Stem Cell Definition, Classification and Research History

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

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" (Stedman's Medical Dictionary, 2002).

2. Stem Cell Classification:

Stem cells can also be classified according to their plasticity. Different types of stem cells vary in their degree of plasticity, or developmental versatility. Stem cells are perhaps best understood in terms of how committed they are to becoming any particular type of cell. The categories into which they fall include: the **totipotent** stem cell, **pluripotent** stem cell, **multipotent** stem cell, and the **adult** stem cell (a certain type of **multipotent** stem cell).

Totipotent Stem Cells

These are the most versatile of the stem cell types. When a sperm cell and an egg cell unite, they form a one-celled fertilized egg. This cell is totipotent, meaning it has the potential to give rise to any and all human cells, such as brain, liver, blood or heart cells. It can even give rise to an entire functional organism. The first few cell divisions in embryonic development produce more totipotent cells. After four days of embryonic cell division, the cells begin to specialize into pluripotent stem cells.

Pluripotent Stem Cells

These cells are like totipotent stem cells in that they can give rise to all tissue types. Unlike totipotent stem cells, however, they cannot give rise to an entire organism. On the fourth day of development, the embryo forms into two layers, an an outer layer which will become the placenta, and an inner mass which will form the tissues of the developing human body. These inner cells, though they can form nearly any human tissue, cannot do so without the outer layer; so are not totipotent, but pluripotent. As these pluripotent stem cells continue to divide, they begin to specialize further.

Multipotent Stem Cells

These are less plastic and more differentiated stem cells. They give rise to a limited range of cells within a tissue type. The offspring of the pluripotent cells become the progenitors of such cell lines as blood cells, skin cells and nerve cells. At this stage, they are multipotent. They can become one of several types of cells within a given organ. For example, multipotent blood stem cells can develop into red blood cells, white blood cells or platelets.

Adult Stem Cells

An adult stem cell is a multipotent stem cell in adult humans that is used to replace cells that have died or lost function. It is an undifferentiated cell present in differentiated tissue. It renews itself and can specialize to yield all cell types present in the tissue from which it originated. So far, adult stem cells have been identified for many different tissue types such as hematopoetic (blood), neural, endothelial, muscle, mesenchymal, gastrointestinal, and epidermal cells

Stem Cell Types

There is More than One Source for Stem Cells

Stem cells are often classified with regard to their origin. Stem cells are present both in the embryo and in the adult. Depending on where the stem cells originate, they have different properties. According to this classification scheme, the three kinds of stem cells are: Embryonic stem cells, embryonic germ cells, and adult stem cells.

Embryonic stem cells are from the inner cell mass, which is part of the early (4-5 day old) embryo called the blastocyst. Once removed, the cells of the

inner cell mass can be cultured into embryonic stem cells.

Embryonic germ cells have characteristics similar to embryonic stem cells. Embryonic germ cells, however, are collected from the fetus later in the developmental process from a region known as the gonadal ridge (which would eventually develop into the sex organs). Though these cells can give rise to the three germ layers that make all the specific organs of the body, the cell types that can develop from embryonic germ cells are slightly more limited than those that develop from embryonic stem cells. This is because embryonic germ cells are further along in the developmental process.

Adult stem cells originate from mature adults. These can also be referred to as **multipotent** stem cells, as the number of cell types which they can differentiate into are limited. Adult stem cells serve as a fresh source of cells in living organisms. They replace cells that need to be replaced on a regular basis in a living organism, such as blood (which has a 120 day lifespan) and other connective tissues. It is generally believed that adult stem cell therapies will complement but not replace embryonic stem cell therapies. One advantage of adult stem cells is that they offer the opportunity to utilize small samples of adult tissues of a patient's own cells for expansion and subsequent implantation. This avoids the ethical issues of embryonic stem cells, as well as the issues that accompany allogeneic donations.

No one type of stem cell is necessarily better than the other, rather the different stem cells have different advantages

3. History of Stem Cells Research:

1878-The first attempts were made to fertilize mammalian eggs outside the body

1959-First animals made by in-vitro fertilization (IVF)

1960s-Teratocarcinomas determined to originate from embryonic germ cells in mice. Embryonal carcoinoma cells (EC) identified as a kind of stem cell.

1968-The first human egg is fertilized in vitro

1970s- EC cells injected into mouse blastocysts make chimeric mice. Cultured SC cells are explored as models of embryonic development in mice.

1978-the first IVF baby is born in England

1981-Mouse ES cells are derived from the inner cell mass of blastocysts. Mouse ES cells are grown in vitro. ES cells injected into mice form teratomas.

1984-88-Pluripotent, clonal cells called embryonal carcinoma (EC) cells are developed. When exposed to retinoic acid these cells differentiate into neuron-like cells and other cell types.

1989-A clonal line of human embryonal carcinoma cells is derived that yields tissues from all three primary germ layers. They have limited replicative and differentiative capacity.

1994-Human blastocysts are generated and the inner cell mass is maintained in culture. ES like cells form in the center and retain stem cell like morphology.

1995-96-Non-human primate ES cells are maintained in vitro from the inner cell mass of monkeys. These cells are pluripotent and differentiate normally into all three primary germ layers

1998-ES cells from the inner cell mass of normal human blastocysts are cultured and maintained normally for many passages. EG cells are also derived and grown in vivo.

2000-Scientists derive human ES cells from the inner cell mass of blastocysts. They proliferate in vitro for a long time and form all three germ layers and teratomas when injected into immune deficient mice.

2001-As human ES cell lines are shared and new lines are derived, more research groups are focusing attention on the differentiation of cells in vitro. Many methods focus on making human tissues for transplantation.

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.

Aiba, K., T. Nedorezov, et al. (2009). "Defining developmental potency and cell lineage trajectories by expression profiling of differentiating mouse embryonic stem cells." DNA Res **16**(1): 73-80.

Biologists rely on morphology, function and specific markers to define the differentiation status of cells. Transcript profiling has expanded the repertoire of these markers by providing the snapshot of cellular status that reflects the activity of all genes. However, such data have been used only to assess relative similarities and differences of these cells. Here we show that principal component analysis of global gene expression profiles map cells in multidimensional transcript profile space and the positions of differentiating cells progress in a stepwise manner along trajectories starting from undifferentiated embryonic stem (ES) cells located in the apex. We present three 'cell lineage trajectories', which represent the differentiation of ES cells into the first three lineages in mammalian development: primitive endoderm, trophoblast and primitive ectoderm/neural ectoderm. The positions of the cells along these trajectories seem to reflect the developmental potency of cells and can be used as a scale for the potential of cells. Indeed, we show that embryonic germ cells and induced pluripotent cells are mapped near the origin of the trajectories, whereas mouse embryo fibroblast and fibroblast cell lines are mapped near the far end of the trajectories. We suggest that this method can be used as the non-operational semi-quantitative definition of cell differentiation status and developmental potency. Furthermore, the global expression profiles of cell lineages provide a framework for the future study of in vitro and in vivo cell differentiation.

Annels, N. E., J. S. Kalpoe, et al. (2006). "Management of Epstein-Barr virus (EBV) reactivation after allogeneic stem cell transplantation by simultaneous analysis of EBV DNA load and EBV-specific T cell reconstitution." Clin Infect Dis **42**(12): 1743-8.

BACKGROUND: Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation and may progress to life-threatening lymphoproliferative disease (EBV-LPD) in the absence of adequate EBV-specific T cell immunity. Quantification of EBV DNA load in asymptomatic individuals who are at risk is a useful (although not entirely predictive) indicator of progression to EBV-LPD and guide for preemptive treatment with CD20 antibodies. METHODS: With the aim of improving the identification of patients at risk, we retrospectively analyzed, within a cohort of 25 consecutive allogeneic stem cell transplant recipients at risk for EBV-LPD, the pattern of T cell reconstitution during EBV reactivation in all preemptively treated patients (8 patients). RESULTS: In 6 of 8 cases, a significant T cell reconstitution (i.e., a CD3+ T cell count of >300 cells/microL) was documented during EBV reactivation, which included an expansion of EBV-specific memory T cells, as shown by human leukocyte antigen class I tetramer analysis. Additional evidence for the antiviral potential of this T cell reconstitution was obtained prospectively from a cohort of 14 consecutive allogeneic stem cell transplant recipients at risk for EBV-LPD. EBV reactivation occurred in 3 patients. Preemptive treatment was successfully withheld for 2 of these patients in light of concurrent (EBV-specific) T cell recovery. CONCLUSION: We conclude that analysis of the level of (EBV-specific) T cell reconstitution during EBV reactivation is an important second parameter, in addition to quantification of EBV DNA load, that will be instrumental in a more accurate definition of patients at risk for EBV-LPD who, given their immunoincompetence, will be most certainly dependent on preemptive interventions.

Bergstraesser, E., H. Hasle, et al. (2007). "Non-hematopoietic stem cell transplantation treatment of juvenile myelomonocytic leukemia: a retrospective analysis and definition of response criteria." Pediatr Blood Cancer **49**(5): 629-33.

BACKGROUND: Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative disease of infancy. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment modality, while the role of anti-leukemic therapy prior to HSCT is uncertain. A comparative evaluation of the efficacy of different clinical protocols and great variety of anti-neoplastic drugs applied pre-HSCT is hampered by the lack of uniform criteria of response. Classification schemas applied in other forms of leukemia are of little value, because in JMML therapy may result in divergent responses in solid organs compared to peripheral blood (PB). PROCEDURE: We therefore defined separate response criteria for white blood count (WBC), platelet count, liver size, and spleen size. We then retrospectively evaluated the efficacy of 129 treatment courses other than HSCT administered to 63 children with JMML. Treatment consisted of intensive therapy according to AML-type chemotherapy, maintenance-type combination therapy, and single agent therapy. To account for the variability observed in the natural course of disease, we also evaluated 32 episodes of "no therapy." RESULTS: Best responses within 3 months of initiation of therapy were highly variable for the four response criteria. In contrast to platelet count and liver size, there was a significant correlation between WBC or spleen size and therapy. Response

rates for WBC and spleen size were best for purine analogs, etoposide, and cytarabine as single agents or for maintenance-type combination therapy. CONCLUSION: To rigorously test future therapeutic strategies in this rare disease an international consensus on the definition of response criteria will be helpful.

Bhatia, V. and D. L. Porter (2001). "Novel approaches to allogeneic stem cell therapy." Expert Opin Biol Ther **1**(1): 3-15.

Traditionally, allogeneic haematopoietic stem cell transplantation (SCT) has involved administration of myeloablative doses of chemotherapy and/or radiation that may cure many patients with haematologic diseases. The high morbidity and mortality associated with the intensive conditioning regimen limits allogeneic SCT to younger and healthier patients. However, it is now known that successful allogeneic SCT is dependent, at least in part, on the antitumour properties of the donor graft independent of the conditioning regimen. This potent 'graft versus tumour' (GVT) effect can now be exploited for clinical benefit. The best evidence of a direct GVT reaction comes from the use of donor leukocyte infusions (DLI). For many patients with relapsed leukaemia after allogeneic SCT, DLI re-establishes complete and durable remissions. This has suggested a novel approach to allogeneic cell therapy (ACT) using non-myeloablative, but immunosuppressive conditioning regimens to permit engraftment of allogeneic stem cells and lymphocytes. Engrafted donor cells would then provide GVT activity in the setting of reduced conditioning regimen toxicity. The ability to minimise toxicity and maximise the immunologic GVT effect will make allogeneic transplantation applicable to patients typically ineligible for conventional allogeneic SCT. Response rates with this strategy have been impressive, although toxicity related to graft versus host disease (GVHD) and other complications remains a concern. Current trials have involved heterogeneous groups of patients using various conditioning regimens. Many issues remain unsettled, including identification of the most appropriate tumour targets and definition of the most effective, least toxic conditioning regimen. In addition, the durability of response is unknown. Nevertheless, the use of non-myeloablative conditioning and ACT may provide a new paradigm for allogeneic cell transplantation and the immunotherapy of cancer.

Boyer, M. J. and T. Cheng (2008). "The CDK inhibitors: potential targets for therapeutic stem cell manipulations?" Gene Ther **15**(2): 117-25.

Therapies involving adult stem cells are dependent upon sufficient expansion of these cells to repopulate or replace the diseased tissue and are consequently hindered by their relatively quiescent phenotype. Cellular proliferation is governed by the cyclin-dependent kinases, which in a complex with a corresponding cyclin, phosphorylate a number of downstream mediators to drive the cell through the cell cycle. In turn, biochemical activities of the cyclin-dependent kinases are regulated by two families of cyclin-dependent kinase inhibitors, which have been shown to be potent cell intrinsic blocks of adult stem cell proliferation in multiple tissue types. In contrast to normal stem cells, inappropriate regulation of the cell cycle in cancer stem cells may underlie tumorigenesis and failure of conventional chemotherapeutics to fully eradicate a tumor. Thus, definition of the roles of the cyclin-dependent kinase inhibitors in normal and cancer stem cells may permit the development of novel strategies for adult stem cell expansion and therapies specifically targeted to cancer stem cells.

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

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

producing progenitor cells that migrate into the neurogenic zones and proliferate to produce new neurons and glia.

Burt, R. K., C. Georganas, et al. (1999). "Autologous hematopoietic stem cell transplantation in refractory rheumatoid arthritis: sustained response in two of four patients." Arthritis Rheum **42**(11): 2281-5.

OBJECTIVE: To investigate the safety and efficacy of immune ablation with subsequent autologous hematopoietic stem cell transplantation (HSCT) in severe rheumatoid arthritis (RA). METHODS: Four patients with refractory RA and poor prognostic indicators were treated. Stem cells were collected and lymphocytes were depleted by 2.3-4.0 logs. The conditioning regimen included cyclophosphamide (200 mg/kg), antithymocyte globulin (90 mg/kg), and, for 1 patient, total body irradiation (TBI) with 400 cGy. Improvement was evaluated according to the American College of Rheumatology (ACR) preliminary definition of improvement in RA (ACR 20), and also according to the ACR 50 and ACR 70 criteria. RESULTS: HSCT was well tolerated. Three patients fulfilled the ACR 70 criteria at 1 month and 3 months post-HSCT. One patient did not fulfill the ACR 20 criteria because of persistent joint tenderness, despite improvement of the joint swelling. At 6 months post-HSCT, 1 patient fulfilled the ACR 70 criteria and 1 fulfilled the ACR 50 criteria, and these 2 patients fulfilled the ACR 70 criteria at 9 months post-HSCT. The other 2 patients (including the patient who received TBI) did not meet the ACR 20 criteria at 6 months and 9 months post-HSCT. The only patient with followup of >9 months fulfilled the ACR 70 criteria at 20 months post-HSCT. CONCLUSION: In this series, autologous HSCT was safe and effective in inducing major clinical response and maintained significant benefit for 2 patients at 9 months and 20 months posttreatment, respectively. Sustained response did not occur for 2 of 4 patients. A regimen dose-response effect may exist, but the addition of TBI did not prevent disease relapse for 1 of the patients. More aggressive T cell depletion of the autograft, use of a myeloablative regimen, or use of an allograft may be necessary to decrease relapse rates.

Calvi, L. M. (2006). "Osteoblastic activation in the hematopoietic stem cell niche." Ann N Y Acad Sci **1068**: 477-88.

Hematopoietic stem cells (HSC) are rare primitive cells capable of reconstituting all blood cell lineages throughout the life of an individual. The microenvironment in which stem cells reside is essential for their survival, self-renewal, and differentiation. This microenvironment, or HSC niche, has been difficult to define in bone and bone marrow, but recent studies from our laboratory and others have shown that osteoblasts, the bone-forming cells, are an essential regulatory component of this complex cellular network. We established that parathyroid hormone (PTH), through activation of the PTH/PTHrP receptor (PTH1R) in osteoblastic cells, could alter the HSC niche resulting in HSC expansion in vivo and in vitro and improving dramatically the survival of mice receiving bone marrow transplants. These findings are of great clinical appeal, because they suggest that a strategy aimed at modifying supportive cells in a stem cell niche can expand HSC. While a number of molecules have been found to be important for hematopoietic/osteoblastic interactions, we have focused on the Jagged1/Notch signaling pathway, which was necessary for the PTH-dependent HSC expansion. Since the Jagged1/Notch signaling pathway has been implicated in the microenvironmental control of stem cell self-renewal in several organ systems, definition of Jagged1 modulation, which is currently poorly understood, should provide additional molecular targets for stem cell regulation and advance the understanding of stem cell-microenvironmental interactions.

