively within live recipients over a long term through the expression of a reporter gene.

Potier, E., E. Ferreira, et al. (2007). "Prolonged hypoxia concomitant with serum deprivation induces massive human mesenchymal stem cell death." *Tissue Eng* **13**(6): 1325-31.

Mesenchymal stem cells (MSCs) have been proposed for the repair of damaged tissue including bone, cartilage, and heart tissue. Upon in vivo transplantation, the MSCs encounter an ischemic microenvironment characterized by reduced oxygen (O₂) tension and nutrient deprivation that may jeopardize viability of the tissue construct. The aim of this study was to assess the effects of serum deprivation and hypoxia on the MSC survival rates in vitro. As expanded MSCs are transferred from plastic to a scaffold in most tissue engineering approaches, possibly inducing loss of survival signals from matrix attachments, the effects of a scaffold shift on the MSC survival rates were also assessed. Human MSCs were exposed for 48 hours to (i) a scaffold substrate shift, (ii) serum deprivation, and (iii) O₂ deprivation. MSCs were also exposed to prolonged (up to 120 hours) hypoxia associated with serum deprivation. Cell death was assessed by Live/Dead staining and image analysis. The MSC death rates were not affected by the shift to scaffold or 48-hour hypoxia, but increased with fetal bovine serum (FBS) starvation, suggesting that between the two components of ischemia, nutrient deprivation is the stronger factor. Long-term hypoxia combined with serum deprivation resulted in the complete death of MSCs (99 +/- 1%), but this rate was reduced by half when MSCs were exposed to hypoxia in the presence of 10% FBS (51 +/- 31%). These results show that MSCs are sensitive to the concurrent serum and O₂ deprivation to which they are exposed when transplanted in vivo, and call for the development of new transplantation methods.

Ramirez, M. A., R. Fernandez-Gonzalez, et al. (2009). "Effect of stem cell activation, culture media of manipulated embryos, and site of embryo transfer in the production of F0 embryonic stem cell mice." *Biol Reprod* **80**(6): 1216-22.

Recently, F0 embryonic stem (ES) cell mice have been produced by injection of ES cells into eight-cell embryos using either laser- or piezo-assisted injection systems. To simplify the injection procedure, we have optimized the conventional blastocyst injection method, free of laser- or piezo-assisted micromanipulation systems, to produce F0 ES cell pups. To increase the efficiency of producing mice from ES cell injection into eight-cell and blastocyst stage embryos, we have tested: 1) the effect of activating ES cell before injection, 2) the effect of in vitro culture in medium optimized for the survival of both ES cells and embryos, and 3) the effect of transferring the micromanipulated embryos into the

oviduct versus into the uterus of CD1 foster mice. Two B6D2 hybrid ES cell lines were used for injection in a multifactorial analysis to evaluate the efficiency of producing live chimeric and F0 ES cell mice. Our results demonstrate that the activation of ES cells and the appropriate culture conditions are crucial parameters influencing the generation of F0 ES cell offspring. Transfer of blastocysts injected with ES cells into the oviduct of 0.5-day postcoitum pseudopregnant females increased the number of live animals with higher chimera proportion. Under these conditions, injections into eight-cell embryos produce a high number of F0 ES mice, and the conventional blastocyst injection method produces a lower number of F0 ES cell pups; however, the efficiency of production of chimeric mice with germline transmission was high. We have developed an economical and efficient technique for producing fully ES cell-derived F0 mice with full germline transmission that can be applied in many laboratories without the use of piezo or laser instruments.

Rauser, G., H. Einsele, et al. (2004). "Rapid generation of combined CMV-specific CD4+ and CD8+ T-cell lines for adoptive transfer into recipients of allogeneic stem cell transplants." *Blood* **103**(9): 3565-72.

Adoptive transfer of cytomegalovirus (CMV)-specific T cells can restore long-lasting, virus-specific immunity and clear CMV viremia in recipients of allogeneic stem cell transplants if CD4(+) and CD8(+) CMV-specific T cells are detected in the recipient after transfer. Current protocols for generating virus-specific T cells use live virus, require leukapheresis of the donor, and are time consuming. To circumvent these limitations, a clinical-scale protocol was developed to generate CMV-specific T cells by using autologous cellular and serum components derived from a single 500-mL blood draw. CMV-specific T cells were stimulated simultaneously with CMV-specific major histocompatibility complex class I (MHC I)-restricted peptides and CMV antigen. Activated T cells were isolated with the interferon-gamma (IFN-gamma) secretion assay and expanded for 10 days. In 8 randomly selected, CMV-seropositive donors, 1.34 x 10⁸ combined CD4(+) and CD8(+) CMV-specific T cells, on average, were generated, as determined by antigen-triggered IFN-gamma production. CMV-infected fibroblasts were efficiently lysed by the generated T cells, and CMV-specific CD4(+) and CD8(+) T cells expanded if they were stimulated with natural processed antigen. On the other hand, CD4(+) and CD8(+) T cell-mediated alloreactivity of generated CMV-specific T-cell lines was reduced compared with that of the starting population. In

conclusion, the culture system developed allowed the rapid generation of allodepleted, highly enriched, combined CD4(+) and CD8(+) CMV-specific T cells under conditions mimicking good manufacturing practice.

