Stem Cell Protocol Literatures

Mark H Smith

Queens, New York 11418, USA
mark20082009@gmail.com

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 protocols.

[Smith MH. **Stem Cell Protocol Literatures.** *Stem Cell* 2012;3(4):180-216] (ISSN 1545-4570). http://www.sciencepub.net/stem. 6

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

Literatures

Abe, Y., K. Kouyama, et al. (2003). "Analysis of neurons created from wild-type and Alzheimer's mutation knock-in embryonic stem cells by a highly efficient differentiation protocol." J Neurosci **23**(24): 8513-25.

It is impossible to obtain and amplify live neurons from Alzheimer's disease (AD) patients. To establish the neurons harboring AD abnormality, we constructed mouse embryonic stem (ES) cells, in which the AD-causative V642I mutation was introduced to the endogenous amyloid precursor protein (APP) gene, in combination with a protocol to efficiently differentiate ES cells into postmitotic neurons without using a cell sorter. By this protocol, ES cells differentiated into >90% of the central type of adult postmitotic neurons. Neurons derived from V642I-APP knock-in ES cells were indistinguishable from wild-type ES-derived neurons, as determined by the expression of various markers for neuronal differentiation. Notably, V642I-APP knock-in ES cell-derived neurons exhibited significantly increased secretion of Abeta42 without AD-related hyperphosphorylation of tau, indicating that the direct output of the AD-causative mutation is increased Abeta42 secretion. In this study, we analyze created neurons with wild-type and AD genotypes and propose a new strategy for generating neurons for any dominantly inherited neurodegenerative diseases. The strategy can be applied to create human neurons with AD or any other neurodegenerative disease by using human ES cells.

Aker, M., G. Varadi, et al. (1999). "Fludarabine-based protocol for human umbilical cord blood transplantation in children with Fanconi anemia." J Pediatr Hematol Oncol **21**(3): 237-9.

PURPOSE: A novel conditioning regimen of fludarabine monophosphate (FLM), anti-T-lymphocyte globulin (ATG), and low-dose cyclophosphamide with no irradiation for human umbilical cord blood transplantation (HUCBT) for the treatment of Fanconi anemia (FA) is described. PATIENT AND METHODS: A 12-year-old girl with FA received a human umbilical cord blood transplant from a fully matched sibling donor. After the HUCBT, the patient was given granulocyte colony stimulating factor in combination with erythropoietin. Pretransplant conditioning consisted of FLM (30 mg/m2/d) from day -10 to day -5, cyclophosphamide (10 mg/kg/d) on day -7 and -6, and rabbit ATG (ATG-Frasenius, 10 mg/kg/d) from day -4 to day -1. Cyclosporin A (3 mg/kg/d) was administered from day -1 as graft-versus-host disease prophylaxis. Cord blood from a sibling donor was used as a source of hematopoietic stem cells. RESULTS: Engraftment was normal and sustained. The regimen was well tolerated with very mild toxicity and no major transplant-related complications or >grade II graft-versus-host disease. Chimerism was 100% donor origin as determined by restriction fragment length polymorphism. CONCLUSIONS: It is possible to achieve sustained engraftment and only mild toxicity in FA after HUCBT with a conditioning regimen of FLM, ATG, and cyclophosphamide with no irradiation. These preliminary results with this novel conditioning protocol are encouraging and should be evaluated in a larger group of patients with FA undergoing HUCBT.

Alvarez, I., A. Sureda, et al. (2006). "Nonmyeloablative stem cell transplantation is an effective therapy for refractory or relapsed hodgkin lymphoma: results of a spanish prospective

cooperative protocol." Biol Blood Marrow Transplant **12**(2): 172-83.