Cancelas, J. A. and D. A. Williams (2009). "Rho GTPases in hematopoietic stem cell functions." Curr Opin Hematol **16**(4): 249-54.

PURPOSE OF REVIEW: Rho GTPases are key molecular switches controlling the transduction of external signals to cytoplasmic and nuclear effectors. In the last few years, the development of genetic and pharmacological tools has allowed a more precise definition of the specific roles of Rho GTPases in hematopoietic stem cells (HSCs) and progeny of these cells. Rho GTPases are now known to be crucial in HSCs response to hematopoietic microenvironment cues. This article will review the known HSC functions, which are regulated by Rho GTPases. RECENT FINDINGS: This review analyzes the latest data on how different Rho GTPases control adhesion, migration, retention, proliferation, survival, senescence and oncogenic transformation of HSCs and relates these new findings to the physiological functions of these cells. SUMMARY: The development of small molecule inhibitors with ability to interfere Rho GTPase activation by guanine nucleotide exchange factors offers new therapeutic strategies to manipulate the function of HSCs.

Cesaro, S., R. Oneto, et al. (2005). "Haematopoietic stem cell transplantation for Shwachman-Diamond disease: a study from the European Group for blood and marrow transplantation." Br J Haematol **131**(2): 231-6.

This report assessed the results of allogeneic stem cell transplantation (allo-SCT) in 26 patients with Shwachman-Diamond disease (SDS) and severe bone marrow abnormalities. The conditioning regimen was based on busulphan (54%), total body irradiation (23%), fludarabine (15%) or other chemotherapy combinations (8%). Standard prevention of graft versus host disease (GVHD) with cyclosporin +/- methotrexate was adopted in 54% of the patients whilst in vivo or in vitro T-cell depletion was used in 17 and four patients respectively. Neutrophil and platelet engraftment were achieved in 21 (81%) and 17 (65%) of 26 patients after a median time of 18 days and 29 days respectively. The incidence of grade III and IV acute GVHD was 24% and of chronic GVHD 29%. Nine patients died after a median time of 70 d, post-SCT. After a median follow-up of 1.1 years, the transplant-related mortality was 35.5% (95% CI 17-54) whilst the overall survival was 64.5% (95% CI 45.7-83.2). Allo-SCT was found to be successful in more than half of SDS patients with severe bone marrow dysfunction. Further improvements would be anticipated by a better definition of the optimum time in the course of disease to transplant and by the adoption of less toxic conditioning regimens.

Cho, B. S., C. K. Min, et al. (2008). "Clinical impact of thrombotic microangiopathy on the outcome of patients with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **41**(9): 813-20.

The impact of thrombotic microangiopathy (TMA) on outcome was studied in 148 patients with acute graft-versus-host disease (GVHD) (> or =grade II). The Blood and Marrow Transplant Clinical Trials Network's definition for TMA was used to diagnose definite TMA. Probable TMA was diagnosed when none of the features of nephropathy and neurologic abnormalities associated with definite TMA were present. Overall, TMA developed in 43 (29%) patients; 16 definite and 27 probable. The occurrence of TMA, the maximum grade of acute GVHD and initial treatment failure were associated with shorter overall and GVHD-specific survival. The development of probable as well as definite TMA affected the survival of patients with acute GVHD adversely. These results show the clinical impact of TMA on patients with acute GVHD, and suggest that the proposed definitions and grading of TMA may need to be modified.

Choi, C. M., A. H. Schmaier, et al. (2009). "Thrombotic microangiopathy in haematopoietic stem cell transplantation: diagnosis and treatment." Drugs **69**(2): 183-98.

Each year in the US, more than 10 000 patients benefit from allogeneic haematopoietic stem cell transplantation (HSCT), a modality that offers an excellent chance of eradicating malignancy but confers a higher risk of treatment-related mortality. An uncommon but devastating consequence of HSCT is transplantation-associated thrombotic microangiopathy (TA-TMA). The incidence of TA-TMA ranges from 0.5% to 76%, with a mortality rate of 60-90% despite treatment. Although there appears to be a consistent treatment approach to idiopathic thrombotic thrombocytopenic purpura (TTP) using plasma exchange, corticosteroids and rituximab, the treatment strategies for TA-TMA are perplexing, in part, because the literature regarding this complex condition does not provide true consensus for incidence, aetiology, diagnostic criteria, classification and optimal therapy. The classic definition of idiopathic TTP includes schistocytes on the peripheral blood smear, thrombocytopenia and increased serum lactate dehydrogenase. Classic idiopathic TTP has been attributed to deficient activity of the metalloproteinase responsible for cleaving ultra-large von Willebrand factor multimers. This protease is a member of the 'a disintegrin and metalloprotease with thrombospondin type 1 motif' family and is subsequently named ADAMTS-13. Severely deficient ADAMTS-13 activity (<5% of normal) is associated with idiopathic TTP in 33-100% of patients. In constrast to the pathophysiology of idiopathic TTP, patients with TA-TMA have >5% ADAMTS-13 serum activity. These data may explain why plasma exchange, a standard treatment modality for idiopathic TTP that restores ADAMTS-13 activity, is not effective in TA-TMA. TA-TMA has a multifactorial aetiology of endothelial damage induced by intensive conditioning therapy, irradiation, immunosuppressants, infection and graft-versus-host disease. Treatment consists of substituting calcineurin inhibitors with an alternative immunosuppressive agent that possesses another mode of action. One candidate may be daclizumab, especially in those with mild to moderate TMA. Rituximab therapy or the addition of defibrotide may also be beneficial. In general, plasma exchange is not recommended.

Comi, G., L. Kappos, et al. (2000). "Guidelines for autologous blood and marrow stem cell transplantation in multiple sclerosis: a consensus report written on behalf of the European Group for Blood and Marrow Transplantation and the European Charcot Foundation. BMT-MS Study Group." J Neurol **247**(5): 376-82.

Recent reports suggest the possible beneficial effects of haemopoietic stem cell transplantation (HSCT) in autoimmune diseases such as multiple

sclerosis (MS). The definition of the risk/benefit ratio for such a treatment is perceived as a major issue for the neurological community worldwide. The First Consensus Conference on Bone Marrow Transplantation in Patients with Multiple Sclerosis was held in Milan, Italy on 21 February 1998. Participants from 16 European, North American, and South American countries discussed the guidelines for performing HSCT in MS. This conference was organized in order to: (a) define criteria for patient selection; (b) define transplantation procedures to maximize efficacy of the treatment and minimize its toxicity; (c) standardize patient outcome evaluation; and (d) establish an international working group to evaluate the efficacy and safety of HSCT in MS and to study the immunological changes related to HSCT in MS patients. During the meeting in Milan agreement was reached on: (a) the preparation and distribution of a consensus report on HSCT in MS and (b) the design of an open trial for an initial assessment of the safety and efficacy of HSCT in MS. The consensus reached during the meeting and the design of the clinical trial are summarized in this contribution.

Conti, L. and E. Cattaneo (2008). "Novel and immortalization-based protocols for the generation of neural CNS stem cell lines for gene therapy approaches." Methods Mol Biol **438**: 319-32.

Transplantation of neural cells engineered to produce growth factors or molecules with antitumor effects have the potential of grafted cells to be used as vectors for protein delivery in animal models of diseases. In this context, neural stem cells (NSCs), since their identification, have been considered an attractive subject for therapeutic applications to the damaged brain. NSCs have been shown to include attributes important for potential successful ex vivo gene therapy approaches: they show extensive in vitro expansion and, in some cases, a particular tropism toward pathological brain areas. Clearly, the challenges for future clinical development of this approach are in the definition of the most appropriate stem cells for a given application, what genes or chemicals can be delivered, and what diseases are suitable targets. Ideally, NSC lines should be homogeneous and well characterized in terms of their in vitro stability and grafting capacity. We discuss two possible approaches to produce homogeneous and stable progenitor and NSC lines that exploit an oncogene-based immortalization, or, in the second case, a novel protocol for growth factor expansion of stem cells with radial glia-like features. Furthermore, we describe the use of retroviral particles for genetic engineering.

Cooke, K. R. and G. Yanik (2004). "Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease?" Bone Marrow Transplant **34**(9): 753-65.

Allogeneic hematopoietic stem cell transplantation (SCT) is an important therapeutic option for a number of malignant and nonmalignant conditions but the broader application of this treatment strategy is limited by several side effects. In particular, diffuse lung injury is a major complication of SCT that responds poorly to standard therapeutic approaches and significantly contributes to transplant-related morbidity and mortality. Historically, approximately 50% of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has tipped the balance of pulmonary complications from infectious to noninfectious causes. This mini review will discuss the definition, risk factors and pathogeneses of noninfectious lung injury that occurs early after allogeneic SCT.

Corso, A., S. Caberlon, et al. (2000). "Blood stem cell collections in multiple myeloma: definition of a scoring system." Bone Marrow Transplant **26**(3): 283-6.

The purpose of the study was to identify factors that could predict good yields of peripheral blood stem cells (PBSC) in multiple myeloma (MM). Fifty-one MM patients, nine with refractory disease and 42 in plateau phase, were mobilized with high-dose cyclophosphamide (HD-Cy) at 4 g/m2 followed by granulocyte colony-stimulating factor (G-CSF) 5 microg/kg/day. Clinical and laboratory parameters at the time of mobilization were analyzed for correlations with the number of CD34+ cells collected, with the colony-forming unit granulocyte-macrophage (CFU-GM) count, and the mononuclear cell (MNC) count. In univariate analysis, low WBC count, low platelet count, prior exposure to melphalan, and an interval >6 months from the start of treatment correlated with poor yields of CD34+ cells. Low platelet count, prior exposure to melphalan or to radiotherapy, and an interval >6 months from the start of treatment were associated with a low CFU-GM count. On the basis of these data, we defined a scoring system able to predict the yield of the mobilizing procedure. According to this system, the presence of more than one risk factor (low WBC and platelet counts, prior exposure to melphalan, interval from first chemotherapy >6 months) was predictive of insufficient collections when a conventional combination of mobilizing measures are used.

Daley, G. Q., M. A. Goodell, et al. (2003). "Realistic prospects for stem cell therapeutics." Hematology Am Soc Hematol Educ Program: 398-418.

Studies of the regenerating hematopoietic system have led to the definition of many of the fundamental principles of stem cell biology. Therapies based on a range of tissue stem cells have been widely touted as a new treatment modality, presaging an emerging new specialty called regenerative medicine that promises to harness stem cells from embryonic and somatic sources to provide replacement cell therapies for genetic, malignant, and degenerative conditions. Insights borne from stem cell biology also portend development of protein and small molecule therapeutics that act on endogenous stem cells to promote repair and regeneration. Much of the newfound enthusiasm for regenerative medicine stems from the hope that advances in the laboratory will be followed soon thereafter by breakthrough treatments in the clinic. But how does one sort through the hype to judge the true promise? Are stem cell biologists and the media building expectations that cannot be met? Which diseases can be treated, and when can we expect success? In this review, we outline the realms of investigation that are capturing the most attention, and consider the current state of scientific understanding and controversy regarding the properties of embryonic and somatic (adult) stem cells. Our objective is to provide a framework for appreciating the promise while at the same time understanding the challenges behind translating fundamental stem cell biology into novel clinical therapies.

Diaz-Ricart, M. (2002). "Stem Cell Plasticity and Tissue Bioengineering: Great Expectations and Some Concerns." Drug News Perspect **15**(2): 93-96.

"Stem cells" are by definition cells that self-renew and that have the capacity to differentiate into several lineages. A recent series of studies has challenged fundamental concepts of stem cell biology by suggesting that the functional potential of stem cells is not restricted to the tissue source from which they are derived. The ability for cells of one tissue to produce cells of other developmentally unrelated tissues has been defined as cellular plasticity. Therefore, the utility of stem cell plasticity in cell replacement therapy should be unlimited. Multipotent stem cells may be used to develop replacement tissues for congenital or degenerative disorders, either on their own or in combination with other therapeutic approaches such us gene therapy. There are, however, several concerns regarding the concept of stem cell plasticity and the methods used to evaluate the starting population.

Dispenzieri, A., M. Q. Lacy, et al. (2008). "Peripheral blood stem cell transplant for POEMS syndrome is associated with high rates of engraftment syndrome." Eur J Haematol **80**(5): 397-406.

Polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes (POEMS) syndrome is a devastating syndrome, characterized by peripheral neuropathy, organomegaly, endocrinopathy, monoclonal plasma cells, skin changes, papilledema, volume overload, sclerotic bone lesions, thrombocytosis and high vascular endothelial growth factor (VEGF). High-dose chemotherapy with autologous peripheral blood stem cell transplantation (ASCT) ultimately yields excellent clinical responses, but there can be considerable peritransplant morbidity. We have treated 30 POEMS patients with ASCT at Mayo Clinic, Rochester. During transplant period, patients had high rates of fever, diarrhea, weight gain and rash (93%, 77%, 53% and 43%, respectively). Only 13% remained outpatient, and median time to discharge from hospital was transplant day 17 (range 0-175). Splenomegaly was the baseline factor that best predicted for a complicated peritransplant course. Depending on the definition used, approximately 50% of patients satisfied criteria for engraftment syndrome. Earlier and more aggressive use of corticosteroids may be associated with less complicated post-transplant courses. Median overall survival has not been reached; the treatment-related mortality was 3%. In addition, important clinical improvements and reductions in plasma VEGF levels can occur in the absence of significant decrease in the monoclonal protein. Unraveling the mechanisms of the syndrome both in the context of ASCT and in general are challenges for the future.

Dreger, P., P. Corradini, et al. (2007). "Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus." Leukemia **21**(1): 12-7.

The aim of this project was to identify situations where allogeneic stem cell transplantation (allo-SCT) might be considered as a preferred treatment option for patients with B-cell chronic lymphocytic leukemia (CLL). Based on a MEDLINE search and additional sources, a consented proposal was drafted, refined and approved upon final discussion by an international expert panel. Key elements of the consensus are (1) allo-SCT is a procedure with evidence-based efficacy in poor-risk CLL; (2) although definition of 'poor-risk CLL' requires further investigation, allo-SCT is a reasonable treatment option for younger patients with (i) non-response or early relapse (within 12 months)

after purine analogues, (ii) relapse within 24 months after having achieved a response with purine-analogue-based combination therapy or autologous transplantation, and (iii) patients with p53 abnormalities requiring treatment; and (3) optimum transplant strategies may vary according to distinct clinical situations and should be defined in prospective trials. This is the first attempt to define standard indications for allo-SCT in CLL. Nevertheless, whenever possible, allo-SCT should be performed within disease-specific prospective clinical protocols in order to continuously refine transplant indications according to new developments in risk assessment and treatment of CLL.

Fuji, S., S. W. Kim, et al. (2007). "Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation." Transplantation **84**(7): 814-20.

BACKGROUND: Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) frequently require support with parenteral nutrition and immunosuppressive drugs, which introduce the risk of hyperglycemia. Van den Berghe et al. showed that the strict glucose control improved the outcome of patients treated in the intensive care unit, and this point was evaluated in this study in a HSCT setting. METHODS: A cohort of 112 consecutive adult patients treated by myeloablative allogeneic HSCT between January 2002 and June 2006 was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, preexisting infectious diseases, preexisting neutropenia or previous allogeneic HSCT. The remaining 91 patients were categorized according to mean fasting blood glucose (BG) level in the neutropenic period after conditioning: normoglycemia (BG <110 mg/dL, n=28), mild hyperglycemia (110 to 150 mg/dL, n=49), and moderate/severe (>150 mg/dL, n=14). The primary endpoint was the occurrence of febrile neutropenia (FN) and documented infection during neutropenia, and the secondary endpoints included organ dysfunction according to the definition used by van den Berghe, acute graft-versus-host disease (GVHD), overall survival, and nonrelapse mortality (NRM). RESULTS: Although the incidence of FN or documented infections was similar between the three groups, hyperglycemia was significantly associated with an increased risk of organ dysfunction, grade II-IV acute GVHD, and NRM. CONCLUSIONS: While the results suggested an association between the degree of hyperglycemia during neutropenia and an increased risk of posttransplant complications and NRM, the possibility that intensive glucose control improves the outcome after HSCT can only be confirmed in a prospective randomized trial.