Reddy, G. V. (2008). "Live-imaging stem-cell homeostasis in the Arabidopsis shoot apex." *Curr Opin Plant Biol* **11**(1): 88-93.

A precise spatio-temporal regulation of growth and differentiation is crucial to maintain a stable population of stem cells in the shoot apical meristems (SAMs) of higher plants. The real-time and simultaneous observations of dynamics of cell identity transitions, growth patterns, and signaling machinery involved in cell-cell communication is crucial to gain a mechanistic view of stem-cell homeostasis. In this article, I review recent advances in understanding the regulatory dynamics of stem-cell maintenance in *Arabidopsis thaliana* and discuss future challenges involved in transforming the static maps of genetic interactions into a dynamic framework representing functional molecular and cellular interactions in living SAMs.

Rhee, W. J. and G. Bao (2009). "Simultaneous detection of mRNA and protein stem cell markers in live cells." *BMC Biotechnol* **9**: 30.

BACKGROUND: Biological studies and medical application of stem cells often require the isolation of stem cells from a mixed cell population, including the detection of cancer stem cells in tumor tissue, and isolation of induced pluripotent stem cells after eliciting the expression of specific genes in adult cells. Here we report the detection of Oct-4 mRNA and SSEA-1 protein in live carcinoma stem cells using respectively molecular beacon and dye-labeled antibody, aiming to establish a new method for stem cells detection and isolation. **RESULTS:** Quantification of Oct-4 mRNA and protein in P19 mouse carcinoma stem cells using respectively RT-PCR and immunocytochemistry confirmed that their levels drastically decreased after differentiation. To visualize Oct-4 mRNA in live stem cells, molecular beacons were designed, synthesized and validated, and the detection specificity was confirmed using control studies. We found that the fluorescence signal from Oct-4-targeting molecular beacons provides a clear discrimination between undifferentiated and retinoic acid-induced differentiated cells. Using deconvolution fluorescence microscopy, Oct-4 mRNAs were found to reside on one side of the cytosol. We demonstrated that, using a combination of Oct-4 mRNA-targeting molecular beacon with SSEA-1 antibody in flow cytometric analysis, undifferentiated stem cells can be clearly distinguished from differentiated cells. We

revealed that Oct-4 targeting molecular beacons do not seem to affect stem cell biology. **CONCLUSION:** Molecular beacons have the potential to provide a powerful tool for highly specific detection and isolation of stem cells, including cancer stem cells and induced pluripotent stem (iPS) cells without disturbing cell physiology. It is advantageous to perform simultaneous detection of intracellular (mRNA) and cell-surface (protein) stem cell markers in flow cytometric analysis, which may lead to high detection sensitivity and efficiency.

Robertson, J. A. (1999). "Ethics and policy in embryonic stem cell research." Kennedy Inst Ethics J **9**(2): 109-36.

Embryonic stem cells, which have the potential to save many lives, must be recovered from aborted fetuses or live embryos. Although tissue from aborted fetuses can be used without moral complicity in the underlying abortion, obtaining stem cells from embryos necessarily kills them, thus raising difficult questions about the use of embryonic human material to save others. This article draws on previous controversies over embryo research and distinctions between intrinsic and symbolic moral status to analyze these issues. It argues that stem cell research with spare embryos produced during infertility treatment, or even embryos created specifically for research or therapeutic purposes, is ethically acceptable and should receive federal funding.

Ross, S., S. D. Spencer, et al. (2002). "Prostate stem cell antigen as therapy target: tissue expression and in vivo efficacy of an immunoconjugate." Cancer Res **62**(9): 2546-53.

We conducted an expression analysis of prostate stem cell antigen (PSCA) in normal urogenital tissues, benign prostatic hyperplasia (n = 21), prostatic intraepithelial neoplasia (n = 33), and primary (n = 137) and metastatic (n = 42) prostate adenocarcinoma, using isotopic in situ hybridization on tissue microarrays. In normal prostate, we observe PSCA expression in the terminally differentiated, secretory epithelium; strong expression was also seen in normal urothelium. Forty-eight percent of primary and 64% of metastatic prostatic adenocarcinomas expressed PSCA RNA. Our studies did not confirm a positive correlation between level of PSCA RNA expression and high Gleason grade. We characterized monoclonal anti-PSCA antibodies that recognize PSCA expressed on the surface of live cells, are efficiently internalized after antigen recognition, and kill tumor cells in vitro in an antigen-specific fashion upon conjugation with maytansinoid. Unconjugated anti-PSCA antibodies demonstrated efficacy against PSCA-positive tumors by delaying progressive tumor growth in vivo.