We report the results of reduced-intensity conditioning allogeneic stem cell transplantation (allo-RIC) in patients with advanced Hodgkin lymphoma (HL). Forty patients with relapsed or refractory HL were homogeneously treated with an RIC protocol (fludarabine 150 mg/m(2) intravenously plus melphalan 140 mg/m(2) intravenously) and cyclosporin A and methotrexate as graft-versus-host disease (GVHD) prophylaxis. Twenty-one patients (53%) had received >2 lines of chemotherapy, 23 patients (58%) had received radiotherapy, and 29 patients (73%) had experienced treatment failure with a previous autologous stem cell transplantation. Twenty patients (50%) were allografted in resistant relapse, and 38 patients received hematopoietic cells from an HLA-identical sibling. Five patients (12%) died from early transplant-related mortality (before day +100 after allo-RIC). One-year transplant-related mortality was 25%. Acute GVHD developed in 18 patients (45%). Chronic GVHD developed in 17 (45%) of the 31 evaluable patients. The response rate 3 months after the allo-RIC was 67% (21 [52%] complete remissions and 6 [15%] partial remissions). Eleven patients received donor lymphocyte infusions (DLIs) for disease relapse. The response rate after DLI was 54% (3 complete remissions and 3 partial remissions). Overall survival (OS) and progression-free survival (PFS) were 48% +/- 10% and 32% +/- 10% at 2 years, respectively. Refractoriness to chemotherapy was the only adverse prognostic factor for both OS (63% +/- 12% versus 35% +/- 13%; P = .05) and PFS (55% +/- 16% versus 10% +/- 9%; P = .006). For patients with failure of a prior autologous hematopoietic stem cell transplantation, results were especially good for those who experienced late relapses (>/=12 months: 2-year OS and PFS were 75% +/- 16% and 70% +/- 18%, respectively). These data suggest that allo-RIC is feasible in heavily pretreated HL patients and has an acceptable early transplant-related mortality. Results are better in patients allografted in sensitive disease. Both responses observed after the development of GVHD and DLI may suggest a graft-versus-HL effect. Allo-RIC has to be considered an effective therapeutic approach for patients who have had treatment failure with a previous autologous hematopoietic stem cell transplantation.

Antonucci, I., I. Iezzi, et al. (2009). "Isolation of osteogenic progenitors from human amniotic fluid using a single step culture protocol." BMC Biotechnol **9**: 9.

BACKGROUND: Stem cells isolated from amniotic fluid are known to be able to differentiate into different cells types, being thus considered as a potential tool for cellular therapy of different human diseases. In the present study, we report a novel single step protocol for the osteoblastic differentiation of human amniotic fluid cells. RESULTS: The described protocol is able to provide osteoblastic cells producing nodules of calcium mineralization within 18 days from withdrawal of amniotic fluid samples. These cells display a complete expression of osteogenic markers (COL1, ONC, OPN, OCN, OPG, BSP, Runx2) within 30 days from withdrawal. In order to test the ability of these cells to proliferate on surfaces commonly used in oral osteointegrated implantology, we carried out cultures onto different test disks, namely smooth copper, machined titanium and Sandblasted and Acid Etching titanium (SLA titanium). Electron microscopy analysis evidenced the best cell growth on this latter surface. CONCLUSION: The described protocol provides an efficient and time-saving tool for the production of osteogenic cells from amniotic fluid that in the future could be used in oral osteointegrated implantology.

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

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

relatively low enrichment in very early uncommitted CD34+/CD38- cells. Conversely, in the absence of IL-3, they are less effective in inducing proliferation yet significantly increase the number of CD34+/CD38- cells. A combination of the above protocols, applied simultaneously to aliquots of the same sample, would allow expansion of both committed and pluripotent HSC. This strategy may represent a significant improvement for clinical applications.

Ayash, L. J., M. Clarke, et al. (2001). "Clinical protocol. Purging of autologous stem cell sources with bcl-x(s) adenovirus for women undergoing high-dose chemotherapy for stage IV breast carcinoma." Hum Gene Ther **12**(16): 2023-5.