Garry, D. J., A. M. Masino, et al. (2003). "Stem cell biology and therapeutic applications." Curr Opin Nephrol Hypertens **12**(4): 447-54.

PURPOSE OF REVIEW: Chronic diseases are common and deadly. Stem cell therapies have received intense interest for the repopulation of damaged or diseased tissues. A detailed understanding of the similarities and differences between embryonic stem cells and somatic stem cells will enhance our understanding of mechanisms of tissue repair or cellular augmentation. In addition, emerging technologies will be useful in the definition of the molecular regulation of the respective stem cell populations. RECENT FINDINGS: A number of postnatal tissues have a population of somatic stem cells, which function in the maintenance and repair of tissues. Using molecular technologies these somatic stem cell populations have been shown to be pluripotent when placed in a permissive environment. Recent studies have utilized emerging technologies to define a molecular signature of embryonic stem cells and selected somatic stem cell populations. These strategies will be useful for the definition of a molecular program that promotes a stem cell phenotype (i.e. stemness phenotype). SUMMARY: Recent studies suggest that embryonic and somatic stem cell populations hold promise as sources for tissue engineering. The use of cell biological and molecular technologies will enhance our understanding of embryonic and somatic stem cell populations and their molecular regulatory events that promote multipotentiation.

Gerna, G., D. Lilleri, et al. (2008). "Validation of a DNAemia cutoff for preemptive therapy of cytomegalovirus infection in adult hematopoietic stem cell transplant recipients." Bone Marrow Transplant **41**(10): 873-9.

A randomized trial comparing a DNAemia cutoff of 10 000 copies per ml whole blood and first pp65 antigenemia positivity for initiation of preemptive therapy of human cytomegalovirus (HCMV) infection in adult hematopoietic stem cell transplant recipients was completed. DNAemia was chosen for cutoff definition since it is more automatable and standardizable than antigenemia, and more closely reflects the actual viral replication. The primary end point of the study was to compare the number of patients treated in the two arms. A total of 83 patients (42 in the DNAemia, and 41 in the antigenemia arm) were enrolled in the study. The incidence of HCMV infection, as detected by the relevant randomization assay (76% in the DNAemia

versus 85% in the antigenemia arm), was comparable in the two arms, whereas the number of patients treated was significantly lower in the DNAemia arm (63 versus 80%, P=0.02). A single patient in the DNAemia arm suffered from biopsy-proven HCMV gastric disease diagnosed in the absence of detectable virus in blood. The incidence of graft-versus-host disease, and transplantation-related mortality did not differ between the two arms. In conclusion, our study shows that the use of a cutoff significantly reduces the number of patients requiring antiviral treatment, thus sparing unnecessary drug administration.

Girmenia, C., G. Barosi, et al. (2009). "Prophylaxis and treatment of invasive fungal diseases in allogeneic stem cell transplantation: results of a consensus process by Gruppo Italiano Trapianto di Midollo Osseo (GITMO)." Clin Infect Dis **49**(8): 1226-36.

In recent years, prospective studies have been conducted to assess the role of prophylaxis and treatment of invasive fungal diseases (IFD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although results of these studies have been encouraging, they have been unable to generate a consensus for optimal prophylaxis and treatment of IFD in the complex scenario of allo-HSCT. A consensus process was undertaken to describe and evaluate current information and practice regarding key questions on IFD management in allo-HSCT recipients; these questions were selected according to the criterion of relevance by group discussion. The Panel produced recommendations for risk stratification, prophylaxis, monitoring, and therapy of IFD and identified top priority issues for further investigation. The definition of the level of risk for IFD associated with the various types and phases of transplantation and the implementation of surveillance and diagnostic strategies are the critical determinants of the antifungal prophylactic and therapeutic approach for allo-HSCT recipients.

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

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

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

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

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

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

Gregori, S., R. Bacchetta, et al. (2005). "Regulatory T cells: prospective for clinical application in hematopoietic stem cell transplantation." Curr Opin Hematol **12**(6): 451-6.

PURPOSE OF REVIEW: Regulatory T cells exert a dominant effect in controlling autoimmunity and maintaining peripheral tolerance. Regulatory T cells are also involved in preventing allograft rejection and graft versus host disease. Cellular therapy with expanded regulatory T cells represents a promising approach to control T-cell mediated pathology. In this review we will summarize the efforts to design new methods for expanding regulatory T cells and exploit their regulatory function as cellular therapy for the treatment of graft versus host disease after hematopoietic stem cell transplantation. RECENT FINDINGS: Among CD4+ T cells, the best described are the naturally occurring CD4+CD25+ regulatory T cells and type 1 regulatory T cells. Recent progress has been made in the characterization of both subsets in terms of isolation and induction, respectively. However, a clear definition of their mechanisms of action has still to be achieved. SUMMARY: Better understanding of the mechanisms of suppression mediated by regulatory T cells might enable their use to modulate specific immune responses. Moreover, the recent development of methods allowing the ex-vivo expansion of regulatory T cells, to provide sufficient number of cells for in-vivo infusion, represents the first step toward the use of these cells as cellular therapy for the treatment of immunologic and hematological diseases.

Gubbins, P. O., J. R. Amsden, et al. (2009). "Pharmacokinetics and buccal mucosal concentrations of a 15 milligram per kilogram of body weight total dose of liposomal amphotericin B administered as a single dose (15 mg/kg), weekly dose (7.5 mg/kg), or daily dose (1 mg/kg) in peripheral stem cell transplant patients." Antimicrob Agents Chemother **53**(9): 3664-74.

The pharmacokinetics and safety of extended-interval dosing of prophylactic liposomal amphotericin B (L-AMB) in peripheral stem cell transplant recipients were evaluated. The patients received L-AMB daily at 1 mg/kg of body weight or weekly at 7.5 mg/kg or received L-AMB as a single dose (15 mg/kg). The buccal mucosal tissue concentrations of L-AMB were measured. Of the 24 patients enrolled, 5 withdrew after the initial dose due to an infusion-related reaction (n = 2) or significant increases in the serum creatinine (Scr) levels (n = 3). Weekly L-AMB dosing (7.5 mg/kg) produced mean plasma concentrations of >0.300 microg/ml for the first 7 days and >0.220 microg/ml for 7 days after the second dose. A single L-AMB dose (15 mg/kg) produced mean plasma concentrations of >0.491 microg/ml for at least 7 seven days. These concentrations are within the range of the MICs reported in the literature for susceptible strains of Candida and are at the lower limits of the MICs for Aspergillus spp. Extended-interval dosing produced buccal mucosal tissue concentrations well in excess of the MICs reported in the literature for susceptible strains of Candida and Aspergillus spp. Infusion-related reactions occurred in 24% of the patients. Baseline and end-of-study Scr, electrolyte (K+, Mg2+, PO4), and serum transaminase levels were similar across the dosage groups. Five (31%) patients met the nephrotoxicity definition prior to completion of the study. Patients in the weekly or single-dose groups

experienced nephrotoxicity significantly faster than the patients in the daily dosing cohort. A weekly L-AMB dose (7.5 mg/kg) or a single L-AMB dose (15 mg/kg) produced sufficient concentrations in plasma and highly vascular tissue to warrant further studies of the safety, efficacy, and practicality of the weekly prophylactic administration of L-AMB.

Guerrero, A., J. A. Perez-Simon, et al. (1999). "Neurological complications after autologous stem cell transplantation." Eur Neurol **41**(1): 48-50.

Introduction: Autologous bone marrow (BMT) or peripheral blood stem cell transplantation (PBSCT) are increasingly used to treat hematological malignancies and solid tumors. The reported incidence of neurological complications varies greatly among the different centers, ranging from 11 to 39%. Patients and Methods: In order to gain insight into the real incidence of early neurological complications (first 6 weeks) after autologous transplantation, we analyzed a series of 254 patients who underwent BMT/PBSCT for hematological malignancies (212 cases) or solid tumors (42 cases). Results: Seven patients died during the early posttransplant period (incidence: 2.4%), and one of these deaths was related to a neurological complication. Eight patients developed neurologic complications (incidence: 2.8%). None of the patients developed cerebral hemorrhage during the early posttransplant period, despite a rather restrictive platelet transfusion support. Two out of 13 patients diagnosed AML and 3 out of 36 patients diagnosed Hodgkin's disease developed early neurological complications. Discussion and Conclusions: The neurological morbidity related to autologous transplantation was very low in our series of patients as compared to that reported in previous studies. This difference could be explained, at least in part, by a more restrictive definition of early neurologic complications.

Hahn, T., D. Wall, et al. (2006). "The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of acute lymphoblastic leukemia in adults: an evidence-based review." Biol Blood Marrow Transplant **12**(1): 1-30.

Evidence supporting the role of hematopoietic stem cell transplantation (SCT) in the therapy of acute lymphoblastic leukemia in adults (> or =15 years) is presented and critically evaluated in this systematic evidence-based review. Specific criteria were used for searching the published medical literature and for grading the quality and strength of the evidence, and the strength of the treatment recommendations. Treatment recommendations based on the evidence are presented and were reached unanimously by a panel of acute lymphoblastic leukemia experts. The priority areas of needed future research for adult acute lymphoblastic leukemia are: definition of patients at high risk in first complete remission, beyond Philadelphia chromosome positive; outcomes of SCT in older (>50 years) adults; determination if reduced intensity versus myeloablative conditioning regimens yield an equivalent graft-versus-leukemia effect with reduced toxicity; monitoring of minimal residual disease to achieve disease control before SCT; and the use of cord blood and other alternative sources of stem cells for use in adult SCT recipients.

Hengstler, J. G., M. Brulport, et al. (2005). "Generation of human hepatocytes by stem cell technology: definition of the hepatocyte." Expert Opin Drug Metab Toxicol **1**(1): 61-74.

Since 1999, numerous articles have reported the generation of hepatocytes from different types of extrahepatic stem or precursor cells. This opens exciting new possibilities for pharmacology and toxicology, as well as for cell therapy. Hepatocyte marker expression, including albumin, cytokeratin 18, c-met, alpha-fetoprotein and cytochrome P450 3A4 and -2B6, has been observed after transplantation of different types of human stem cells into the liver of laboratory animals or in vitro after incubation with cytokines. These intriguing observations have prompted scientists to classify stem cell-derived cell populations as hepatocytes. However, this conclusion may be premature. It has been shown that factors of the liver microenvironment can induce expression of a limited number of hepatocyte marker genes in nonhepatic cell types. To conclude on the grounds of a limited number of markers that these cells are true hepatocytes is not indicated. In this case one should carefully evaluate crucial hepatocyte-defining enzymatic properties. The present article: i) reviews studies describing the fate of extrahepatic human stem and precursor cells in livers of laboratory animals, including the possibility of cell fusion; and ii) critically discusses the phenotype of stem cells after application of various differentiation protocols aimed at generating human hepatocytes. In addition, the necessary criteria needed for defining a true hepatocyte are suggested. Establishing the necessary properties for stem cell-derived hepatocytes is timely and reasonable, and thus avoids further misleading semantic confusion. Finally, it is essential to understand that the definition of a bona fide hepatocyte should not be limited to qualitative assays, such as reverse transcriptase polymerase chain reaction and immunohistochemistry, but has to include a quantitative analysis of enzymatic activities, which allows direct comparison with primary hepatocytes. Although the stem cell-derived-

hepatocyte does not yet exist there is a good chance that this aim may be achieved in the future.

Herbst, R., M. S. Shearman, et al. (1995). "Formation of signal transfer complexes between stem cell and platelet-derived growth factor receptors and SH2 domain proteins in vitro." Biochemistry **34**(17): 5971-9.

Cellular growth and differentiation signals are generated and defined by the interaction of specific phosphotyrosine residues of activated receptor tyrosine kinases (RTKs) and src homology-2 (SH2) domain-containing intracellular signal transducers. This appears to involve for both the p145c-kit and beta platelet-derived growth factor receptor (PDGF-R) cytoplasmic domains the formation of multiprotein signal transfer complexes, which include combinations of noncatalytic and enzymatically active subunits of phosphatidylinositol 3'-kinase (PI3'-K), phospholipase C-gamma (PLC gamma), and guanosine trisphosphatase activating protein (GAP). In vitro association experiments indicate that PLC gamma and PI3'-K bind the beta PDGF-R simultaneously, while these two SH2 proteins compete for association to p145c-kit binding sites, with p85/PI3'-K exhibiting higher affinity. Interestingly, GAP and p85/PI3'-K binding to distinct p145c-kit phosphotyrosines is cooperative, enhancing formation of a heterotetrameric signaling complex, which may include different combinations of p85 alpha and p85 beta with p110, p112, and p116 by interaction with the same tyrosine 721 docking site. The diversity of molecular interactions observed for PDGF-R and p145c-kit suggests a new mode of signal definition and modulation.

Ho, V. T., C. Cutler, et al. (2005). "Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation." Biol Blood Marrow Transplant **11**(8): 571-5.

The syndrome of microangiopathic hemolysis associated with renal failure, neurologic impairment, or both is a recognized complication of hematopoietic stem cell transplantation. This entity is often called hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP), yet it is clear that the pathophysiology of transplant-associated HUS/TTP is different from that of classic HUS or TTP. Furthermore, the incidence of this syndrome varies from 0.5% to 76% in different transplant series, primarily because of the lack of a uniform definition. The toxicity committee of the Blood and Marrow Transplant Clinical Trials Network has reviewed the current literature on transplant-related HUS/TTP and recommends that it be henceforth renamed posttransplantation thrombotic microangiopathy (TMA). An operational definition for TMA based on the presence of microangiopathic hemolysis and renal and/or neurologic dysfunction is proposed. The primary intervention after diagnosis of TMA should be withdrawal of calcineurin inhibitors. Plasma exchange, although frequently used in this condition, has not been proven to be effective. In the absence of definitive trials, plasma exchange cannot be considered a standard of care for TMA. It is hoped that these positions will improve the identification and reporting of this devastating complication after hematopoietic stem cell transplantation and facilitate future clinical studies for its prevention and treatment.

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

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

(CTLp) frequencies, the allogeneic impact of class II and class I HLA mismatches between a donor and graft recipient can be assessed and permissive disparities defined.

Kalambakas, S. A., T. B. Moore, et al. (2004). "Megatherapy and stem cell transplantation for Ewing's family of tumors: a critical review of current literature." Pediatr Transplant **8 Suppl 5**: 83-8.

Although a multimodal approach consisting of chemotherapy and local control with surgery and/or radiation has improved survival in children with Ewing's sarcoma family of tumors, the prognosis for patients with high-risk disease remains poor. More aggressive treatments with high-dose myeloablative chemotherapy followed by stem cell rescue have been utilized in an attempt to improve survival in these patients. Although many studies have been published, it is difficult to interpret the data since patient populations were heterogeneous with respect to disease stage, prior therapy, conditioning and stem cell source. Furthermore, there was no uniform definition of high risk, the sample sizes were small and most studies lacked appropriate control groups. Assessment of the utility of megatherapy will require prospective controlled studies.

Kearney, E. M., P. J. Prendergast, et al. (2008). "Mechanisms of strain-mediated mesenchymal stem cell apoptosis." J Biomech Eng **130**(6): 061004.

Mechanical conditioning of mesenchymal stem cells (MSCs) has been adopted widely as a biophysical signal to aid tissue engineering applications. The replication of in vivo mechanical signaling has been used in in vitro environments to regulate cell differentiation, and extracellular matrix synthesis, so that both the chemical and mechanical properties of the tissue-engineered construct are compatible with the implant site. While research in these areas contributes to tissue engineering, the effects of mechanical strain on MSC apoptosis remain poorly defined. To evaluate the effects of uniaxial cyclic tensile strain on MSC apoptosis and to investigate mechanotransduction associated with strain-mediated cell death, MSCs seeded on a 2D silicone membrane were stimulated by a range of strain magnitudes for 3 days. Mechanotransduction was investigated using the stretch-activated cation channel blocker gadolinium chloride, the L-type voltage-activated calcium channel blocker nicardipine, the c-jun NH(2)-terminal kinase (JNK) blocker D-JNK inhibitor 1, and the calpain inhibitor MDL 28170. Apoptosis was assessed through DNA fragmentation using the terminal deoxynucleotidyl transferase mediated-UTP-end nick labeling method. Results demonstrated that tensile strains of 7.5% or greater induce apoptosis in MSCs. L-type voltage-activated calcium channels coupled mechanical stress to activation of calpain and JNK, which lead to apoptosis through DNA fragmentation. The definition of the in vitro boundary conditions for tensile strain and MSCs along with a proposed mechanism for apoptosis induced by mechanical events positively contributes to the development of MSC biology, bioreactor design for tissue engineering, and development of computational methods for mechanobiology.