Maytansinoid-conjugated antibodies caused complete regression of established tumors in a large proportion of animals. Our results strongly suggest that maytansinoid-conjugated anti-PSCA monoclonal antibodies should be evaluated as a therapeutic modality for patients with advanced prostate cancer.

Scadden, D. T. (2007). "The stem cell niche in health and leukemic disease." Best Pract Res Clin Haematol **20**(1): 19-27.

That adult stem cells live in a highly specialized complex microenvironment, also known as a niche, is a pedestrian concept about 30 years old. It may, however, represent a relatively novel approach to being able to modify either normal or abnormal stem cells. Our emphasis in the past has been focused on identifying autonomous regulators of the stem cells and in attempting to modify them through the use of exogenous agents like cytokines. The body modulates these cells largely through the complex system that is embodied in the niche. This report discusses studies in which the niche components are modified to observe their effect on stem cells. The niches being investigated lie in the gut, skin, brain and bone. Other sites for hematopoiesis exist in the body, but these specific microenvironments can be localized and each component can be carefully evaluated using mouse models. Studies are ongoing as to how the stem cell microenvironment can support or propagate malignancies. By understanding the signals of this particular microenvironment, we may be able to adapt them to achieve a therapeutic benefit.

Shepherd, B. E., H. P. Kiem, et al. (2007). "Hematopoietic stem-cell behavior in nonhuman primates." Blood **110**(6): 1806-13.

Little is known about the behavior of hematopoietic stem cells (HSCs) in primates because direct observations and competitive-repopulation assays are not feasible. Therefore, we used 2 different and independent experimental strategies, the tracking of transgene expression after retroviral-mediated gene transfer (N = 11 baboons; N = 7 rhesus macaques) and quantitation of the average telomere length of granulocytes (N = 132 baboons; N = 14 macaques), together with stochastic methods, to study HSC kinetics in vivo. The average replication rate for baboon HSCs is once per 36 weeks according to gene-marking analyses and once per 23 weeks according to telomere-shortening analyses. Comparable results were derived from the macaque data. These rates are substantially slower than the average replication rates previously reported for HSCs in mice (once per 2.5 weeks) and cats (once per 8.3 weeks). Because baboons and macaques live for 25 to 45 years, much longer than mice (approximately 2 years) and cats

(12-18 years), we can compute that HSCs undergo a relatively constant number (approximately 80-200) of lifetime replications. Thus, our data suggest that the self-renewal capacity of mammalian stem cells in vivo is defined and evolutionarily conserved.

Shields, L. E., L. K. Gaur, et al. (2003). "In utero hematopoietic stem cell transplantation in nonhuman primates: the role of T cells." *Stem Cells* **21**(3): 304-14.

In utero transplantation of hematopoietic stem cells is a promising treatment for immune and hematologic diseases of fetuses and newborns. Unfortunately, there are limited data from nonhuman primates and humans describing optimal transplantation conditions. The purpose of this investigation was to determine the effect of T-cell number on engraftment and the level of chimerism after in utero transplantation in nonhuman primates. CD34(+) allogeneic adult bone marrow cells, obtained from the sire after G-CSF and stem cell factor administration, were transplanted into female fetal recipients. The average CD34(+) cell dose was 3.0×10^9 /kg (range, 9.9×10^8 to 4.4×10^9) and the T-cell dose ranged from 2.6×10^5 to 1.1×10^8 /kg. Chimerism was determined in peripheral blood subsets (CD2, CD13, and CD20) and in progenitor cell populations by using polymerase chain reaction. Chimerism was noted in seven of eight live-born animals. The level of chimerism in the progenitor population was related to the fetal T-cell dose ($r = 0.64$, $p < 0.02$). At the lowest T-cell dose (2.6×10^5 /kg), no chimerism was detected. As the T-cell dose increased to $10(6-7)$ /kg, the level of chimerism increased. Adjusting the T-cell dose to 1.1×10^8 /kg resulted in fatal graft-versus-host disease (GVHD). The results of this study emphasize the importance of T cells in facilitating donor cell engraftment and in producing GVHD in fetal nonhuman primates. Some animals achieved levels of chimerism in the marrow hematopoietic progenitor cell population that would likely have clinical relevance. However, the levels of chimerism in peripheral blood were too low for therapeutic benefit. Further studies are needed to test methods that are likely to enhance donor cell engraftment and peripheral blood levels of donor cells.

Singhal, S. and J. Mehta (1999). "Reimmunization after blood or marrow stem cell transplantation." *Bone Marrow Transplant* **23**(7): 637-46.