High-dose chemotherapy (HDCT) and autologous bone marrow transplantation (BMT) is frequently used to treat patients with metastatic cancer including breast cancer and neuroblastoma. However, the bone marrow of such patients is often contaminated with tumor cells. Recently, we have found that a recombinant adenovirus vector that contains a bcl-x, minigene (a dominant negative inhibitor of the bcl-2 family), called the bcl-x(s) adenovirus, is lethal to cancer cells derived from epithelial tissues, but not to normal human hematopoietic cells. To determine the mechanism, by which this virus spares normal hematopoietic cells, we isolated normal mouse hematopoietic stem cells and infected them with an adenovirus that contains a beta-galactosidase minigene. Such cells do not express beta-galactosidase, indicating that hematopoietic stem cells do not express transgene encoded by adenovirus vectors based upon the RSV-AD5 vector system. When breast cancer cells mixed with hematopoietic cells were infected with the bcl-x(s) adenovirus, cancer cells were selectively killed by the suicide adenoviruses. Hematopoietic cells exposed to the suicide vectors were able to reconstitute the bone marrow of mice exposed to lethal doses of y-irradiation. These studies suggest that adenovirus suicide vectors may provide a simple and effective method to selectively eliminate cancer cells derived from epithelial tissue that contaminate bone marrow to be used for autologous BMT. We therefore propose to initiate a phase I clinical trial to test the safety of this virus in women with breast cancer undergoing high does chemotherapy and autologous BMT.

Balint, B., Z. Ivanovic, et al. (1999). "The cryopreservation protocol optimal for progenitor recovery is not optimal for preservation of marrow repopulating ability." Bone Marrow Transplant **23**(6): 613-9.

The efficiency of five different cryopreservation protocols (our original controlled-rate and noncontrolled-rate protocols) was evaluated on the basis of the recovery after thawing of very primitive pluripotent hemopoietic stem cells (MRA(CFU-GM), pluripotent progenitors (CFU-Sd12) and committed granulocyte-monocyte progenitors (CFU-GM) in mouse bone marrow. Although the nucleated cell recovery and viability determined immediately after the thawing and washing of the cells were found to be similar, whether controlled-rate or noncontrolled-rate cryopreservation protocols were used, the recovery of MRA(CFU-GM), CFU-Sd12 and CFU-GM varied depending on the type of protocol and the cryoprotector (DMSO) concentrations used. It was shown that the controlled-rate protocol was more efficient, enabling better MRA(CFU-GM), CFU-Sd12 and CFU-GM recovery from frozen samples. The most efficient was the controlled-rate protocol of cryopreservation designed to compensate for the release of fusion heat, which enabled a better survival of CFU-Sd12 and CFU-GM when combined with a lower (5%) DMSO concentration. On the contrary, a satisfactory survival rate of very primitive stem cells (MRA(CFU-GM)) was achieved only when 10% DMSO was included with a five-step protocol of cryopreservation. These results point to adequately used controlled-rate freezing as essential for a highly efficient cryopreservation of some of the categories of hematopoietic stem and progenitor cells. At the same time, it was obvious that a higher DMSO concentration was necessary for the cryopreservation of very primitive stem cells, but not, however, for more mature progenitor cells (CFU-S, CFU-GM). These results imply the existence of a mechanism that decreases the intracellular concentration of DMSO in primitive MRA cells, which is not the case for less primitive progenitors.

Balint, B., M. Ljubenov, et al. (2008). "Stem cell harvesting protocol research in autologous transplantation setting: large volume vs. conventional cytapheresis." Vojnosanit Pregl **65**(7): 545-51.

BACKGROUND/AIM: The use of peripheral blood as a source of hematopoietic stem cells (SCs) is progressively increasing and has nearly supplanted bone marrow transplantation. Interpatient variability in the degree and kinetics of SC mobilization into peripheral blood is an expected event after conventional chemotherapy-based treatment, followed by sequential administration of recombinant granulocyte-colony-stimulating factor (rHu-CSF). In this study, specific factors associated with the application of two different SC-harvesting approaches, including the use of large volume leukapheresis (LVL) vs. repetitive conventional apheresis (RCA), were analyzed. The basic goal of the study was to