Kellinsalmi, M., H. Monkkonen, et al. (2005). "In vitro comparison of clodronate, pamidronate and zoledronic acid effects on rat osteoclasts and human stem cell-derived osteoblasts." Basic Clin Pharmacol Toxicol **97**(6): 382-91.

In the present study we compared the first generation non-nitrogen-containing bisphosphonate, clodronate with second and third generation nitrogen-containing bisphosphonates, pamidronate and zoledronic acid in dynamic rat osteoclast resorption and apoptosis assays and in human mesenchymal stem cell-derived osteoblast assay. We found that due to high bisphosphonate-bone binding affinity, bone surface exposure to clodronate for 3 min. had maximal resorption inhibition. The mechanism of action of both clodronate and zoledronic acid involved osteoclast apoptosis, whereas pamidronate had only minor apoptotic effect at dosages, which readily inhibited resorption. Zoledronic acid was not metabolised into an intracellular ATP-analogue in vitro in contrast to clodronate. All bisphosphonates had a dose-dependent inhibitory effect on the human bone marrow mesenchymal stem cell (hMSC)-derived osteoblast calcium deposition. None of the compounds had inhibitory effect on hMSC differentiation. Zoledronic acid was the most potent of all three bisphosphonates in terms of both apoptosis induction and resorption inhibition. Zoledronic acid efficacy might thus use its capacity to trigger osteoclast apoptosis in an unknown, but similar manner to that of the non-nitrogen-containing bisphosphonates. It appears that zoledronic acid has properties of both bisphosphonate classes and could well be the first member of a new class of bisphosphonates, by definition.

Kiatpongsan, S., Y. Tannirandorn, et al. (2006). "Introduction to stem cell medicine." J Med Assoc Thai **89**(1): 111-7.

Embryonic stem cell is the promising novel therapeutic tool for various degenerative diseases and tissue injuries. With the concept of cell and tissue therapy, many chronic disorders will be curable. The present article provides basic knowledge of stem cell

in areas of definition, classification and future clinical applications. In addition, stem cell application is not only focusing on regenerative purpose, but also concentrating on more understanding about the early human development and the pathophysiology of genetic diseases at the cellular level. However, there are some technical problems and ethical concern that should be resolved before applying stem cells into clinical practice.

Kibbler, C. (2005). "Defining invasive fungal infections in neutropenic or stem cell transplant patients." J Antimicrob Chemother **56 Suppl 1**: i12-i16.

Consistent definition of invasive fungal infection is important for managing individual patients, for conducting clinical trials and for evaluating diagnostic tests. However, a recent systematic review of the literature found that at least 25 adverbs have been used to categorize infections and when the criteria in these papers were applied to a single database of patients with fungal infections, there was little agreement. This is the consequence of the varying sensitivity and specificity of different clinical features and investigations in different patient groups and an inconsistency in their application. This review examines the clinical presentation of invasive fungal infections in neutropenic patients and those receiving stem cell transplants, as well as the performance of currently available investigations, in order to consider their value as invasive fungal infection criteria. The recent publication of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions has provided an international standard for the performance of clinical research in this group of patients. The definitions committee has now been reconvened to consider some of the criticisms of the original criteria and these are likely to evolve further in the future.

Kim, K. C., I. H. Lee, et al. (2002). "Autologous stem cell transplantation in the treatment of refractory rheumatoid arthritis." J Korean Med Sci **17**(1): 129-32.

The concept of using high-dose immunosuppressive treatment (HDIT) with autologous stem cell transplantation (ASCT) to treat patients with refractory rheumatoid arthritis has been provided by animal studies and anecdotal case reports. Over the past five years, an increasing number of patients with refractory rheumatoid arthritis have received HDIT with ASCT as an adjunct to intense immunosuppression. Here, we present a case of refractory rheumatoid arthritis in a 54-yr-old woman using HDIT with ASCT. Peripheral blood stem cells were mobilized with cyclophosphamide (4 g/m(2)) followed by G-CSF (5 microg/kg/day). Leukapheresis continued daily until the number of harvested progenitor cells reached 2 x 10(6) CD34+ cells/kg after CliniMax CD34+ positive selection. For HDIT, high-dose cyclophosphamide (total dose 200 mg/kg) and antithymocyte globulin (total dose 90 mg/kg) were administered and CD34+ cells were infused 24 hr after HDIT. The patient tolerated the treatment well but experienced an episode of neutropenic fever. She achieved an early dramatic improvement of joint symptoms during therapy. Fifty percent of improvement of rheumatoid arthritis by the American College of Rheumatology (ACR 50) preliminary definition was fulfilled during the 6 months following ASCT. Although further long-term follow-up is required, the patient's activity of arthritis has been stable since receiving HDIT with ASCT.

Kojouri, K. and J. N. George (2007). "Thrombotic microangiopathy following allogeneic hematopoietic stem cell transplantation." Curr Opin Oncol **19**(2): 148-54.

PURPOSE OF REVIEW: The aim of this article is to assess the current understanding and uncertainties about the evaluation and management of thrombotic microangiopathy that occurs following allogeneic hematopoietic stem cell transplantation. RECENT FINDINGS: Current data may not be sufficient to establish posttransplantation thrombotic microangiopathy as a discrete clinical or pathologic entity, distinct from other well recognized transplant-related complications. Analysis of case series of posttransplantation thrombotic microangiopathy illustrates uncertainties regarding incidence, risk factors, diagnosis, treatment, and survival. These studies have suggested the lack of efficacy of plasma exchange treatment and have identified other transplant-related complications, such as acute graft-versus-host disease and opportunistic infections, as the predominant causes of death in patients who had been diagnosed with posttransplantation thrombotic microangiopathy. Recently consensus diagnostic criteria were proposed by two independent groups to provide more uniform identification of patients with posttransplantation thrombotic microangiopathy; these criteria may result in a clearer definition of this syndrome. SUMMARY: Posttransplantation thrombotic microangiopathy remains a diagnostic and therapeutic challenge. Further studies are required to determine if it is a specific entity and to define its relation to other transplant-related complications.

Lakshmipathy, U. and C. Verfaillie (2005). "Stem cell plasticity." Blood Rev **19**(1): 29-38.

The central dogma in stem cell biology has been that cells isolated from a particular tissue can renew and differentiate into lineages of the tissue it resides in. Several studies have challenged this idea by demonstrating that tissue specific cell have considerable plasticity and can cross-lineage restriction boundary and give rise to cell types of other lineages. However, the lack of a clear definition for plasticity has led to confusion with several reports failing to demonstrate that a single cell can indeed differentiate into multiple lineages at significant levels. Further, differences between results obtained in different labs has cast doubt on some results and several studies still await independent confirmation. In this review, we critically evaluate studies that report stem cell plasticity using three rigid criteria to define stem cell plasticity; differentiation of a single cell into multiple cell lineages, functionality of differentiated cells in vitro and in vivo, robust and persistent engraft of transplanted cells.

Leist, M., S. Bremer, et al. (2008). "The biological and ethical basis of the use of human embryonic stem cells for in vitro test systems or cell therapy." Altex **25**(3): 163-90.

Human embryonic stem cells (hESC) are now routinely cultured in many laboratories, and differentiation protocols are available to generate a large variety of cell types. In an ongoing ethical debate opinions of different groups are based on varying sets of religious, historical, cultural and scientific arguments as well as on widely differing levels of general information. We here give an overview of the biological background for non-specialists, and address all is- sues of the current stem cell debate that are of concern in different cultures and states. Thirty-five chapters address embryo definition, potential killing and the beginning of human life, in addition to matters of human dignity, patenting, commercialisation, and potential alternatives for the future, such as induced pluripotent (reprogrammed) stem cells. All arguments are compiled in a synopsis, and compromise solutions, e.g. for the definition of the beginning of personhood and for assigning dignity to embryos, are suggested. Until recently, the major application of hESC was thought to be transplantation of cells derived from hESC for therapeutic use. We discuss here that the most likely immediate uses will rather be in vitro test systems and disease models. Major and minor pharmaceutical companies have entered this field, and the European Union is sponsoring academic research into hESC-based innovative test systems. This development is supported by new testing strategies in Europe and the USA focussing on human cell-based in vitro systems for safety evaluations, and shifting the focus of toxicology away from classical animal experiments towards a more mechanistic understanding.

Lenoir, N. (2000). "Europe confronts the embryonic stem cell research challenge." Science **287**(5457): 1425-7.

Europe's historic plurality and the lack of a commonly accepted definition of the moral status of the embryo have led to varying regulation in European countries. Council of Europe and European Union legislation, based on fundamental ethical principles, does exist for specific issues, such as prohibition against producing embryos solely for research. Such principles have recently been elucidated by the European Group on Ethics in Science and New Technologies. Newly emerging research techniques are beginning to cause reconsideration of the regulation of embryo research in Europe.

Linker, C. (2000). "Thrombopoietin in the treatment of acute myeloid leukemia and in stem-cell transplantation." Semin Hematol **37**(2 Suppl 4): 35-40.

Recent studies indicate that thrombopoietin (TPO) may be highly effective in mobilizing autologous peripheral blood stem cells (PBSCs) for transplantation in patients undergoing intensive chemotherapy. The yield of CD34+ progenitor cells can be increased as can the percentage of patients achieving adequate grafts for use in transplantation. However, the effect of TPO in patients with hematologic malignancies undergoing induction or postremission chemotherapy or in the stem-cell transplantation setting has not been demonstrated. Further study is warranted for better definition of the role of TPO in the treatment of severe thrombocytopenia in these settings.

Mackillop, W. J., A. Ciampi, et al. (1983). "A stem cell model of human tumor growth: implications for tumor cell clonogenic assays." J Natl Cancer Inst **70**(1): 9-16.

A simple stem cell model of human tumor growth is presented. Three tumor cell populations are predicted: stem, transitional, and end cells. The properties of these cells are discussed in terms of their behavior in currently available technologies for investigation of cell kinetics and for their influence on clinical outcome. Stem cell renewal, transitional cell proliferation, and cell loss are analyzed mathematically to define their influence on the relative proportions of cell populations; it is demonstrated that stem cell renewal has a central role in determining the growth properties of tumors. The impact of a stem cell model on the use of tumor clonogenic assays as predictors of clinical outcome is

discussed; opinions are expressed as to the definition of reasonable expectations for current experimental procedures.

Maertens, J., J. Verhaegen, et al. (2001). "Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation." Blood **97**(6): 1604-10.

The diagnosis of invasive aspergillosis (IA) in patients with hematologic disorders is not straightforward; lack of sensitive and specific noninvasive diagnostic tests remains a major obstacle for establishing a precise diagnosis. In a series of 362 consecutive high-risk treatment episodes that were stratified according to the probability of IA based on recently accepted case definition sets, the potential for diagnosis of serial screening for circulating galactomannan (GM), a major aspergillar cell wall constituent was validated. After incorporating postmortem findings to allow a more accurate final analysis, this approach proved to have a sensitivity of 89.7% and a specificity of 98.1%. The positive and negative predictive values equaled 87.5% and 98.4%, respectively. False-positive reactions occurred at a rate of 14%, although this figure might be overestimated due to diagnostic uncertainty. More or less stringent criteria of estimation could highly influence sensitivity, which ranged from 100% to 42%; the impact on other test statistics was far less dramatic. All proven cases of IA, including 23 cases confirmed after autopsy only, had been detected before death, although serial sampling appeared to be necessary to maximize detection. The excellent sensitivity and negative predictive value makes this approach suitable for clinical decision making. Unfortunately, given the species-specificity of the assay, some emerging non-Aspergillus mycoses were not detected. In conclusion, serial screening for GM, complemented by appropriate imaging techniques, is a sensitive and noninvasive tool for the early diagnosis of IA in high-risk adult hematology patients.

Maiolino, A., I. Biasoli, et al. (2003). "Engraftment syndrome following autologous hematopoietic stem cell transplantation: definition of diagnostic criteria." Bone Marrow Transplant **31**(5): 393-7.

Engraftment syndrome (ES) is an increasingly reported complication of hematopoietic stem cell transplantation (HSCT). In order to better characterize the clinical criteria for the diagnosis of ES, we retrospectively analyzed 125 autologous HSCT recipients. ES was first defined as the presence of noninfectious fever plus skin rash. Patients with and without these findings were compared (univariate and multivariate analyses) regarding the presence of weight gain, hypoalbuminemia, pulmonary infiltrates, diarrhea, neurological manifestations and jaundice. The variables that are significantly more frequent in patients with fever and skin rash were incorporated in the definition criteria. The final diagnostic criteria were noninfectious fever plus any of the following: skin rash, pulmonary infiltrates or diarrhea. The incidence of ES was 20%. The single risk factor for ES by multivariate analysis was a diagnosis other than Hodgkin's disease (odds ratio 6.17, 95% confidence interval 1.38-27.78). Patients with ES received empirical antifungal therapy more frequently than patients without the syndrome (40 vs 19%, P=0.03), and had a longer duration of hospitalization (P=0.0007). The prospective application of these diagnostic criteria may have a favorable impact on the early diagnosis of the syndrome, with the initiation of corticosteroids and a reduction in the unnecessary use of antimicrobial agents.

Majolino, I., R. Scime, et al. (1990). "Autologous blood stem cell transplantation in hematologic malignancies." Haematologica **75**(6): 555-66.

Circulating stem cells (CSC) are well documented in animals and humans. Though their function in normal conditions remains obscure, autologous CSC seem capable of restoring hemopoiesis after myeloablative treatment. With cell separators CSC may be harvested in adequate number, and collection may be further improved giving chemotherapy and/or GM-CSF that mobilize stem cells into the circulation. Due to the high number of progenitor cells infused, hematologic reconstitution is more rapid with CSC than with marrow cells. Autologous blood stem cell transplantation (ABSCT) is increasingly employed in a variety of hematologic malignancies and in some solid tumors. CSC allow transplantation in patients previously irradiated on the sites of harvest or with marrow tumor involvement, and probably decrease the risk of infection by shortening the duration of post-graft aplasia. Their use is also encouraged by a belief that, along with CSC, a large number of immunocompetent cells are infused that may exert an anti-tumor effect. A lower tumor contamination of CSC as compared to marrow is an attractive matter, but remains to be demonstrated. Standardization of cell cloning assays, identification of monoclonal antibodies to recognize the surface antigens expressed on progenitor cells, and definition of advantages of ABSCT are items of future work.

Marone, M., D. De Ritis, et al. (2002). "Cell cycle regulation in human hematopoietic stem cells: from isolation to activation." Leuk Lymphoma **43**(3): 493-501.

Hematopoietic stem cells (HSCs) reside mostly in the bone marrow and are defined by their ability to self-renew and to give rise by proliferation and differentiation to all blood lineages. Despite this strict definition HSCs cannot be unequivocally identified in the hematopoietic cell pool. Despite innumerable studies over the years, which focused on the search of the ideal phenotypic marker to selectively isolate stem cells, most of the known markers still define heterogeneous populations in different stages of commitment. Functional features attributed to stem cells have also been investigated, and among these the use of fluorescent markers which allow tracking of the cell division record of each cell. A second issue, after the initial isolation process, is the expansion ex vivo in order to obtain production of large numbers of homogeneous cell populations for both biological studies and clinical applications. Expansion ex vivo is difficult to modulate and normally occurs only along with commitment and consequent loss of multipotentiality. Moreover expansion obtained ex vivo is significantly reduced to that achievable in vivo. One of the key features of HSCs is a very slow proliferation rate, but when the appropriate stimuli are delivered, the proliferation rate can drastically increase. In normal physiological conditions a strict balance is maintained between the number of cells that maintain the original pool and those that proliferate and differentiate. Numerous data in recent years are providing some clue to elucidate the key steps in this tightly controlled process, but the dynamics that regulate which and how many cells self-renew to maintain the pool, and which proliferate and become committed to give rise to the mature blood elements, are still unclear.