Protective immunity to diseases preventable by routine vaccination is lost over time following allogeneic and autologous blood and marrow transplantation. Adoptive transfer of immunity from donors to recipients after allogeneic transplantation is not sufficient to prevent this decline. Systematic

reimmunization is necessary at appropriate time intervals following transplantation to re-establish immunity. Response to vaccination depends upon the type of transplant, the source of cells, the immune status of the patient, and the vaccine being used. While inactivated or subunit vaccines are safe in all transplant recipients, live vaccines are generally contraindicated. Reimmunization practices vary widely amongst transplant centers. This comprehensive review summarizes published data on post-transplant vaccination, and based upon these, suggests guidelines which may be used as a framework for development of reimmunization protocols.

Sun, Y., D. Xiao, et al. (2007). "Generation of human/rat xenograft animal model for the study of human donor stem cell behaviors in vivo." *World J Gastroenterol* **13**(19): 2707-16.

AIM: To accurately and realistically elucidate human stem cell behaviors in vivo and the fundamental mechanisms controlling human stem cell fates in vivo, which is urgently required in regenerative medicine and treatments for some human diseases, a surrogate human-rat chimera model was developed. METHODS: Human-rat chimeras were achieved by in utero transplanting low-density mononuclear cells from human umbilical cord blood into the fetal rats at 9-11 d of gestation, and subsequently, a variety of methods, including flow cytometry, PCR as well as immunohistochemical assay, were used to test the human donor contribution in the recipients. RESULTS: Of 29 live-born recipients, 19 had the presence of human CD45(+) cells in peripheral blood (PB) detected by flow cytometry, while PCR analysis on genomic DNA from 11 different adult tissues showed that 14 selected from flow cytometry-positive 19 animals possessed of donor-derived human cell engraftment in multiple tissues (i.e. liver, spleen, thymus, heart, kidney, blood, lung, muscle, gut and skin) examined at the time of tissue collection, as confirmed by detecting human beta2-microglobulin expression using immunohistochemistry. In this xenogeneic system, the engrafted donor-derived human cells persisted in multiple tissues for at least 6 mo after birth. Moreover, transplanted human donor cells underwent site-specific differentiation into CK18-positive human cells in chimeric liver and CD45-positive human cells in chimeric spleen and thymus of recipients. CONCLUSION: Taken together, these findings suggest that we successfully developed human-rat chimeras, in which xenogeneic human cells exist up to 6 mo later. This humanized small animal model, which offers an in vivo environment more closely resembling to the situations in human, provides an

invaluable and effective approach for in vivo investigating human stem cell behaviors, and further in vivo examining fundamental mechanisms controlling human stem cell fates in the future. The potential for new advances in our better understanding the living biological systems in human provided by investigators in humanized animals will remain promising.

Thomson, K. J., K. S. Peggs, et al. (2008). "Superiority of reduced-intensity allogeneic transplantation over conventional treatment for relapse of Hodgkin's lymphoma following autologous stem cell transplantation." *Bone Marrow Transplant* **41**(9): 765-70.

This study compares outcome of reduced-intensity conditioned transplant (RIT) with outcome of conventional non-transplant therapy in patients with Hodgkin's lymphoma relapsing following autograft. There were 72 patients in two groups who had relapsed, and received salvage therapy with chemotherapy+/-radiotherapy. One group (n=38) then underwent alemtuzumab-containing RIT. The second group-historical controls (n=34), relapsing before the advent of RIT-had no further high-dose therapy. This group was required to respond to salvage therapy and live for over 12 months post-relapse, demonstrating potential eligibility for RIT, had this been available. Overall survival (OS) from diagnosis was superior following RIT (48% at 10 years versus 15%; $P=0.0014$), as was survival from autograft (65% at 5 years versus 15%; $P<0.0001$). For the RIT group, OS at 5 years from allograft was 51%, and in chemoresponsive patients was 58%, with current progression-free survival of 42%. Responses were seen in 8 of 15 patients receiving donor lymphocyte infusions (DLI) for relapse/progression, with durable remission in five patients at median follow-up from DLI of 45 months (28-55). These data demonstrate the potential efficacy of RIT in heavily pre-treated patients whose outlook with conventional therapy is dismal, and provide evidence of a clinically relevant graft-versus-lymphoma effect.

Tsai, J. W., Y. Chen, et al. (2005). "LIS1 RNA interference blocks neural stem cell division, morphogenesis, and motility at multiple stages." *J Cell Biol* **170**(6): 935-45.