evaluate the influence of apheresis protocol (collection timing, processed blood volume and cell yield) upon the clinical outcome of transplantation. METHODS: Results obtained by LVL (76 pts) and RCA (20 pts--control group) were compared. The SC mobilizing regimen used was cyclophosphamide (4-7 g/m2) or polychemotherapy and rHuG-CSF 10-16 microg/kg of body mess (bm) per day. Cell harvesting was performed using Caridian-BCT Spectra (Gambro, USA). The volume of processed blood in LVL setting was > or = 3.5-fold of the patient's circulating blood quantity (ranged from 12.7 to 37.8 l). All patients tolerated well the use of intensive treatment, without any side or adverse effects. Our original controlled-rate cryopreservation was carried out with 10% dimethyl sulfoxide (DMSO) using Planer R203/200R or Planer 560-16 equipments (Planer Products Ltd, UK). Total nucleated cell (NC) and mononuclear cell (MNC) counts were examined by flow cytometry (Advia-2120 Bayer, Germany; Technicon H-3 System, USA). The CD34+ cell surface antigen was investigated by the EPICS XL-MCL device (Coulter, Germany). RESULTS: Performing LVL-apheresis, high-level MNC and CD34+ cell yields (7.6 +/- 4.6 x 10(8)/kg bm and 11.8 +/- 6.5 x 10(6)/kg bm, respectively) were obtained. As a result, rapid hematopoietic reconstitution ("graft-healing")--on the 9.4th and 12.4th day for granulocytes and platelets, respectively was achieved. Using repetitive conventional apheresis (2-3 procedures), the total MNC count was high (8.2 +/- 7.0 x 10(8)/kg bm), but the total CD34+ yield was lower 10.8 +/- 9.9 due to inferior CD34+ vs. MNC ratio. CONCLUSION: The results obtained suggest that well-timed LVL-apheresis increased SC-yield in cell harvest, resulting in faster bone marrow repopulation and hematological reconstitution, as well as better overall clinical outcome of transplantation. These results necessitate additional examinations of CD34+ subsets ratio in cell harvest.

Brice, P., D. Simon, et al. (2000). "High-dose therapy with autologous stem-cell transplantation (ASCT) after first progression prolonged survival of follicular lymphoma patients included in the prospective GELF 86 protocol." Ann Oncol **11**(12): 1585-90.

BACKGROUND: Among the 566 patients with follicular lymphomas (FL) included in the GELF 86 prospective trials from October 1986 to September 1995, 372 with progressive/relapsing disease were analyzed retrospectively to identify prognostic factors at first relapse. PATIENTS AND METHODS: For progressive FL, patients received mono- (22%) or polychemotherapy (78%) followed by high-dose therapy (HDT) with ASCT for 83 patients (22%). The median time to progression from initial treatment was 23 months (range 3-102 months) and 24% of documented patients (52 of 217) had histological transformation (HT). Salvage therapy produced an overall response in 64% of patients and the five-year survival from progression was 42%. RESULTS: For patients who underwent HDT with ASCT compared to standard treatment, five-year freedom from second failure was at 42% vs. 16% (P = 0.0001) and five-year survival was 58% vs. 38% (P = 0.0005), respectively. The benefit of HDT and ASCT remained if we consider only patients less than 65 years (five-year survival at 60% vs. 40%; P = 0.001). Multivariate analysis of parameters significant according to univariate analysis found that no ASCT at first progression, age at relapse > 50 years, progression on-therapy were adversely significant on survival. CONCLUSIONS: HDTwith ASCT compared to standard treatment prolonged remission and survival after first progression of FL patients.

Budak-Alpdogan, T., M. Przybylowski, et al. (2006). "Functional assessment of the engraftment potential of gammaretrovirus-modified CD34+ cells, using a short serum-free transduction protocol." Hum Gene Ther **17**(7): 780-94.

The successful transduction and engraftment of human mobilized peripheral blood (MBP) CD34(+) cells are determined to a large extent by the ex vivo cell-processing conditions. In preparation for upcoming clinical trials, we investigated essential culture parameters and devised a short and efficient gammaretroviral transduction protocol entailing minimal manipulation of MBP CD34(+) cells. The engraftment potential and in vivo transgene expression in the progeny of repopulating CD34(+) cells were measured to assess the functionality of CD34(+) cells transduced under these conditions. Using a competitive in vivo repopulation assay in nonobese diabetic/severe combined immunodeficient mice, we demonstrate equivalent engraftment of CD34(+) cells transduced under serum-free conditions as compared with CD34(+) cells cultured with serum. We also took advantage of this in vivo model to demonstrate that ex vivo manipulation of CD34(+) cells can be shortened to 60 hr, using 36 hr of prestimulation and two cycles of transduction 12 hr apart. These minimally manipulated CD34(+) cells engraft in a manner similar to cells transduced under longer protocols and the vector-encoded transgene is expressed at the same frequency in cells derived from repopulating CD34(+) cells in vivo. We have thus developed a short and efficient human MBP CD34(+) transduction protocol under serum-free conditions that is suitable and broadly applicable for phase I clinical trials.