McCune, J. S., T. Gooley, et al. (2002). "Busulfan concentration and graft rejection in pediatric patients undergoing hematopoietic stem cell transplantation." Bone Marrow Transplant **30**(3): 167-73.

We retrospectively analyzed the relationship between busulfan average steady-state plasma concentration (C(SS)) and graft rejection in 53 children receiving busulfan/cyclophosphamide (BU/CY) preparative regimens prior to hematopoietic stem cell transplantation (HSCT). Patients received a total oral busulfan dose of 11 to 28 mg/kg followed by a total cyclophosphamide dose of 120 to 335 mg/kg in preparation for allogeneic grafts (HLA-matched or HLA partially matched sibling, parent or unrelated donor). Graft rejection occurred in eight (15%) patients. Busulfan C(SS) (P = 0.0024) was the only statistically significant predictor of rejection on univariate logistic regression analysis, with the risk of rejection decreasing with an increase in busulfan C(SS). Severe (grade 3 or 4) regimen-related toxicity (RRT) occurred in four patients. Ten patients (19%) had a busulfan C(SS) higher than 900 ng/ml, one of whom had severe RRT. Higher and variable doses of cyclophosphamide may explain the lack of a relationship between busulfan C(SS) and RRT in children. It may be possible to improve the outcome of HSCT in pediatric patients receiving the BU/CY regimen through optimization of busulfan C(SS) and better definition of the contribution of activated cyclophosphamide metabolites to toxicity.

Metsuyanim, S., O. Harari-Steinberg, et al. (2009). "Expression of stem cell markers in the human fetal kidney." PLoS One **4**(8): e6709.

In the human fetal kidney (HFK) self-renewing stem cells residing in the metanephric mesenchyme (MM)/blastema are induced to form all cell types of the nephron till 34(th) week of gestation. Definition of useful markers is crucial for the identification of HFK stem cells. Because wilms' tumor, a pediatric renal cancer, initiates from retention of renal stem cells, we hypothesized that surface antigens previously up-regulated in microarrays of both HFK and blastema-enriched stem-like wilms' tumor xenografts (NCAM, ACVRIIB, DLK1/PREF, GPR39, FZD7, FZD2, NTRK2) are likely to be relevant markers. Comprehensive profiling of these putative and of additional stem cell markers (CD34, CD133, c-Kit, CD90, CD105, CD24) in mid-gestation HFK was performed using immunostaining and FACS in conjunction with EpCAM, an epithelial surface marker that is absent from the MM and increases along nephron differentiation and hence can be separated into negative, dim or bright fractions. No marker was specifically localized to the MM. Nevertheless, FZD7 and NTRK2 were preferentially localized to the MM and emerging tubules (<10% of HFK cells) and were mostly present within the EpCAM(neg) and EpCAM(dim) fractions, indicating putative stem/progenitor markers. In contrast, single markers such as CD24 and CD133 as well as double-positive CD24(+)CD133(+) cells comprise >50% of HFK cells and predominantly co-express EpCAM(bright), indicating they are mostly markers of differentiation. Furthermore, localization of NCAM exclusively in the MM and in its nephron progenitor derivatives but also in stroma and the expression pattern of significantly elevated renal stem/progenitor genes Six2, Wt1, Cited1, and Sall1 in NCAM(+)EpCAM(-) and to a lesser extent in NCAM(+)EpCAM(+) fractions confirmed regional identity of cells and assisted us in pinpointing the presence of subpopulations that are putative MM-derived progenitor cells (NCAM(+)EpCAM(+)FZD7(+)), MM stem cells (NCAM(+)EpCAM(-)FZD7(+)) or both

(NCAM(+)FZD7(+)). These results and concepts provide a framework for developing cell selection strategies for human renal cell-based therapies.

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

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

Parker, M. A., J. K. Anderson, et al. (2005). "Expression profile of an operationally-defined neural stem cell clone." Exp Neurol **194**(2): 320-32.

Neural stem cells (NSCs) are the most primordial and least committed cells of the nervous system, the cells that exist before regional specification develops. Because immunocytochemically-detectable markers that are sufficiently specific and sensitive to define an NSC have not yet been fully defined, we have taken the strong view that, to be termed a "stem cell" in the nervous system--in contrast to a "progenitor" or "precursor" (whose lineage commitment is further restricted)--a single neuroectodermally-derived cell must fulfill an operational definition that is essentially similar to that used in hematopoiesis. In other words, it must possess the following functional properties: (1) "Multipotency", i.e., the ability to yield mature cells in all three fundamental neural lineages throughout the nervous system--neurons (of all subtypes), astrocytes (of all types), oligodendrocytes--in multiple regional and developmental contexts and in a region and developmental stage-appropriate manner. (2) The ability to populate a developing region and/or repopulate an ablated or degenerated region of the nervous system with appropriate cell types. (3) The ability to be serially transplanted. (4) "Self-renewal", i.e., the ability to produce daughter cells (including new NSCs) with identical properties and potential. Having identified a murine neural cell clone that fulfills this strict operational definition--in contrast to other studies that used less rigorous or non-operational criteria for defining an NSC (e.g., the "neurosphere" assay)--we then examined, by comparing gene expression profiles, the relationship such a cell might have to (a) a multipotent somatic stem cell from another organ system (the hematopoietic stem cell [HSC]); (b) a pluripotent stem cell derived from the inner cell mass and hence without organ assignment (an embryonic stem cell); (c) neural cells isolated and maintained primarily as neurospheres but without having been subjected to the above mentioned operational screen ("CNS-derived neurospheres"). ESCs, HSCs, and operationally-defined NSCs--all of which have been identified not only by markers but by functional assays in their respective systems and whose state of differentiation could be synchronized--shared a large number of genes. Although, as expected, the most stem-like genes were expressed by ESCs, NSCs and HSCs shared a number of genes. CNS-derived neurospheres, on the other hand, expressed fewer "stem-like" genes held in common by the other operationally-defined stem cell populations. Rather they displayed a profile more consistent with differentiated neural cells. (Genes of neural identity were shared with the NSC clone.) Interestingly, when the operationally-defined NSC clone was cultured as a neurosphere (rather than in monolayer), its expression pattern shifted from a "stem-like" pattern towards a more "differentiated" one, suggesting that the neurosphere, without functional validation, may be a poor model for predicting stem cell attributes because it consists of heterogeneous populations of cells, only a small proportion of which are truly "stem-like". Furthermore, when operational definitions are employed, a common set of stem-like genes does emerge across both embryonic and somatic stem cells of various organ systems, including the nervous system.

Patel, P. (2006). "A natural stem cell therapy? How novel findings and biotechnology clarify the ethics of stem cell research." J Med Ethics **32**(4): 235-9.

The natural replacement of damaged cells by stem cells occurs actively and often in adult tissues, especially rapidly dividing cells such as blood cells. An exciting case in Boston, however, posits a kind of natural stem cell therapy provided to a mother by her fetus-long after the fetus is born. Because there is a profound lack of medical intervention, this therapy seems natural enough and is unlikely to be morally suspect. Nevertheless, we feel morally uncertain when we consider giving this type of therapy to patients who would not naturally receive it. Much has been written about the ethics of stem cell research and therapy; this paper will focus on how recent advances in biotechnology and biological understandings of development narrow the debate. Here, the author briefly reviews current stem cell research practices, revisits the natural stem cell therapy case for moral evaluation, and ultimately demonstrates the importance of permissible stem cell research and therapy, even absent an agreement about the definition of when embryonic life begins. Although one promising technology, blighted ovum utilisation, uses fertilised but developmentally bankrupt eggs, it is argued that utilisation of unfertilised eggs to derive totipotent stem cells obviates the moral debate over when life begins. There are two existing technologies that fulfil this criterion: somatic cell nuclear transfer and parthenogenic stem cell derivation. Although these technologies are far from therapeutic, concerns over the morality of embryonic stem cell derivation should not hinder their advancement.

Petersdorf, E. W. (2007). "Risk assessment in haematopoietic stem cell transplantation: histocompatibility." Best Pract Res Clin Haematol **20**(2): 155-70.

Consideration of potential donors for transplantation includes a rigorous assessment of the availability and HLA-match status of family members, and the identification of suitable unrelated donors when related donors are not available. Because HLA gene products provoke host-versus-graft and graft-versus-host alloimmune responses, HLA matching serves a critical preventive role in lowering risks of graft failure and graft-versus-host disease (GVHD). At the same time, graft-versus-leukemia effects associated with HLA mismatching may provide an immunological means to lower the recurrence of post-transplant disease in high-risk patients. The definition of a suitable allogeneic donor is ever changing, shaped not only by current typing technology for the known HLA genes but also by the specific transplant procedure. Increased safety of alternative donor hematopoietic cell transplantation (HCT) has been achieved in part through advances in the field of immunogenetics. Increased availability of HCT through the use of HLA-mismatched related and unrelated donors is feasible with a more complete understanding of permissible HLA mismatches and the role of NK-KIR genes in transplantation.

Phinney, D. G. (2007). "Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy." Cell Cycle **6**(23): 2884-9.

Mesenchymal stem cells (MSCs) were initially identified by their capacity to differentiate into connective tissue cell types. In the past decade MSCs were also shown to exhibit unexpected plasticity, which was thought to account for their broad therapeutic efficacy in animal models of disease and human clinical trials. More recent evidence indicates that their capacity to alter the microenvironment via secretion of soluble factors contributes more significantly than their plasticity in effecting tissue repair. However, the production by MSCs of a diverse array of trophic factors is inconsistent with their designation as stem cells, which by definition lie at the apex of a hierarchy of cellular differentiation and lineage specification. Analysis of the MSC transcriptome has led to the identification of sub populations that express a variety of regulatory proteins that function in angiogenesis, hematopoiesis, neural activities, and immunity and defense. These activities reflect the varied functions of distinct stromal subtypes in marrow that play important roles in tissue homeostasis. Evidence is provided that the biochemical heterogeneity of these subpopulations contributes more significantly to the therapeutic potential of MSCs than their stem-like characteristics.

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

The capacity of adult bone marrow cells to convert to cells of other tissues, referred to by many as stem cell plasticity, was the focus of the meeting in Providence entitled "Challenges in the Era of Stem Cell Plasticity". The meeting provided a showcase for the many impressive positive results on tissue restoration including the capacity of purified marrow stem cells to restore heart, skin, and liver function in impaired mice or humans. This area of research has become a center of controversy, although it is not clear why. Calls for clonality, robustness, and function have been shown to be erroneous or premature. A call for clonality (which has been shown nicely in one study) is meaningless on a predefined stem cell

population which is intrinsically heterogeneous, as they all are. Robustness means nothing; it all depends on the details of the situation. Function on an organ level is, of course, the goal of many investigators and should not be raised as a limiting consideration. Lastly, fusion has been highlighted as undermining studies with adult stem cells. It, of course, does not. Fusion is simply a means to a final goal, which occurs in certain settings of marrow conversions (transdifferentiation) and not in others. We hypothesize that the conversion phenomena may, in fact, be due to one or several marrow stem cells with broad differentiation potential which can be expressed when the cell is placed in an environment with the appropriate inductive signals. Furthermore, initial events may be relatively rare and significant conversion numbers may be obtained with massive or ongoing selection. Fusion appears in an initial mechanism in some cases and not in others. Overall, the therapeutic potential of adult marrow stem cells is very intriguing, and successful use therapeutically will probably depend on definition of the most appropriate transplant model and tissue injury.

Rak, J. (2006). "Is cancer stem cell a cell, or a multicellular unit capable of inducing angiogenesis?" Med Hypotheses **66**(3): 601-4.

Cancer stem cells are presently viewed as carriers of the growth initiating potential, repopulation capability and drug resistance in tumors. However, many of these fundamental properties of cancer are host-related, modified by cell-cell interactions and/or dependent on angiogenesis. Indeed, it is well established that co-injection of cancer cells with their irradiated (mitotically dead) counterparts, or with Matrigel can significantly increase their tumor forming capacity (i.e. the attribute presently associated with cancer cell 'stemness'). Similarly transfection of angiogenic factors (e.g., VEGF/VPF) can promote such capacity in certain cell lines. Moreover, injection site (e.g., orthotopic vs. ectopic) may significantly modulate tumor take in experimental settings. These observations cannot be reconciled with the paradigm that tumor initiation potential is a fixed, constitutive and cell autonomous feature of a subset of cancer cells expressing stem cell markers (e.g., CD133, Sca1 and other). Instead, it is proposed here that 'stemness' in cancer (perhaps unlike in normal self renewing tissues), rather then being assigned to a particular readily identifiable cell subset, could be a property of interactive clusters of cancer cells (perhaps including, but not limited to cells with stem cell markers). Such 'multicellular units' would become equipped with properties experimentally perceived as 'stemness', i.e. the capacity to initiate tumor growth, when they express the capacity to induce angiogenesis. It is also postulated here that, while the pursuit of subsets of cancer cells harbouring stem cell markers has been fascinating and revealing, due to aforementioned limitations of the present stem cell concept and presumed intractability (e.g., mutability) of such cells, further therapeutic promise may reside in a better definition of 'multicellular angiogenic cancer stem units'.

Rao, M. S. (2006). "Mired in the quagmire of uncertainty: The "catch-22" of embryonic stem cell research." Stem Cells Dev **15**(4): 492-6.

Pluripotent human embryonic stem (ES) cells hold remarkable therapeutic potential, but their use is fraught with moral, ethical, scientific, and political concerns. In this essay, I discuss how an odd combination of patent issues, presidential policy, market uncertainties, and evolving Food and Drug Administration regulations have together hindered the progress of ES cell research in the United States of America. This coalescence of issues is unique. I suggest that these factors explain why the United States has not been a dominant player in advancing ES research. I predict that small, noncontroversial changes would go far in ameliorating many of the roadblocks that now exist. Most of these changes would not require a change in policy or even action by the U.S. government; a simple clarification and definition would suffice. The reason these changes have met solid resistance is suggested to derive from financial rather than moral, ethical, or scientific issues.

Richardson, P. G., C. Murakami, et al. (2002). "Multi-institutional use of defibrotide in 88 patients after stem cell transplantation with severe veno-occlusive disease and multisystem organ failure: response without significant toxicity in a high-risk population and factors predictive of outcome." Blood **100**(13): 4337-43.

Veno-occlusive disease (VOD) is the most common regimen-related toxicity accompanying stem cell transplantation (SCT). Severe VOD complicated by multisystem organ failure (MOF) remains almost uniformly fatal. Preliminary experience with defibrotide (DF), a single-stranded polydeoxyribonucleotide with fibrinolytic, antithrombotic, and anti-ischemic properties, in the treatment for severe VOD has suggested safety and activity. Eighty-eight patients who developed severe VOD after SCT were treated with DF under a defined treatment plan. At diagnosis, median bilirubin was 76.95 microM (4.5 mg/dL), median weight gain was 7%, ascites was present in 84%, and abnormal hepatic portal venous flow was present in 35%. At DF

initiation, median bilirubin had increased to 215.46 microM (12.6 mg/dL), and MOF was present in 97%. DF was administered intravenously in doses ranging from 5 to 60 mg/kg per day for a median of 15 days. No severe hemorrhage or other serious toxicity related to DF was reported. Complete resolution of VOD was seen in 36%, with 35% survival at day +100. Predictors of survival included younger age, autologous SCT, and abnormal portal flow, whereas busulfan-based conditioning and encephalopathy predicted worse outcome. Decreases in mean creatinine and plasminogen activator inhibitor 1(PAI-1) levels during DF therapy predicted better survival. The complete response rate, survival to day +100, and absence of significant DF-associated toxicity in this largest patient cohort reported to date confirm the results of earlier studies. Certain features associated with successful outcome may correlate with DF-related treatment effects, and prospective evaluation of DF therapy for severe VOD should allow better definition of predictors of response or failure.

Roeder, I. and M. Loeffler (2002). "A novel dynamic model of hematopoietic stem cell organization based on the concept of within-tissue plasticity." Exp Hematol **30**(8): 853-61.