Mutations in the human LIS1 gene cause the smooth brain disease classical lissencephaly. To understand the underlying mechanisms, we conducted in situ live cell imaging analysis of LIS1 function throughout the entire radial migration pathway. In utero electroporation of LIS1 small interference RNA and short hairpin dominant negative LIS1 and dynactin cDNAs caused a dramatic accumulation of

multipolar progenitor cells within the subventricular zone of embryonic rat brains. This effect resulted from a complete failure in progression from the multipolar to the migratory bipolar state, as revealed by time-lapse analysis of brain slices. Surprisingly, interkinetic nuclear oscillations in the radial glial progenitors were also abolished, as were cell divisions at the ventricular surface. Those few bipolar cells that reached the intermediate zone also exhibited a complete block in somal translocation, although, remarkably, process extension persisted. Finally, axonal growth also ceased. These results identify multiple distinct and novel roles for LIS1 in nucleokinesis and process dynamics and suggest that nuclear position controls neural progenitor cell division.

Valero, A., J. N. Post, et al. (2008). "Gene transfer and protein dynamics in stem cells using single cell electroporation in a microfluidic device." *Lab Chip* **8**(1): 62-7.

There is great interest in genetic modification of bone marrow-derived mesenchymal stem cells (MSC), not only for research purposes but also for use in (autologous) patient-derived-patient-used transplantations. A major drawback of bulk methods for genetic modifications of (stem) cells, like bulk-electroporation, is its limited yield of DNA transfection (typically then 10%). This is even more limited when cells are present at very low numbers, as is the case for stem cells. Here we present an alternative technology to transfect cells with high efficiency (>75%), based on single cell electroporation in a microfluidic device. In a first experiment we show that we can successfully transport propidium iodide (PI) into single mouse myoblastic C2C12 cells. Subsequently, we show the use of this microfluidic device to perform successful electroporation of single mouse myoblastic C2C12 cells and single human MSC with vector DNA encoding a green fluorescent-erk1 fusion protein (EGFP-ERK1 (MAPK3)). Finally, we performed electroporation in combination with live imaging of protein expression and dynamics in response to extracellular stimuli, by fibroblast growth factor (FGF-2). We observed nuclear translocation of EGFP-ERK1 in both cell types within 15 min after FGF-2 stimulation. Due to the successful and promising results, we predict that microfluidic devices can be used for highly efficient small-scale 'genetic modification' of cells, and biological experimentation, offering possibilities to study cellular processes at the single cell level. Future applications might be small-scale production of cells for therapeutic application under controlled conditions.

Vigneau, C., K. Polgar, et al. (2007). "Mouse embryonic stem cell-derived embryoid bodies generate progenitors that integrate long term into renal proximal tubules in vivo." *J Am Soc Nephrol* **18**(6): 1709-20.

The metanephric kidney is a mesodermal organ that develops as a result of reciprocal interactions between the ureteric bud and the blastema. The generation of embryonic stem (ES) cell-derived progenitors offers potential for regenerative therapies but is often limited by development of tumor formation. Because brachyury (T) denotes mesoderm specification, a mouse ES cell line with green fluorescence protein (GFP) knocked into the functional T locus as well as lacZ in the ROSA26 locus (LacZ/T/GFP) was used in cell selection and lineage tracing. In the absence of leukemia inhibitory factor, mouse ES cells give rise to embryoid bodies that can differentiate into mesoderm. Culture conditions were optimized (4 d, 10 ng/ml Activin-A) to generate maximal numbers of renal progenitor populations identified by expression of the specific combination of renal markers cadherin-11, WT-1, Pax-2, and Wnt-4. LacZ/T/GFP+ cells were further enriched by FACS selection. Five days after injection of LacZ/T/GFP+ cells into embryonic kidney explants in organ culture, beta-galactosidase immunohistochemistry showed incorporation into blastemal cells of the nephrogenic zone. After a single injection into developing live newborn mouse kidneys, co-localization studies showed that the LacZ/T/GFP+ cells were stably integrated into proximal tubules with normal morphology and normal polarization of alkaline phosphatase and aquaporin-1 for 7 mo, without teratoma formation. It is concluded that defined differentiation of ES cells into embryoid bodies with Activin-A and selection for T expression provides a means to isolate and purify renal proximal tubular progenitor cells with the potential for safe use in regenerative therapies.

Viswanathan, S., T. Benatar, et al. (2003). "Supplementation-dependent differences in the rates of embryonic stem cell self-renewal, differentiation, and apoptosis." *Biotechnol Bioeng* **84**(5): 505-17.