Cavaleri, F., L. Gentile, et al. (2006). "Recombinant human albumin supports development of somatic cell nuclear transfer embryos in mice: toward the establishment of a chemically defined cloning protocol." Cloning Stem Cells **8**(1): 24-40.

Culturing embryos in different media is a useful approach to characterize their nature in regard to "memory" of the donor nucleus and its "reprogramming" after somatic cell nuclear transfer (SCNT). However, efforts to elucidate the mechanisms of reprogramming are seriously undermined when embryo culture conditions are not completely defined. Using recombinant human albumin (rHA) is a step toward establishing defined culture conditions for mouse cloning. Recombinant HA supports blastocyst formation of cumulus cell-derived clones at a rate comparable with two types of bovine serum albumin (BSA); following transfer of blastocysts to the genital tract, rates of development to midgestation (10.5 dpc) were indistinguishable. rHA also supports the derivation of germline competent embryonic stem (ES) cells from SCNT blastocysts at a substantial rate compared with BSA counterparts and with zygotic blastocysts. Unlike the developmental parameters, the gene expression patterns of clones cultured in rHA or BSA were not superimposed; identical patterns were observed for zygotic blastocysts in the two albumins. In summary, the present study demonstrates that (1) rHA can replace BSA, proving a defined protein source for SCNT in mice; (2) although using rHA is similar to BSA, it is not equal (rHA leaves a mark on gene expression of clones but not zygotes). Future studies that investigate reprogramming after SCNT will need to consider not only the implications of culture media for cloning but also the supplement choice.

Chapple, P., H. M. Prince, et al. (2000). "Comparison of three methods of CD34+ cell enumeration in peripheral blood: dual-platform ISHAGE protocol versus single-platform, versus microvolume fluorimetry." Cytotherapy **2**(5): 371-6.

BACKGROUND: Quantitation of peripheral blood (PB) CD34(+) cells is now an established method for timing PBPC harvesting. Recent refinements to the dual-platform ISHAGE gating strategy for CD34(+) cells has seen the introduction of microbeads to enable absolute counting of cells on a single instrument platform. This eliminates the need for total WBCC performed on an automated hematology analyzer and potentially increases the analytical precision of the methodology. At the same time, alternative methods for CD34(+) cell enumeration have started to emerge, notably microvolume fluorimetry, which forms the basis of the fully-automated STELLer CD34 method using the Imagn 2000. METHODS: We performed a three-way evaluation of these methods. Sixty-eight samples of PB from 42 patients undergoing PBPC mobilization were analyzed by all three methods and correlations between all three calculated. The two-platform ISHAGE method was used as the reference method. RESULTS: Precision and linearity of the single-platform and STELLer CD34 assays were excellent. Correlation with the dual-platform reference method was also excellent (single-platform method slope = 1.03, intercept = -0.03 and R(2) = 0.9325, STELLer CD34 assay slope = 0.827, intercept = 4.27, R(2) =0.8215). Bias, determined by Bland-Altman analysis, was 1.16 and -1.62 for single platform and STELLer CD34 assay respectively. CONCLUSION: The three methods of CD34(+) cell enumeration gave equivalent results. The single-platform methodology negated the need for a separate white cell analyzer, while the STELLer CD34 methodology was technically the simplest.

Coleman, B., N. A. Rickard, et al. (2009). "A protocol for cryoembedding the adult guinea pig cochlea for fluorescence immunohistology." J Neurosci Methods **176**(2): 144-51.