OBJECTIVE: At present, no dynamic quantitative models of stem cell organization are available that fulfill all criteria of the prevalent functional definition of hematopoietic stem cells and, at the same time, provide a consistent explanation of cell kinetic and functional stem cell heterogeneity, reversibility of cellular properties, self-organized regeneration after damage, fluctuating activity and competition of stem cell clones, and microenvironment dependency of stem cell quality. To solve this problem, we propose a new, comprehensive model concept. MATERIALS AND METHODS: A single cell-based stochastic model is described. It makes the novel concept of within-tissue plasticity operational. Within a range of potential options, individual cells may reversibly change their actual set of properties depending on the influence of the local growth environment. Stochastic switching between the growth environments introduces fluctuations that eventually generate heterogeneity. Extensive model simulations are compared with experimental data. RESULTS: Although stemness is not an explicit cellular model property, the system behavior is consistent with the functional definition of stem cells and explains a large set of experimental observations on stem cell function in vivo and in vitro on the level of cell populations and individual cells. Classic results such as the colony-forming unit spleen assay, as well as recent experimental observations on stem cell kinetics, individual clone tracking, and fluctuating clonal contribution, are discussed. CONCLUSIONS: This concept introduces a fundamentally new perspective on stem cell organization treating stemness not as an explicit cellular property but as the result of a dynamic process of self-organization. The model needs to be extended to incorporate lineage specification and tissue plasticity.

Roeder, I. and R. Lorenz (2006). "Asymmetry of stem cell fate and the potential impact of the niche: observations, simulations, and interpretations." Stem Cell Rev **2**(3): 171-80.

Asymmetric cell division is a common concept to explain the capability of stem cells to simultaneously produce a continuous output of differentiated cells and to maintain their own population of undifferentiated cells. Whereas for some stem cell systems, an asymmetry in the division process has explicitly been demonstrated, no evidence for such a functional asymmetry has been shown for hematopoietic stem cells (HSC) so far. This raises the question regarding whether asymmetry of cell division is a prerequisite to explain obvious heterogeneity in the cellular fate of HSC. Through the application of a mathematical model based on self-organizing principles, we demonstrate that the assumption of asymmetric stem cell division is not necessary to provide a consistent account for experimentally observed asymmetries in the development of HSC. Our simulation results show that asymmetric cell fate can alternatively be explained by a reversible expression of functional stem cell potentials, controlled by changing cell-cell and cell-microenvironment interactions. The proposed view on stem cell organization is pointing to the potential role of stem cell niches as specific signaling environments, which induce developmental asymmetries and therefore, generate cell fate heterogeneity. The self-organizing concept is fully consistent with the functional definition of tissue stem cells. It naturally includes plasticity phenomena without contradicting a hierarchical appearance of the stem cell population. The concept implies that stem cell fate is only predictable in a probabilistic sense and that retrospective categorization of stem cell potential, based on individual cellular fates, provides an incomplete picture.

Ruck, P., J. C. Xiao, et al. (1997). "Hepatic stem-like cells in hepatoblastoma: expression of cytokeratin 7, albumin and oval cell associated antigens detected by OV-1 and OV-6." Histopathology **31**(4): 324-9.

AIMS: In a recent study we described a population of small epithelial cells (SEC) in human hepatoblastoma that exhibit ultrastructural features of

the oval cell of rodents. Both SEC and oval cells are immunoreactive for cytokeratin 7, a marker of biliary differentiation, and it was postulated that SEC, like oval cells, are closely related to hepatic stem cells. This study was undertaken to investigate whether SEC also exhibit immunolabelling for albumin, a marker of hepatocytic differentiation, and to determine whether other antigens typical of oval cells are detectable in hepatoblastoma. METHODS AND RESULTS: Hepatoblastomas of various subtypes were investigated by electron microscopy, and by immunohistochemistry with the monoclonal antibodies OV-1 and OV-6, which recognize antigens associated with oval cells. Double-labelling for cytokeratin 7 and albumin was carried out by immuno-electron microscopy. OV-1 stained scattered cells in seven of 12 tumours investigated and OV-6 in nine. On immunoelectron-microscopic investigation, SEC exhibited labelling for both cytokeratin 7 and albumin. CONCLUSIONS: The results demonstrate that antigens associated with oval cells are found in certain cells in hepatoblastoma. SEC, like oval cells, co-express markers for hepatocytic and biliary differentiation. The findings further support the hypothesis that SEC are closely related to the putative bipotent hepatic stem cell, which, by definition, gives rise to both hepatocytes and biliary epithelial cells.

Ruutu, T., G. Barosi, et al. (2007). "Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group." Haematologica **92**(1): 95-100.

BACKGROUND AND OBJECTIVES: There are no widely accepted criteria for the definition of hematopoietic stem cell transplant -associated microangiopathy (TAM). An International Working Group was formed to develop a consensus formulation of criteria for diagnosing clinically significant TAM. DESIGN AND METHODS: The participants proposed a list of candidate criteria, selected those considered necessary, and ranked those considered optional to identify a core set of criteria. Three obligatory criteria and four optional criteria that ranked highest formed a core set. In an appropriateness panel process, the participants scored the diagnosis of 16 patient profiles as appropriate or not appropriate for TAM. Using the experts' ratings on the patient profiles as a gold standard, the sensitivity and specificity of 24 candidate definitions of the disorder developed from the core set of criteria were evaluated. A nominal group technique was used to facilitate consensus formation. The definition of TAM with the highest score formed the final PROPOSAL: RESULTS: The Working Group proposes that the diagnosis of TAM requires fulfilment of all of the following criteria: (i) >4% schistocytes in blood; (ii) de novo, prolonged or progressive thrombocytopenia (platelet count <50 x 109/L or 50% or greater reduction from previous counts); (iii) sudden and persistent increase in lactate dehydrogenase concentration; (iv) decrease in hemoglobin concentration or increased transfusion requirement; and (v) decrease in serum haptoglobin. The sensitivity and specificity of this definition exceed 80%. INTERPRETATION AND CONCLUSIONS: The Working Group recommends that the presented criteria of TAM be adopted in clinical use, especially in scientific trials.

Rzepecki, P., J. Barzal, et al. (2007). "Which parameters of nutritional status should we choose for nutritional assessment during hematopoietic stem cell transplantation?" Transplant Proc **39**(9): 2902-4.

Since changes in nutritional indices after hematopoietic stem cell transplantation (HSCT) have not been well studied, there is no definition of risk factors for the development of malnutrition, and the inception of total parenteral nutrition (TPN). We sought to analyze changes in nutritional status parameters and acute phase protein levels as qualifications for TPN. Nutritional status was assessed in 54 patients during autologous (n = 30) on allogeneic (n = 24) transplantations. Eight of 15 patients who had to be treated with TPN, needed prolonged hospitalization (>5 weeks). We assessed biochemical and anthropometric indices of nutritional status, body fat and resting energy expenditure, and acute phase protein levels on the day before starting a conditioning regimen, after chemotherapy completion, and every 7 days until engraftment, which was at least three times after stem cell infusion. Wilcoxon test and canonical analysis were used for statistical analyses. The measurement of body weight and retinol binding protein or transferrin may be useful for nutritional assessment during autologous or allogeneic HSCT, respectively. Prealbumin level, measured 8 days after the end of the conditioning regimen was helpful to make a decision about starting TPN.

Schaap, N., A. Schattenberg, et al. (2002). "Long-term follow-up of persisting mixed chimerism after partially T cell-depleted allogeneic stem cell transplantation." Leukemia **16**(1): 13-21.

Using red cell phenotyping (RCP) and/or cytogenetics (CYT) we identified 19 patients with persisting mixed chimerism (MC) among 231 patients transplanted with partially T cell-depleted stem cell grafts from HLA-identical siblings. Persisting MC is defined as MC for more than 2 years in patients without any evidence of relapse. Median leukemia-free survival in these patients was 150 (range, 50-218)

months. Diagnoses were ALL (n= 10); AML (n = 2); CML (n = 2); NHL (n = 2); MDS (n= 1); MM (n = 1) and SAA (n = 1). Purpose of this study was the long-term follow-up of MC and definition of patterns of chimerism in the various subsets of PBMCs and granulocytes. Using a PCR-STR technique CD3(+)/CD4(+) (T4 lymphocytes), CD3(+)/CD8(+) (T8 lymphocytes), CD45(+)/CD19(+) (B lymphocytes), CD45(+)/CD14(+) (monocytes), CD45(+)/CD15(+) (granulocytes) and CD3(-)/CD56(+) (NK-cells) were analyzed. The majority of patients with persisting MC were conditioned with a less intensive conditioning regimen and had little GVHD. Sequential monitoring of the chimerism resulted in a group of patients (n = 7) with very slow transient mixed chimerism that resulted in complete DC after median 7 years. Another nine patients had a relatively high percentage of persisting autologous cells for a median of 12 years and in three patients we observed a stable low percentage of autologous cells. Only two out of 19 patients (AML-CR1, CML-CP1) relapsed during follow-up. Both patients had a relatively high percentage of autologous cells. Chimerism in granulocytes and PBMC subsets was analyzed at a median of 8 years after SCT in nine patients. In five patients mixed chimerism simultaneously detected by RCP and CYT was associated with MC in all subsets. Within each individual patient the percentages of donor and recipient cells were very different between the different subsets. Two CML-CP1 patients were mixed chimera in only two subsets and in one patient these subsets represented pending relapse. In another two patients mixed chimerism with a very low number of autologous red cells was not found in the PBMCs because of the different sensitivity level of the RCP and the PCR-STR technique. We conclude that in patients with persisting mixed chimerism after partially T cell-depleted SCT a remarkable number of patients had lymphoid malignancies, the majority of the patients were conditioned with less intensive conditioning regimens and the mixed chimerism was not correlated with relapse. Chimerism in granulocytes and PBMC subsets did show great intra-individual differences in the subsets and these data correlated well with RCP and CYT data with the exception of the NK cells.

Schmitt, A., C. Bechter, et al. (2009). "Cytomegalovirus vaccination of leukemia and lymphoma patients after allogeneic stem cell transplantation--validation of a peptide vaccine." J Immunol Methods **343**(2): 140-7.

Peptide vaccination constitutes a novel immunotherapeutical approach for the treatment of patients with solid tumors, lymphoma and leukemia. Moreover it might be of use in hematooncological patients for the prevention and therapy of infections like cytomegalovirus (CMV) reactivation due to immunosuppression. To meet good manufacturing practice (GMP) criteria, we introduce here a bio-assay to validate peptide vaccines for peptide content and bio-activity. As a paradigm for peptide vaccine preparation the immunogenic CMV peptide 495-503 NLVPMVATV lyophilisate was resolubilized in dimethyl sulfoxide, phosphate buffered saline and admixed with Montanide. Addition of different amounts of peptide (10-80 microg) to a mixed lymphocyte peptide culture (MLPC) resulted in the generation of interferon (IFN) gamma and granzyme B releasing CD8(+) CMV tetramer(+) T cells in a dose dependent manner. The combination of FACS and ELISPOT results allowed the definition of the peptide amount in a vaccine preparation. Storage at +/-4 degrees C over 24 h did not result in a significant change of the immunogenicity of the vaccine. In contrast, cryopreservation of the vaccine at -20 degrees C resulted in a loss of immunogenicity. Quantitation of tumor/viral antigen peptides admixed with adjuvants, such as incomplete Freund's adjuvant (IFA), is feasible through bio-assays as the modified ELISPOT/FACS assay described here, meeting GMP criteria for multi-center trials.

Schulz-Kindermann, F., A. Mehnert, et al. (2007). "Cognitive function in the acute course of allogeneic hematopoietic stem cell transplantation for hematological malignancies." Bone Marrow Transplant **39**(12): 789-99.

The aim of the study was to assess cognitive performance in patients with hematological malignancies before, and 3 months after, allogeneic hematopoietic stem cell transplant (HSCT). A consecutive sample of 39 patients was assessed before admission with a comprehensive neuropsychological test battery and health-related quality-of-life (HRQoL) questionnaires; 19 of these patients were retested around 100 days post HSCT. Test results were compared with normative data and revealed minimal differences at both time points in the level of group-means. One parameter - simple reaction time - was significantly worse (prolonged) at second measurement after HSCT. According to the definition of an impairment score (more than three impaired functions), 26% of patients were classified as impaired before as well as after HSCT. Neuropsychological test results did not vary systematically according to medical variables such as extent of pretreatment, graft-versus-host-disease (GvHD) and kind of conditioning protocol. As a dimension of HRQoL, self-rated cognitive function was in the normal range before and after HSCT.

Significant correlations between HRQoL and neuropsychological parameters were related to symptom scales. This study showed impairments of neuropsychological performance for a subgroup of patients before and after allogeneic HSCT. Systematic effects of conditioning, medical variables or self-rated HRQoL could not be observed.

Sharp, J. G., B. O. Murphy, et al. (2005). "Promises and pitfalls of stem cell therapy for promotion of bone healing." Clin Orthop Relat Res(435): 52-61.

There is promise in combining stem cells with allogeneic bone matrix to promote bone healing. Murine bone marrow, peripheral blood, and compact bone cells were transplanted ectopically under the kidney capsule in mice, alone or in combination with allogeneic matrix products: powder and putty to determine their bone forming potential in comparison to transplanted femoral bone fragments and long-term cultured bone marrow cells. The end point was the amount of bone formed as determined by quantitative histology. Mononuclear cells from marrow, peripheral blood, or bone alone transplanted under the kidney capsule did not form bone. Mononuclear cell populations did not combine readily with matrix products and there was in vivo migration of the transplanted combinations. Kidney subcapsular transplanted cultured bone marrow cells formed bone in proportion to the culture period, but after 9 weeks, the extent was only 20% by area of that of similarly transplanted femoral bone fragments. An inductive stimulus for bone formation seemed necessary. Osteoprogenitor cells were not detected in significant numbers in blood unless high doses of cytokines were administered. A better definition of the optimal cell populations and manipulations required for promotion of bone healing is needed along with new (transplant) models that allow for cell tracking. Much work remains to overcome current pitfalls in the use of stem cells to promote allograft integration and bone healing. LEVEL OF EVIDENCE: Therapeutic study, Level V (expert opinion). See the Guidelines for Authors for a complete description of levels of evidence.

Silani, V. and L. Cova (2008). "Stem cell transplantation in multiple sclerosis: safety and ethics." J Neurol Sci **265**(1-2): 116-21.

Stem cell therapy is considered a promising strategy aiming at neuronal and glial cell replacement or neuroprotection in neurological diseases affecting the brain and spinal cord. Multiple Sclerosis (MS), characterized by inflammation-induced destruction of the myelin sheath surrounding axons leading to conduction deficits and variability of clinical signs, is not an exception. MS is considered an autoimmune disease and, in the last few years, an intense immunodepletion followed by autologous hematopoietic-stem-cell transplant (HSCT) is being assessed as potential therapeutical strategy for severe patients unresponsive to the immunomodulatory and immunosuppressive treatment. Partially supported by evidence in animal models and by anecdotal reports on the beneficial effects on MS patients with concomitant malignant diseases, HSCT programs for MS have been initiated worldwide and follow-up data are accumulating. A Consensus Meeting has been held in Milano (1998) providing a document that defined criteria for patient selection, transplantation procedures, and outcome evaluations. Nowadays the high number of patients already treated allows us to draw initial conclusions related to clinical efficacy. After careful monitoring of the available data and improvement of the procedure, safety seems not to be anymore an issue. Ethics of HSCT deserve, on the contrary, a profound evaluation: the procedure is a multistep process with manifold options, each step with different ethical implications. Even more difficult appears the definition of the MS patient selection criteria for HSCT. The informed consensus needs to be exhaustive for the full comprehension of a complex procedure. In conclusion, although HCST is today an established therapeutical option for MS patients, safety and ethical issues need to be further clarified.

Singh, H. P., S. Gupta, et al. (2001). "Redefining 'self': the role of microflora (commensals) mismatch in the development of GvHD after allogeneic stem cell transplantation and some possible remedies." Med Hypotheses **56**(4): 448-50.

The discovery of human leukocyte antigen (HLA) molecules and their role in allorecognition has facilitated the initiation of allogeneic stem cell transplantation in human beings. HLA mismatch to a large extent explains the phenomenon of graft rejection and graft versus host disease (GvHD). Incidence of GvHD even in syngeneic transplants suggests a role for extra genetic factors in the causation of GvHD. We hereby propose a hypothesis that the definition of 'self' (in the immunological sense) should be broadened to include both genetically determined molecules (e.g. HLA) and the microbial flora that colonize an individual. This hypothesis explains several observations about GvHD which can not fully be accounted for by the HLA mismatch theory and gives some clues towards circumventing GvHD.