Although it is known that leukemia inhibitory factor (LIF) supports the derivation and expansion of murine embryonic stem (ES) cells, it is unclear whether this is due to inhibitory effects of LIF on ES cell differentiation or stimulatory effects on ES cell survival and proliferation. Using an ES cell line transgenic for green fluorescent protein (GFP) expression under control of the Oct4 promoter, we were able to simultaneously track the responses of live Oct4-GFP-positive (ES) and -negative (differentiated) fractions to LIF, serum, and other growth factors. Our

findings show that, in addition to inhibiting differentiation of undifferentiated cells, the administration of LIF resulted in a distinct dose-dependent survival and proliferation advantage, thus enabling the long-term propagation of undifferentiated cells. Competitive responses from the differentiated cell fraction could only be elicited upon addition of serum, fibroblast growth factor-4 (FGF-4), or insulin-like growth factor-1 (IGF-1). The growth factors did not induce additional differentiation of ES cells, but rather they significantly improved the proliferation of already differentiated cells. Our analyses show that, by adjusting culture conditions, including the type and amount of growth factors or cytokines present, the frequency of media exchange, and the presence or absence of serum, we could selectively and specifically alter the survival, proliferation, and differentiation dynamics of the two subpopulations, and thus effectively control population outputs. Our findings therefore have important applications in engineering stem cell culture systems to predictably generate desired stem cells or their derivatives for various regenerative therapies.

Wang, H. and X. Chen (2008). "Imaging mesenchymal stem cell migration and the implications for stem cell-based cancer therapies." *Future Oncol* **4**(5): 623-8.

Mesenchymal stem cells are promising cellular vehicles for the delivery of therapeutic proteins to sites of cancer growth upon transplantation. To better understand the physiology and biology of the transplanted stem cells, it is necessary and desirable to track the fate of stem cells noninvasively and longitudinally. Reporter gene imaging is a powerful tool to monitor live stem cells in vivo. In this special report, we review currently investigated reporter genes used for tracking stem cells in vivo by optical, radionuclide, magnetic resonance and multimodality imaging techniques. We also discuss the possibility and feasibility of applying reporter gene imaging to monitor stem-cell-based therapeutic gene delivery efficiency and treatment efficacy.

Ward, C. M., A. M. Eastham, et al. (2006). "Cell surface 5T4 antigen is transiently upregulated during early human embryonic stem cell differentiation: effect of 5T4 phenotype on neural lineage formation." *Exp Cell Res* **312**(10): 1713-26.

The 5T4 oncofoetal antigen is a cell surface glycoprotein that is transiently expressed during mouse ES cell differentiation and correlates with decreased pluripotency of such cells. We show that 5T4 antigen is transiently unregulated during HES4 and H1 human ES cell differentiation and its

expression correlates with loss of the pluripotent markers OCT-4 and Tra-1-60 and upregulation of transcript markers associated with the three primary germ layers. To confirm that absence of cell surface 5T4 antigen represents a pluripotent hES cell phenotype, we performed mechanical transfer of either 5T4-ve or 5T4+ve HES4 colonies identified using live cell staining. 5T4-ve transfers maintained expression of OCT-4 in over 90% of resultant colonies, whereas 5T4+ve transfers exhibited significantly lower numbers of OCT-4-expressing colonies (92 +/- 1.4 vs. 2.9 +/- 2.0%). Interestingly, low cell density 5T4-ve colony transfers exhibited increased numbers of OCT-4-expressing colonies compared to large 5T4-ve transfers (92 +/- 1.4 vs. 63.2 +/- 1.9%). 5T4-ve and 5T4+ve HES4 and H1 ES cell lines expressed markers representative of neuroectoderm lineages, and we assessed the formation of neural lineages from these phenotypes in serum-containing medium and N2B27 medium. Expression of 5T4 was found to be inversely related to the yield of tyrosine-hydroxylase (TH⁺)-expressing neurons in N2B27 medium, with additional mesoderm and endoderm transcript markers detected. Homogeneous glial cell populations were derived from low cell density 5T4-ve colony transfers cultured in serum-containing medium, with TH⁺ neuronal formation inhibited in a cell-density-dependent manner. We conclude that the 5T4 antigen is a transient marker of hES cell differentiation and that 5T4 phenotype, colony seeding density and culture conditions significantly influence the maintenance of pluripotent hES cells and their differentiation to neural lineages.

Watson, D. J., R. M. Walton, et al. (2006). "Structure-specific patterns of neural stem cell engraftment after transplantation in the adult mouse brain." *Hum Gene Ther* 17(7): 693-704.

Transplantation of neural stem cells (NSCs) may be useful for delivering exogenous gene products to the diseased CNS. When NSCs are transplanted into the developing mouse brain, they can migrate extensively and differentiate into cells appropriate to the sites of engraftment, in response to the normal signals directing endogenous cells to their appropriate fates. Much of the prior work on NSC migration in the adult brain has examined directed migration within or toward focal areas of injury such as ischemia, brain tumors, or 6-hydroxydopamine (6-OHDA) lesions. However, treatment of many genetic disorders that affect the CNS will require widespread dissemination of the donor cells in the postnatal brain, because the lesions are typically distributed globally. We therefore tested the ability of NSCs to migrate in the unlesioned adult mouse brain after stereotaxic transplantation into