Green fluorescent protein (GFP) has been used extensively to label cells in vitro and to track them following their transplantation in vivo. During our studies using the mouse embryonic stem cell line R1 B5-EGFP, we observed variable levels of fluorescence intensity of the GFP within these transfected cells. The variable fluorescence of this protein coupled with the innately autofluorescent nature of several structures within the cochlea collectively made the in vivo identification of these transplanted stem cells difficult. We have modified previously published protocols to enable the discrimination of an authentic GFP signal from autofluorescence in the adult guinea pig cochlea using fluorescence-based immunohistochemistry. The protocol described can also be used to label tissues of the cochlea using a chromogen, such as 3,3'-diaminobenzidine tetrahydrochloride (DAB). Moreover, the described method gives excellent preservation of structural morphology making the tissues useful for both morphological and quantitative studies in combination with robust immunohistochemistry in the adult guinea pig cochlea.

Corso, A., M. G. Hogeweg-Platenburg, et al. (1993). "A protocol for the enrichment of different types of CFU-S from fetal mouse liver." Haematologica **78**(1): 5-11.

A method is described for purifying hemopoietic stem cells from fetal mouse liver. It

entails three steps. First, fetal liver cell obtained from 14- and 15- days-old fetuses are centrifuged on a discontinuous metrizamide gradient and the low density fraction is removed. These cells are then incubated with monoclonal antibodies directed against late differentiation antigens, and the positive cells are removed by immunomagnetic beads. The negative cells are labelled with fluorescein-conjugated pokeweed mitogen (FITC-PWM) and sorted by a fluorescence- activated cell sorter (FACS) on the basis of differences in fluorescence intensity. The number of stem cells is determined by spleen colony assay (CFU-S) for the various sorted fractions. We observed that, as shown for the bone marrow, the different cell fractions are responsible for CFU-S heterogeneity in the kinetics of spleen colony formation. The PWM dull day-12 CFU-S, which originate from cells with a high enrichment factor, would probably be a closer measure of pluripotent hemopoietic stem cells (PHSC), while the PWM bright day-8 and day-12 CFU-S likely originate from committed stem cells. Furthermore, the PWM dull sorted cells had a better capacity for protecting lethally irradiated mice than did the bright cells, although the latter yielded good numbers of day-8 and day-12 CFU-S that displayed, as in the bone marrow, a discrepancy between the enrichment for day-12 CFU-S and radioprotection.

Dal Negro, G., L. Vandin, et al. (2006). "A new experimental protocol as an alternative to the colony-forming unit-granulocyte/macrophage (CFU-GM) clonogenic assay to assess the haematotoxic potential of new drugs." Toxicol In Vitro **20**(5): 750-6.

In this work, a first attempt to set-up a new in vitro experimental protocol with culture in liquid medium and flow cytometry analysis of bone marrow progenitors is described. This protocol is proposed as an alternative to the colony-forming unit-granulocyte/macrophage (CFU-GM) clonogenic in vitro assay currently used to assess the toxic potential of new drugs in the bone marrow. This new experimental approach should enable to speed up the procedure of the in vitro haematotoxic potential assessment, to reduce inter-experimental variability and to enhance result accuracy. Preliminary results obtained demonstrated that the progenitor cell count by flow cytometry replacing the light microscopy granulocyte/macrophage colony count represents a tremendous improvement in terms of accuracy and standardisation. Moreover, differential counts of cell sub-populations can be performed by using specific monoclonal antibodies. Furthermore, this method demonstrated to be time-saving, since 4 day cell incubation period is required instead of 7-14 day incubation in the CFU-GM clonogenic assay. On the basis of results obtained so far, the new experimental protocol proposed looks a promising alternative to the CFU-GM clonogenic assay currently used.

Dalbagni, G., W. Han, et al. (1997). "Evaluation of the telomeric repeat amplification protocol (TRAP) assay for telomerase as a diagnostic modality in recurrent bladder cancer." Clin Cancer Res **3**(9): 1593-8.