Souza, B. S., R. C. Nogueira, et al. (2009). "Current status of stem cell therapy for liver diseases." Cell Transplant **18**(12): 1261-79.

Liver failure is one of the main causes of death worldwide and is a growing health problem. Since the discovery of stem cell populations capable of differentiating into specialized cell types, including hepatocytes, the possibility of their utilization in the regeneration of the damaged liver has been a focus of intense investigation. A variety of cell types were tested both in vitro and in vivo, but the definition of a more suitable cell preparation for therapeutic use in each type of liver lesions is yet to be determined. Here we review the protocols described for differentiation of stem cells into hepatocytes, the results of cell therapy in animal models of liver diseases, as well as the available data of the clinical trials in patients with advanced chronic liver disease.

Spangrude, G. J. (1992). "Characteristics of the hematopoietic stem cell compartment in adult mice." Int J Cell Cloning **10**(5): 277-85.

Mouse hematopoietic stem cells can be enriched from adult bone marrow by a number of methods. The resulting cell populations are heterogeneous in function, suggesting a complex organizational structure within the stem cell compartment. Several assays can be applied to the study of early stages of hematopoiesis; however clonal assays for long-term repopulation, the most critical operational definition of hematopoietic stem cells, are lacking. Further complicating the prospect of understanding early hematopoiesis is the finding that genetic variations among laboratory strains of mice lead to major differences in phenotypic and functional characteristics of hematopoietic stem cells. Application to the human situation of the methodology developed for stem cell isolation and characterization in the mouse will be hampered by the possibility of genetic variations among human subjects and the lack of a well-characterized assay system to detect and quantify cells capable of long-term repopulation of irradiated recipients.

Spitzer, T. R. (2001). "Engraftment syndrome following hematopoietic stem cell transplantation." Bone Marrow Transplant **27**(9): 893-8.

During neutrophil recovery following hematopoietic stem cell transplantation, a constellation of symptoms and signs including fever, erythrodermatous skin rash, and noncardiogenic pulmonary edema often occur. These clinical findings have usually been referred to as engraftment syndrome, or, reflecting the manifestations of increased capillary permeability, capillary leak syndrome. While described most often following autologous stem cell transplantation, a similar clinical syndrome has been observed followed allogeneic stem cell transplantation. Distinction from graft-versus-host disease in the allogeneic setting however, has been difficult. Recent experience with non-myeloablative conditioning for stem cell transplantation, however, reveals that an engraftment syndrome independent of GVHD may occur. In some cases, this engraftment syndrome may be a manifestation of a host-versus-graft reaction (graft rejection). While cellular and cytokine interactions are believed to be responsible for these clinical findings, a distinct effector cell population and cytokine profile have not been defined. Engraftment syndromes are likely associated with an increased transplant-related mortality, mostly from pulmonary and associated multi-organ failure. Corticosteroid therapy is often dramatically effective for engraftment syndrome, particularly for the treatment of the pulmonary manifestations. A proposal for a more uniform definition of engraftment syndrome has been developed in order to allow for a reproducible method of reporting of this complication and for evaluating prophylactic and therapeutic strategies.

Spooncer, E., N. Brouard, et al. (2008). "Developmental fate determination and marker discovery in hematopoietic stem cell biology using proteomic fingerprinting." Mol Cell Proteomics **7**(3): 573-81.

In hematopoiesis, co-expression of Sca-1 and c-Kit defines cells (LS(+)K) with long term reconstituting potential. In contrast, poorly characterized LS(-)K cells fail to reconstitute lethally irradiated recipients. Relative quantification mass spectrometry and transcriptional profiling were used to characterize LS(+)K and LS(-)K cells. This approach yielded data on >1200 proteins. Only 32% of protein changes correlated to mRNA modulation demonstrating post-translational protein regulation in early hematopoietic development. LS(+)K cells had lower expression of protein synthesis proteins but did express proteins associated with mature cell function. Major increases in erythroid development proteins were observed in LS(-)K cells; based on this assessment of erythroid potential we showed them to be principally erythroid progenitors, demonstrating effective use of discovery proteomics for definition of primitive cells.

Stewart, A. K., C. I. Chen, et al. (2004). "Results of a multicenter randomized phase II trial of thalidomide and prednisone maintenance therapy for multiple myeloma after autologous stem cell transplant." Clin Cancer Res **10**(24): 8170-6.

We report a multicenter, randomized phase II trial conducted to assess the tolerability of combined thalidomide and prednisone maintenance in multiple myeloma. Eligibility required administration of

melphalan (200 mg/m2) with blood stem cell support within 1 year of treatment onset and initiation of maintenance within 60 to 100 days after stem cell infusion. All patients received 50 mg of prednisone by mouth on alternate days and thalidomide at a starting dose of either 200 or 400 mg daily by mouth. The primary end point was the incidence of dropout or dose reduction due to treatment toxicity within 6 months. Sixty-seven patients were enrolled. Median follow-up is 36.8 months. The primary end point was reached by 31% of patients on the 200 mg of thalidomide arm and 64% of patients on the 400 mg of thalidomide arm. Allowing for dose reduction, 76% of patients assigned to the 200 mg of thalidomide arm and 41% of patients assigned to the 400 mg of thalidomide arm remained on any maintenance therapy 18 months after registration. Eighty-eight percent of all patients dose-reduced thalidomide and 72% of all patients dose-reduced prednisone within 2 years of beginning maintenance. The median progression-free survival post-transplant is 32.3 months, or 42.2 months from diagnosis. Only the 200 mg of thalidomide arm of this trial met our definition of a tolerable maintenance therapy, defined as no dose reductions or discontinuation due to toxicity in at least 65% of patients for a minimum of 6 months, thus establishing a dosing schedule for phase III trials.

Stewart, F. A. and W. Dorr (2009). "Milestones in normal tissue radiation biology over the past 50 years: from clonogenic cell survival to cytokine networks and back to stem cell recovery." Int J Radiat Biol **85**(7): 574-86.

PURPOSE: To illustrate the progress in normal tissue radiation biology over the last five decades and its impact on radiotherapy. MATERIALS AND METHODS: Major milestones over the last 50 years and their consequences for radiation oncology are described: The identification of clonogenic cell survival and the (target) stem cell concept, the dissociation between early and late responding tissues with regard to dose fractionation and development of the linear-quadratic model, characterisation of the effect of overall treatment time, the definition of retreatment tolerance. Current knowledge of mechanisms of radiation pathogenesis is a basis for most recent approaches for amelioration of normal tissue effects. RESULTS: Advances in radiobiological research in normal tissues in the last 50 years have had a major impact on radiation oncology. This includes the linear-quadratic model to adjust doses in altered fractionation protocols, and quantitation of repopulation processes to avoid toxicities in accelerated regimen. Based on new insights into the pathogenesis of normal tissue radiation effects, promising strategies for their modulation, e.g., with cytokines or by stem cell therapy, have been developed. CONCLUSIONS: Research on radiobiology with relevant in vivo models, and relevant treatment protocols is essential for the further progress in radiation oncology.

Storey, J. A., R. F. Connor, et al. (2009). "The transplant iron score as a predictor of stem cell transplant survival." J Hematol Oncol **2**: 44.

Recent studies have suggested that the presence of iron overload prior to stem cell transplantation is associated with decreased survival. Within these studies, the criteria used to define iron overload have varied considerably. Given the lack of consensus regarding the definition of iron overload in the transplant setting, we sought to methodically examine iron status among transplant patients. We studied 78 consecutive patients at risk for transfusion-related iron overload (diagnoses included AML, ALL, MDS, and aplastic anemia) who received either autologous or allogeneic stem cell transplant. Multiple measures of iron status were collected prior to transplantation and examined for their association with survival. Using this data, three potentially prognostic iron measures were identified and incorporated into a rational and unified scoring system. The resulting Transplant Iron Score assigns a point for each of the following variables: (1) greater than 25 red cell units transfused prior to transplantation; (2) serum ferritin > 1000 ng/ml; and (3) a semi-quantitative bone marrow iron stain of 6+. In our cohort, the score (range 0 to 3) was more closely associated with survival than any available single iron parameter. In multivariate analysis, we observed an independent effect of iron overload on transplant survival ($p = 0.01$) primarily attributable to an increase in early treatment-related deaths ($p = 0.02$) and lethal infections. In subgroup analysis, the predictive power of the iron score was most pronounced among allogeneic transplant patients, where a high score (> or = 2) was associated with a 50% absolute decrease in survival at one year. In summary, our results lend further credence to the notion that iron overload prior to transplant is detrimental and suggest iron overload may predispose to a higher rate of lethal infections.

Sugrue, M. W., K. Williams, et al. (2000). "Characterization and outcome of "hard to mobilize"' lymphoma patients undergoing autologous stem cell transplantation." Leuk Lymphoma **39**(5-6): 509-19.

A "hard to mobilize" patient was defined as one in whom >or= 1x10(6) CD 34+ cells/kg cannot be obtained after two consecutive large volume aphereses. Forty-four consecutive Hodgkin's and non-Hodgkin's lymphoma patients who underwent

autologous peripheral blood stem cell (PBSC) transplant treatment between June 1996 and June 1998 were included in this study. Twenty-one patients (48%) met the definition of "hard to mobilize" (Group I). All the rest of the patients (n=23) were the good mobilizers (Group II). The initial mobilization protocol for most patients was 10 microg/kg of G-CSF alone for both groups. For Group I, 7/21 (33%) patients were unable to achieve a minimal dose of >or= 1x10(6) CD34+ cells/kg even after a second mobilization attempt and/or bone marrow (BM) harvest (n=5). Overall, 11/21 (52%) required an additional mobilization and/or BM harvest. Only 3/21 (14%) patients were able to meet the target cell dose of >or= 2.5x10(6) CD34+ cells/kg (median of 4 apheresis). In contrast, 87% of Group II achieved the target dose with a median of 2 aphereses. Predictors of poor mobilization were greater than two prior treatment regimens (p=0.038) and the WBC count (<25,000/microL) on the first day of apheresis (p=0.053). Nineteen patients in Group I and all Group II completed treatment with a median time to engraftment of ANC>500/microl of 12 and 11 days, and platelet >20x10(3)/microl of 31 and 13 days, respectively. Outcome analysis revealed that 6/19 patients in Group I died of relapse within one year from transplant compared with only 2/23 of Group II who died of relapse (p=0.005, log rank test). There were no treatment related deaths in either group. Independent predictive features for "hard to mobilize" patients are a lack of significant increase in WBC count on the first day of apheresis and the number of prior treatment regimens. Poor mobilization appears to predict a worse outcome after autografting for lymphoma patients.

Surbek, D. V., A. Gratwohl, et al. (1999). "In utero hematopoietic stem cell transfer: current status and future strategies." Eur J Obstet Gynecol Reprod Biol **85**(1): 109-15.

Successful prenatal treatment of severe immunodeficiencies by allogeneic hematopoietic stem cell transplantation in utero has been reported. Though other diseases like hemoglobinopathies or storage diseases are potentially amenable to this novel therapeutic approach, no success has yet been achieved in recipients without severe immunodeficiency. Graft rejection by the developing fetus and/or lack of selective, competitive advantage of donor versus host stem cells preventing stable engraftment seem to be the major obstacles. Several strategies to overcome these hurdles are being explored in preclinical settings, including timing and repeated dosing of stem cell administration to the fetus, ex vivo modification of the transplant, using different fetal compartments as targets for early stem cell transfer, or inducing microchimerism for postnatal transplantation from the same donor. In addition, the exact definition of the basic concept of early fetal immunologic naivete and the understanding of the molecular basics of migration and homing in fetal hematopoiesis system seem mandatory for a successful approach. Gene therapy using ex vivo transduced autologous cord blood cells or direct gene targeting in utero are other potential means to correct hematopoietic and immunologic single gene disorders in utero, though this approach is still away from the stage of clinical trials.

Tocci, A. and L. Forte (2003). "Mesenchymal stem cell: use and perspectives." Hematol J **4**(2): 92-6.

Studies on hematopoiesis have focused on the function and composition of human bone marrow stroma. Stroma function gives hematopoietic stem cells the microenvironment appropriate for self-renewal and/or prompt differentiation into hematopoietic progenitor cells, then into terminal specialized cells. Human bone marrow stroma has been dissected into hematopoietic and nonhematopoietic components. The former includes hematopoietic-derived cells, mainly macrophages, while the latter, still poorly characterized, is composed mainly of endothelial and mesenchymal stem cells and their derivatives (adipocytes, chondrocytes, cells of the osteogenic lineage). Isolation of bone marrow mesenchymal stem cells has made available a population of adherent cells, belonging to the non-hematopoietic stroma, which are morphologically and phenotypically homogeneous. This review will focus on: (i) definition of bone marrow stroma and mesenchymal stem cells; (ii) methods of mesenchymal stem cell isolation, morphological and phenotypic characterization; (iii) mesenchymal stem cell functional and differentiation properties and (iv) therapeutic applications of mesenchymal stem cells.

Toh, H. C., S. L. McAfee, et al. (1999). "Late onset veno-occlusive disease following high-dose chemotherapy and stem cell transplantation." Bone Marrow Transplant **24**(8): 891-5.

The original definition of hepatic veno-occlusive disease (VOD), which is still widely accepted, includes onset of the clinical syndrome before day +20 following high-dose chemotherapy (HDC) and stem cell transplantation (SCT). We retrospectively identified four patients following HDC and SCT presenting with late onset VOD occurring at day +24, day +27, day +34 and day +42 post SCT. All patients had moderate VOD, with successful resolution of the VOD before day +100 with optimal supportive therapy. Common risk factors for VOD

shared by all four patients included an older age (median age: 60 years), and use of a busulphan-containing regimen. Mean and maximum bilirubin levels for all patients during the VOD syndrome were 2.02, 1.76, 5.09, 2.87 mg/dl and 2.5, 2.2, 8.9 and 4.1 mg/dl, respectively, which correlated well with duration of VOD. All patients encountered platelet transfusion-dependent thrombocytopenia during VOD. Ursodeoxycholic acid was used as VOD prophylaxis beginning at a mean of 33 days prior to onset of VOD. As the cellular target of hepatic VOD is as yet unidentified, it is uncertain whether ursodiol or other common characteristics of patients with late onset VOD influence the pathogenesis and natural history of this disease. We believe that the uncommon clinical entity of late onset VOD, a potentially fatal regimen-related toxicity, should not be ignored as a diagnosis of liver disease after 3 or more weeks following HDC and SCT.

Tonti, G. A. and F. Mannello (2008). "From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera?" Int J Dev Biol **52**(8): 1023-32.

Bone marrow-derived mesenchymal stem cells are a multipotent adult cellular population endowed with broad differentiation potential. Their regeneration capability, ease to undergo gene modifications, and immuno-suppressive capacity makes them optimal tools for tissue engineering, gene- and immuno-therapy. Due to the ever-increasing number of studies on the clinical applications of mesenchymal stem cells in regenerative medicine, these cells have become attractive targets in clinical transplantation. However, the identification and definition of mesenchymal stem cell culture media for their clinical application in cell therapy is currently a matter of strong discussion. Up to now, clinical studies have been conducted with mesenchymal stem cells cultured in foetal calf serum, and the chance of contamination or immunological reaction towards xenogeneic compounds must be taken into consideration. On the other hand, a serum-free medium without the addition of growth factors is not able to expand these cells in vitro; so the evaluation of which is best, among foetal calf serum, human serum (whether autologous or allogeneic) and platelet-rich plasma, is a hot topic urgently needing further research efforts. The need for the establishment of standardized protocols for mesenchymal stem cell preparations, in order not to interfere with their self-renewal and differentiation processes, assuring durable engraftment and long-term therapeutic effects, is evidently crucial. Therefore, the search for optimal culture conditions for the effective clinical-scale production of vast numbers of mesenchymal stem cells for cellular therapy is of paramount importance and the need for a robust passage from basic to translational research is fundamental.

Tuzuner, N., C. Cox, et al. (1994). "Bone marrow cellularity in myeloid stem cell disorders: impact of age correction." Leuk Res **18**(8): 559-64.