several structures including the cortex and hippocampus. NSC engraftment was monitored in live animals by magnetic resonance imaging (MRI) after superparamagnetic iron oxide (SPIO) labeling of cells. Histological studies demonstrated that the cells engrafted in significantly different patterns within different regions of the brain. In the cerebral cortex, donor cells migrated in all directions from the injection site. The cells maintained an immature phenotype and cortical migration was enhanced by trypsin treatment of the cells, indicating a role for cell surface proteins. In the hippocampus, overall cell survival and migration were lower but there was evidence of neuronal differentiation. In the thalamus, the transplanted cells remained in a consolidated mass at the site of injection. These variations in pattern of engraftment should be taken into account when designing treatment approaches in nonlesion models of neurologic disease.

Wright, J., B. Purdy, et al. (2007). "E-Clinic: an innovative approach to complex symptom management for allogeneic blood and stem cell transplant patients." *Can Oncol Nurs J* 17(4): 187-92.

The allogeneic blood and stem cell program (ABSCP) at Princess Margaret Hospital, Toronto, performs 75 transplants annually. Many patients live greater than 100 kilometres from the centre and require frequent visits to the hospital for posttransplant care. The weekly travel to clinic, combined with complex symptom issues and the overwhelming desire to be cared for in their home community, is a major burden to patients and care providers. Our team of oncology health professionals, led by the nurse practitioner on service, sought to determine whether telehealth videoconferencing would be a viable option as a care delivery model to meet the complex needs of our remote patients and care partners. We introduced telehealth into the ambulatory clinic as a pilot project in early 2005. Patients were selected based upon symptoms, therapeutic plan and geographical remoteness. Patient progress was monitored with a goal of transitioning patients from posttransplant hospital-based care to partnered self-care in their home communities. The purpose of this article is to illustrate the ABSCP telehealth program development using a patient case study, and to detail the clinical process improvements and overall program successes that have led to the integration of telehealth into everyday clinical practice as a viable service delivery option for patient-centred symptom management and treatment compliance with a geographically remote patient population.

Wurmser, A. E., K. Nakashima, et al. (2004). "Cell fusion-independent differentiation of neural stem cells to the endothelial lineage." *Nature* **430**(6997): 350-6.

Somatic stem cells have been claimed to possess an unexpectedly broad differentiation potential (referred to here as plasticity) that could be induced by exposing stem cells to the extracellular developmental signals of other lineages in mixed-cell cultures. Recently, this and other experimental evidence supporting the existence of stem-cell plasticity have been refuted because stem cells have been shown to adopt the functional features of other lineages by means of cell-fusion-mediated acquisition of lineage-specific determinants (chromosomal DNA) rather than by signal-mediated differentiation. In this study we co-cultured mouse neural stem cells (NSCs), which are committed to become neurons and glial cells, with human endothelial cells, which form the lining of blood vessels. We show that in the presence of endothelial cells six per cent of the NSC population converted to cells that did not express neuronal or glial markers, but instead showed the stable expression of multiple endothelial markers and the capacity to form capillary networks. This was surprising because NSCs and endothelial cells are believed to develop from the ectoderm and mesoderm, respectively. Experiments in which endothelial cells were killed by fixation before co-culture with live NSCs (to prevent cell fusion) and karyotyping analyses, revealed that NSCs had differentiated into endothelial-like cells independently of cell fusion. We conclude that stem-cell plasticity is a true characteristic of NSCs and that the conversion of NSCs to unanticipated cell types can be accomplished without cell fusion.

Xie, Y., T. Yin, et al. (2009). "Detection of functional haematopoietic stem cell niche using real-time imaging." *Nature* **457**(7225): 97-101.

Haematopoietic stem cell (HSC) niches, although proposed decades ago, have only recently been identified as separate osteoblastic and vascular microenvironments. Their interrelationships and interactions with HSCs in vivo remain largely unknown. Here we report the use of a newly developed ex vivo real-time imaging technology and immunoassaying to trace the homing of purified green-fluorescent-protein-expressing (GFP(+)) HSCs. We found that transplanted HSCs tended to home to the endosteum (an inner bone surface) in irradiated mice, but were randomly distributed and unstable in non-irradiated mice. Moreover, GFP(+) HSCs were more frequently detected in the trabecular bone area compared with compact bone area, and this was validated by live imaging bioluminescence driven by the stem-cell-leukaemia (Scl) promoter-enhancer.

HSCs home to bone marrow through the vascular system. We found that the endosteum is well vascularized and that vasculature is frequently localized near N-cadherin(+) pre-osteoblastic cells, a known niche component. By monitoring individual HSC behaviour using real-time imaging, we found that a portion of the homed HSCs underwent active division in the irradiated mice, coinciding with their expansion as measured by flow assay. Thus, in contrast to central marrow, the endosteum formed a special zone, which normally maintains HSCs but promotes their expansion in response to bone marrow damage.