There is a need for the identification of an accurate test for the detection of recurrent bladder cancer. In this study, we evaluate the telomeric repeat amplification protocol (TRAP) assay for detection of telomerase as a potential new method for bladder cancer detection and compare it to voided urine cytology. A urine sample and a bladder wash were obtained from 63 patients with a history of bladder cancer. Cytological evaluation was performed on voided urine, and the TRAP assay was performed on voided urine and bladder wash. The overall clinical sensitivity of the TRAP assay, as defined by the ability to identify correctly the patients with pathologically confirmed bladder cancer, was 35% in voided urine and 50% in bladder wash, whereas the overall clinical sensitivity of voided urine cytology was 71%. The sensitivity of voided urine cytology for the papillary and noninvasive tumors (Ta) was 50%, compared to 92% for the superficially invasive tumors (T1), 62% for the muscle-invasive tumors (T2+), and 100% for the high-grade flat carcinoma in situ (Tis). The clinical sensitivity of the TRAP assay using voided urine was 46% for Ta, 50% for T1, 18% for T2+, and 20% for Tis. The sensitivity of the TRAP assay in Ta disease was similar to that of cytology (50% for cytology versus 46% for the TRAP assay). There was a strong association between the total number of exfoliated malignant cells and the sensitivity of the assay. The sensitivity of the TRAP assay in bladder washes was 44% for Ta, 67% for T1, 46% for T2+, and 43% for Tis. The TRAP assay is reproducible, highly specific, and not dependent on the expertise of the cytopathologist. These results suggest that this assay should be further investigated as a diagnostic tool for bladder cancer.

Davis, R. P., M. Costa, et al. (2008). "A protocol for removal of antibiotic resistance cassettes from human embryonic stem cells genetically modified by homologous recombination or transgenesis." Nat Protoc **3**(10): 1550-8.

The first step in the generation of genetically tagged human embryonic stem cell (HESC) reporter lines is the isolation of cells that contain a stably integrated copy of the reporter vector. These cells are identified by their continued growth in the presence of a specific selective agent, usually conferred by a cassette encoding antibiotic resistance. In order to mitigate potential interference between the regulatory

elements driving expression of the antibiotic resistance gene and those controlling the reporter gene, it is advisable to remove the positive selection cassette once the desired clones have been identified. This report describes a protocol for the removal of loxP-flanked selection cassettes from genetically modified HESCs by transient transfection with a vector expressing Cre recombinase. An integrated procedure for the clonal isolation of these genetically modified lines using single-cell deposition flow cytometry is also detailed. When performed sequentially, these protocols take approximately 1 month.

de la Fuente, J., S. Reiss, et al. (2003). "Non-TBI stem cell transplantation protocol for Fanconi anaemia using HLA-compatible sibling and unrelated donors." Bone Marrow Transplant **32**(7): 653-6.

Allogeneic haemopoietic stem cell transplantation (SCT) is the only curative option for severe bone marrow (BM) failure in patients with Fanconi anaemia (FA). We have developed a non total body irradiation (TBI) conditioning protocol consisting of fludarabine (120-150 mg/m(2)), low dose of cyclophosphamide (40 mg/kg) and antilymphocyte globulin (45 mg/kg). Graft-versus-host disease (GVHD) prophylaxis was with cyclosporin alone for sibling allografts but also included Campath-1 H (days 1-5 post SCT) for the unrelated allografts. We have performed two sibling and two unrelated BM transplants with a follow-up of 11-51 months. All patients experienced minimal toxicity and were discharged from hospital 28-32 days post SCT. Neutrophil and platelet engraftment occurred from days 11 to 19 and 15 to 34, respectively. All patients achieved stable full donor haemopoiesis with normalisation of the peripheral blood count despite one of them having myelodysplasia (MDS) with 8% blasts prior to the SCT. The only site of acute GVHD was in the skin (grade I-II) and only one patient progressed to limited chronic GVHD. This protocol is associated with reduced toxicity and prompt engraftment in FA patients with AA and/or MDS undergoing SCT using sibling or unrelated donors.

Deola, S., S. Scaramuzza, et al. (2004). "Mobilized blood CD34+ cells transduced and selected with a clinically applicable protocol reconstitute lymphopoiesis in SCID-Hu mice." Hum Gene Ther **15**(3): 305-11.