We have reviewed the initial diagnostic bone marrow aspirate and biopsy specimens performed on the same date on 92 patients with acute myeloid leukaemia (AML), 100 patients with myelodysplastic syndrome (MDS), 24 patients with chronic granulocytic leukaemia (CGL), 19 patients with polycythemia vera (PV) and essential thrombocythemia (ET). An excellent assessment of cellularity by aspirate and biopsy was found. The estimation of BM cellularity for each group was utilized with and without age adjustment based on normal marrow biopsies. Without correcting the BM cellularity for age it was observed that the median BM cellularity was > 50% in AML, CGL, PV and ET. In contrast, the median BM cellularity was estimated at 40% for MDS. In the age group 70 years and beyond the median BM cellularity was not changed in CGL, PV and ET, and only slightly decreased (35%) in MDS. However, a trend from hypercellularity to normocellularity was observed in patients with AML in this age group. By utilizing anatomic comparisons with normal age the corrected data disclosed that all patients with CGL, PV and ET, 63% of patients with AML and only 35% of patients with MDS had hypercellular BM according to their age, while only two patients with AML and seven patients with MDS were found to be truly hypocellular by age. The optimal cut-off value for definition of hypocellular AML and hypocellular MDS, and differences between MDS and other myeloid stem cell disorders in terms of BM cellularity have been discussed.

Unwin, R. D., S. J. Gaskell, et al. (2003). "The potential for proteomic definition of stem cell populations." Exp Hematol **31**(12): 1147-59.

Embryonic and adult stem cell populations have great potential value in medicine, and hematopoietic stem cells are already being used in transplantation. Definition of these populations to increase our understanding of the programs that control differentiation, self-renewal, and possibly plasticity would be of great interest. The relative quantitation of transcriptional activity in stem cells and other populations has defined a profile of gene expression activity in stem cells. Confirmation that these differences have an impact on protein levels within stem cells via their complete protein

complement and protein interactions will enable further understanding of regulatory processes in these cells. The recent developments in proteomics and their potential application to the definition of the stem cell proteome are discussed, and examples are given. Advances in mass spectrometry, subcellular prefractionation protocols, and electrophoresis that make stem cell proteomics a tractable problem are discussed. Beyond the proteome per se, advances in post-translational modification profiling mean that comparative analysis of phosphorylation patterns between stem cells and other populations can be approached.

van Dartel, D. A., J. L. Pennings, et al. (2009). "Early gene expression changes during embryonic stem cell differentiation into cardiomyocytes and their modulation by monobutyl phthalate." Reprod Toxicol **27**(2): 93-102.

The Embryonic Stem cell Test (EST) is an in vitro alternative test designed for the prediction of embryotoxicity. The endpoint of the test is the interference with mesoderm-derived cardiac muscle differentiation observed under the microscope as beating muscle foci. The relative subjectivity of this endpoint, as well as the applicability domain and related predictivity need further to be defined to facilitate implementation of the EST into regulatory strategies. The use of transcriptomics techniques to monitor differentiation-related gene expression changes in the EST might improve the EST in each of these aspects. Therefore, we studied the gene expression profile in embryonic stem cells (ESC) in the early phase of differentiation and its modulation by exposure to the well known embryotoxicant monobutyl phthalate (MBP). Cells were exposed from the early embryoid body stage onwards and RNA was collected after 6, 12 and 24h of exposure. Samples were hybridized to spotted microarrays, containing 21,997-mer oligonucleotides. Differential gene expression patterns were analyzed. A total number of 43 genes that were found to be upregulated in this study as a consequence of induction of cardiomyocyte differentiation were combined in a gene set, named 'VAN_DARTEL_HEARTDIFF_24H'. Gene Set Enrichment Analysis (GSEA) comparative analysis using multiple gene set collections clearly showed that temporal changes in gene expression were functionally related to cardiomyocyte differentiation. Furthermore, exposure of embryoid bodies (EB) to MBP increased expression of pluripotency-, proliferation- and nonmesodermal differentiation-related gene sets, which indicates inhibition of mesodermal differentiation. The inhibition of mesoderm-derived cardiomyocyte differentiation by MBP exposure was most obvious through the downregulation of our novel gene set identified in this study, 'VAN_DARTEL_HEARTDIFF_24H', which specifically describes the niche of early cardiomyocyte differentiation. The gene set defined in this study might serve as a starting point for defining a dedicated gene set for early detection of embryotoxicity in the EST. Such a gene set may serve as an improved endpoint in the EST as compared to morphology, and will allow a more detailed definition of the applicability domain and predictivity of EST.

van Os, R., L. M. Kamminga, et al. (2004). "Stem cell assays: something old, something new, something borrowed." Stem Cells **22**(7): 1181-90.

Numerous assays exist that measure the function of stem cells. In this article, we review in detail the history and future of existing stem cell assays. Hematopoietic stem cells (HSCs) are historically the most well studied, but new developments in stem cell research, including the claim of stem cell plasticity, have caused controversies related to technical issues, as well as to semantics. Stem cell research requires proper definitions, and utilization of stem cell assays, especially since research on non-HSCs that lack solid stem cell assays, is rapidly evolving. These emerging fields may benefit from what has been learned from HSC assays: most important, that the true potential of stem cells can only be assessed retrospectively. This also relates to new developments in HSC research, when limiting numbers of in vitro-manipulated stem cells are transplanted. The most conflicting results arise when cells express stem cell characteristics in one assay but not in another. Should we adjust our definition of a stem cell? If so, when do we decide a claim of stem cell activity to be justified? We therefore recommend using multiple stem cell assays, preferably at least one in vivo assay. These assays should measure functionality of the putative stem cell population.

Vescovi, A. L. and E. Y. Snyder (1999). "Establishment and properties of neural stem cell clones: plasticity in vitro and in vivo." Brain Pathol **9**(3): 569-98.

The study of the basic physiology of the neural precursors generated during brain development is driven by two inextricably linked goals. First, such knowledge is instrumental to our understanding of how the high degree of cellular complexity of the mature central nervous system (CNS) is generated, and how to dissect the steps of proliferation, fate commitment, and differentiation that lead early pluripotent neural progenitors to give rise to mature CNS cells. Second, it is hoped that the isolation, propagation, and manipulation of brain precursors

and, particularly, of multipotent neural stem cells (NSCs), will lead to therapeutic applications in neurological disorders. The debate is still open concerning the most appropriate definition of a stem cell and on how it is best identified, characterized, and manipulated. By adopting an operational definition of NSCs, we review some of the basic findings in this area and elaborate on their potential therapeutic applications. Further, we discuss recent evidence from our two groups that describe, based on that rigorous definition, the isolation and propagation of clones of NSCs from the human fetal brain and illustrate how they have begun to show promise for neural cell replacement and molecular support therapy in models of degenerative CNS diseases. The extensive propagation and engraftment potential of human CNS stem cells may, in the not-too-distant-future, be directed towards genuine clinical therapeutic ends, and may open novel and multifaceted strategies for redressing a variety of heretofore untreatable CNS dysfunctions.

Vezzoni, L. and G. Parmiani (2008). "Limitations of the cancer stem cell theory." Cytotechnology **58**(1): 3-9.

Cancer stem cells (CSCs) can be operationally defined as a subset of neoplastic cells which are responsible for the growth and re-growth of primary and metastatic tumors. Although the existence of perpetually dividing cells is a logical necessity to explain the malignant properties of human tumors, experimental data supporting their existence have only recently been obtained. New knowledge in basic stem cell biology and the availability of several cell surface markers for the definition and isolation of small subsets of immature cells coupled to the use of the classical model of xenotransplantation in immune deficient mice has identified putative CSCs in several solid tumors such as mammary, colon, brain, pancreas, prostate, melanoma and others. However, the theory must be considered as still in its infancy, since tumors grown in mice only partially recapitulate the biology of human cells. In addition, whether the "transformed" cell is the neoplastic counterpart of a normal stem cell or whether complete malignant behaviour can occur in a more differentiated cell has still to be demonstrated. In spite of these difficulties, the CSC hypothesis could be of clinical relevance, especially in the definition of new ways to assay drug sensitivity of primary human tumors.

von Tigerstrom, B. J. (2008). "The challenges of regulating stem cell-based products." Trends Biotechnol **26**(12): 653-8.

Appropriate regulation of stem cell-based products is essential to ensure public safety and trust while minimising unnecessary barriers to product development, but presents numerous challenges. Weaknesses of existing legal frameworks include variation between jurisdictions and poor fit between product categories and new technologies. The new European Regulation on advanced therapy medicinal products is an important attempt to provide a consolidated regulatory framework for novel products. Others can learn from issues encountered in its development, including definition of product categories, ethical concerns, and the application of regulations to small-scale production. Several aspects of the Regulation will be useful models, but some larger questions remain unresolved. As reform efforts move forward, harmonisation and sharing of expertise will be vital to effective regulation.

Wagner, W., R. E. Feldmann, Jr., et al. (2006). "The heterogeneity of human mesenchymal stem cell preparations--evidence from simultaneous analysis of proteomes and transcriptomes." Exp Hematol **34**(4): 536-48.

OBJECTIVE: Mesenchymal stem cells (MSC) raise high hopes in clinical applications. However, the lack of common standards and a precise definition of MSC preparations remains a major obstacle in research and application of MSC. Whereas surface antigen markers have failed to precisely define this population, a combination of proteomic data and microarray data provides a new dimension for the definition of MSC preparations. METHODS: In our continuing effort to characterize MSC, we have analyzed the differential transcriptome and proteome expression profiles of MSC preparations isolated from human bone marrow under two different expansion media (BM-MSC-M1 and BM-MSC-M2). RESULTS: In proteomics, 136 protein spots were unambiguously identified by MALDI-TOF-MS and corresponding cDNA spots were selected on our "Human Transcriptome cDNA Microarray." Combination of datasets revealed a correlation in differential gene expression and protein expression of BM-MSC-M1 vs BM-MSC-M2. Genes involved in metabolism were more highly expressed in BM-MSC-M1, whereas genes involved in development, morphogenesis, extracellular matrix, and differentiation were more highly expressed in BM-MSC-M2. Interchanging culture conditions for 8 days revealed that differential expression was retained in several genes whereas it was altered in others. CONCLUSION: Our results have provided evidence that homogeneous BM-MSC preparations can reproducibly be isolated under standardized conditions, whereas culture conditions

exert a prominent impact on transcriptome, proteome, and cellular organization of BM-MSC.

Wilpshaar, J., E. C. Joekes, et al. (2002). "Magnetic resonance imaging of fetal bone marrow for quantitative definition of the human fetal stem cell compartment." Blood **100**(2): 451-7.

Magnetic resonance imaging (MRI) can be used to distinguish bone marrow (BM) from cartilage and may therefore be used to measure BM volume in intact bones. We used MRI to measure the total human fetal BM volume in intact fetuses during the second trimester of pregnancy and determined the contribution of the individual bones to the total compartment. The total BM volume ranged from 934 microL at 17 to 18 weeks to 4563 microL at 22 to 23 weeks of gestation. The largest contributor to the total BM volume was the spine, constituting 26.4% +/- 2.7% of the total volume. By analyzing leukocyte content and percentages of CD34+ cells, lymphocytes, granulocytes, and monocytes of determined volumes, absolute numbers of these cell populations in BM could be measured. The cellular composition of the BM compartment did not significantly change throughout the second trimester of gestation. Absolute white blood cell counts per fetus increased from 111 x 10(6) at 16 to 17 weeks to 1229 x 10(6) at 21 to 22 weeks. The absolute numbers of CD34+ cells increased from 25 x 10(6) at 16 to 17 weeks to 256 x 10(6) at 21 to 22 weeks. Similar analysis of liver and spleen revealed comparable absolute numbers of CD34+ cells in BM and liver throughout the second trimester of gestation. In fetal liver, CD34+ cells differentiate into red cells, myeloid cells, and platelets, while lymphopoiesis mainly occurs in BM or spleen. Combining MRI and cell counts provides a method to quantify specific cell populations in fetal compartments. This study may enable better evaluation of fetal diagnostics and therapies.

Wulffraat, N. M., P. J. Haas, et al. (2003). "Myeloid related protein 8 and 14 secretion reflects phagocyte activation and correlates with disease activity in juvenile idiopathic arthritis treated with autologous stem cell transplantation." Ann Rheum Dis **62**(3): 236-41.

OBJECTIVES: To determine whether myeloid related proteins (MRP8/MRP14), a complex of two S100 proteins related to neutrophil and monocyte activation, might be used as a marker for disease activity, and as an early indicator of relapse in juvenile idiopathic arthritis. PATIENTS AND METHODS: A group of 12 patients who underwent an autologous haematopoietic stem cell transplantation (ASCT) for refractory juvenile idiopathic arthritis (JIA) were studied. MRP8/MRP14 serum concentrations were determined by a sandwich enzyme linked immunosorbent assay (ELISA) as described. Improvement from baseline was described by a definition of improvement employing a core set of criteria as detailed previously by Giannini. RESULTS: After ASCT, MRP8/MRP14 serum concentrations in JIA showed a positive correlation with the Child Health Assessment Questionnaire (CHAQ; r=0.80) and erythrocyte sedimentation rate (r=0.45), but not with the total leucocyte count (r=0.26). Mean MRP8/MRP14 serum concentrations dropped markedly in the first three months after ASCT (p=0.0039) and clinical parameters of disease activity such as CHAQ markedly improved (p=0.0039). During a transient relapse there was an increase in MRP8/MRP14. CONCLUSIONS: MRP8/MRP14 serum concentration can be used as a marker for disease activity in patients who receive an ASCT for refractory JIA. This indicates a role of macrophage activation in the pathogenesis of JIA. The occurrence of MAS in three patients in this study was not preceded by significant changes in MRP8/MRP14 concentration.

Zandstra, P. W. and A. Nagy (2001). "Stem cell bioengineering." Annu Rev Biomed Eng **3**: 275-305.

Tissue engineering and cellular therapies, either on their own or in combination with therapeutic gene delivery, have the potential to significantly impact medicine. Implementation of technologies based on these approaches requires a readily available source of cells for the generation of cells and tissues outside a living body. Because of their unique capacity to regenerate functional tissue for the lifetime of an organism, stem cells are an attractive "raw material" for multiple biotechnological applications. By definition they are self-renewing because on cell division they can generate daughter stem cells. They are also multipotent because they can differentiate into numerous specialized, functional cells. Recent findings have shown that stem cells exist in most, if not all, tissues, and that stem cell tissue specificity may be more flexible than originally thought. Although the potential for producing novel cell-based products from stem cells is large, currently there are no effective technologically relevant methodologies for culturing stem cells outside the body, or for reproducibly stimulating them to differentiate into functional cells. A mechanistic understanding of the parameters important in the control of stem cell self-renewal and lineage commitment is thus necessary to guide the development of bioprocesses for the ex vivo culture of stem cells and their derivates.

Zino, E., G. Frumento, et al. (2004). "A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines

nonpermissive mismatches for hematologic stem cell transplantation." Blood **103**(4): 1417-24.

The importance of HLA-DPB1 matching for the outcome of allogeneic hematologic stem cell (HSC) transplantation is controversial. We have previously identified HLA-DPB1*0901 as a target of cytotoxic T cells mediating in vivo rejection of an HSC allograft. Here we show that HLA-DPB1*0901 encodes a T-cell epitope shared by a subset of DPB1 alleles that determines nonpermissive mismatches for HSC transplantation. Several T-cell clones obtained from the patient at the time of rejection showed HLA-DP restricted recognition of allogeneic targets expressing HLA-DPB1*0901, *1001, *1701, *0301, *1401, and *4501, but not other alleles. Based on these findings, we developed an algorithm for prediction of nonpermissive HLA-DPB1 mismatches. Retrospective evaluation of 118 transplantations showed that the presence of nonpermissive HLA-DPB1 mismatches was correlated with significantly increased hazards of acute grade II to IV graft-versus-host disease (HR = 1.87, P =.046) and transplantation-related mortality (HR = 2.69, P =.027) but not relapse (HR = 0.98, P =.939), as compared with the permissive group. There was also a marked but statistically not significant increase in the hazards of overall mortality (HR = 1.64, P =.1). These data suggest that biologic characterization of in vivo alloreactivity can be a tool for definition of clinically relevant nonpermissive HLA mismatches for unrelated HSC transplantation.

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