Yan, J., L. Xu, et al. (2006). "Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice." *Stem Cells* **24**(8): 1976-85.

Amyotrophic lateral sclerosis (ALS) is a target for cell-replacement therapies, including therapies based on human neural stem cells (NSCs). These therapies must be first tested in the appropriate animal models, including transgenic rodents harboring superoxide dismutase (SOD1) mutations linked to familial ALS. However, these rodent subjects reject discordant xenografts. In the present investigation, we grafted NSCs from human embryonic spinal cord into the ventral lumbar cord of 2-month-old SOD1-G93A transgenic mice. Animals were immunosuppressed with FK506, FK506 plus rapamycin, FK506 plus rapamycin plus mycophenolate mofetil, or CD4 antibodies. With FK506 monotherapy, human NSC grafts were rejected within 1 week, whereas combinations of FK506 with one or two of the other agents or CD4 antibodies protected grafts into end-stage illness (i.e., more than 2 months after grafting). The combination of FK506 with rapamycin appeared to be optimal with respect to efficacy and simplicity of administration. Graft protection was achieved via the blockade of CD4- and CD8-cell infiltration and attenuation of the microglial phagocytic response from the host. Surviving NSCs differentiated extensively into neurons that began to establish networks with host nerve cells, including alpha-motor neurons. Immunosuppressed animals with live cells showed later onset and a slower progression of motor neuron disease and lived longer compared with immunosuppressed control animals with dead NSC grafts. Our findings indicate that combined immunosuppression promotes the survival of human NSCs grafted in the spinal cord of SOD1-G93A mice and, in doing so, allows the differentiation of NSCs into neurons and leads to the improvement of key parameters of motor neuron disease.

Yang, Z. J. and R. J. Wechsler-Reya (2007). "Hit 'em where they live: targeting the cancer stem cell niche." *Cancer Cell* **11**(1): 3-5.

Cancer stem cells (CSCs) are thought to be critical for initiation and propagation of many types of cancer. Because these cells are resistant to conventional therapies, they have been very difficult to eliminate. A study in this issue of *Cancer Cell* suggests that brain tumor CSCs live in a "vascular niche" that promotes their long-term growth and self-renewal. Disrupting this niche impairs CSC self-renewal and thereby significantly inhibits the growth of tumors. Targeting the unique microenvironment of CSCs may be the key to effective cancer therapy.

Yoshida, S. (2008). "Spermatogenic stem cell system in the mouse testis." *Cold Spring Harb Symp Quant Biol* **73**: 25-32.

Mouse spermatogenesis represents a highly potent and robust stem cell system. Decades of research have made it one of the most intensively studied mammalian tissue stem cell systems. These studies include detailed morphological examinations, posttransplantation colony formation, and in vitro culture of the stem cells; however, the nature of the stem cells as well as their niche are mostly to be elucidated in the context of homeostatic spermatogenesis. Our group has been challenging this issue by means of transgenic and live-imaging approaches that enable the investigation of live behaviors of "undifferentiated spermatogonia," the candidate stem cell population. A pulse-label experiment has suggested a hierarchical composition of the stem cell functional compartments, unlike the general idea. In addition, live imaging revealed the preferential localization of undifferentiated spermatogonia in the area adjacent to the blood vessel, leading to the proposal of a vasculature-associated niche. These results have suggested the idea of "flexibility" in the mouse spermatogenic stem cell system, which makes a good contrast to the "strict" stem-cell-niche system observed, for example, in the *Drosophila* germ line. This flexible nature seems to be advantageous for mammals.

Zhao, C., R. Yao, et al. (2007). "Establishment of customized mouse stem cell lines by sequential nuclear transfer." *Cell Res* **17**(1): 80-7.

Therapeutic cloning, whereby embryonic stem cells (ESCs) are derived from nuclear transfer (NT) embryos, may play a major role in the new era of regenerative medicine. In this study we established forty nuclear transfer-ESC (NT-ESC) lines that were derived from NT embryos of different donor cell types or passages. We found that NT-ESCs were capable of forming embryoid bodies. In addition, NT-ESCs

expressed pluripotency stem cell markers in vitro and could differentiate into embryonic tissues in vivo. NT embryos from early passage R1 donor cells were able to form full term developed pups, whereas those from late passage R1 ES donor cells lost the potential for reprogramming that is essential for live birth. We subsequently established sequential NT-R1-ESC lines that were developed from NT blastocyst of late passage R1 ESC donors. However, these NT-R1-ESC lines, when used as nuclear transfer donors at their early passages, failed to result in live pups. This indicates that the therapeutic cloning process using sequential NT-ESCs may not rescue the developmental deficiencies that resided in previous donor generations.

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