We developed a clinically applicable gene transfer procedure into mobilized peripheral blood (MPB) CD34(+) hematopoietic progenitor cells, based on single viral exposure and selection of engineered cells. CD34(+) cells were transduced with a retroviral vector carrying the truncated form of the nerve growth factor receptor (Delta NGFR) marker gene, and immunoselected for Delta NGFR expression. Optimal time and procedure for viral exposure, length of culture, and transgene expression of MPB CD34(+) cells were determined using in vitro assays. The multipotent capacity of MPB CD34(+)-transduced cells was demonstrated in the SCID-hu bone/liver/thymus mouse model. Transduced Delta NGFR(+) cells retained 50% of long-term culture-colony forming cells (LTC-CFC) compared to unmanipulated CD34(+) cells. In SCID-hu mice, 52% of CD45(+) cells, 27% of CD34(+) cells, 49% of B cells, and more than 50% of T cells were derived from transplanted CD34(+)/Delta NGFR(+) cells. Furthermore, transplantation of purified transduced cells greatly reduced the competition with untransduced progenitors occurring in unselected grafts. These data demonstrate that MPB CD34(+) cells, transduced with a single viral exposure and selected by transgene expression, retain multilineage reconstitution capacity and remarkable transgene expression.

Duda, D. G., K. S. Cohen, et al. (2007). "A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood." Nat Protoc **2**(4): 805-10.

Blood circulating endothelial cells (CECs) and circulating hematopoietic progenitor cells (CPCs) represent two cell populations that are thought to play important roles in tissue vascularization. CECs and CPCs are currently studied as surrogate markers in patients for more than a dozen pathologies, including heart disease and cancer. However, data interpretation has often been difficult because of multiple definitions, methods and protocols used to evaluate and count these cells by different laboratories. Here, we propose a cytometry protocol for phenotypic identification and enumeration of CECs and CPCs in human blood using four surface markers: CD31, CD34, CD133 and CD45. This method allows further phenotypic analyses to explore the biology of these cells. In addition, it offers a platform for longitudinal studies of these cells in patients with different pathologies. The protocol is relatively simple, inexpensive and can be adapted for multiple flow cytometer types or software. The procedure should take 2-2.5 h, and is expected to detect 0.1-6.0% viable CECs and 0.01-0.20% CPCs within blood mononuclear cell population.

Eduardo, F. P., L. Bezinelli, et al. (2009). "Severity of oral mucositis in patients undergoing hematopoietic cell transplantation and an oral laser phototherapy

protocol: a survey of 30 patients." Photomed Laser Surg **27**(1): 137-44.

BACKGROUND DATA AND OBJECTIVE: Oral mucositis (OM) is one of the worst cytotoxic effects of chemotherapy and radiotherapy in patients undergoing hematopoietic cell transplantation (HCT), and it causes severe morbidity. Laser phototherapy has been considered as an alternative therapy for prevention and treatment of OM. The aim of this study was to describe the incidence and severity of OM in HCT patients subjected to laser phototherapy, and to discuss its effect on the oral mucosa. PATIENTS AND METHODS: Information concerning patient age and gender, type of basic disease, conditioning regimen, type of transplant, absence or presence of pain related to the oral cavity, OM grade, and adverse reactions or unusual events were collected from 30 patients undergoing HCT (allogeneic or autologous). These patients were given oral laser phototherapy with a InGaAIP laser (660 nm and 40 mW) daily. The data were tabulated and their frequency expressed as percentages. RESULTS: In the analysis of those with OM, it was observed that 33.4% exhibited grade I, 40% grade II, 23.3% grade III, and 3.3% grade IV disease. On the most critical post-HCT days (D+5 and D+8), it was observed that 63.3% of patients had grade I and 33.3% had grade II disease; no patients had grade III or IV disease in this period. This severity of OM was similar to that seen in other studies of laser phototherapy and OM. CONCLUSION: The low grades of OM observed in this survey show the beneficial effects of laser phototherapy, but randomized clinical trials are necessary to confirm these findings.

Federico, M., V. Clo, et al. (1996). "High-dose therapy autologous stem cell transplantation vs conventional therapy for patients with advanced Hodgkin's disease responding to first-line therapy: analysis of clinical characteristics of 51 patients enrolled in the HD01 protocol. EBMT/ANZLG/Intergroup HD01 Trial." Leukemia **10 Suppl 2**: s69-71.

Whether high-dose therapy (HDT) plus autologous stem cell transplantation (ASCT) ought to be included in the initial treatment plan for those patients with unfavourable Hodgkin's disease, a wide cooperative study (HD01 protocol) was approved, comparing HDT followed by ASCT vs conventional chemotherapy (CT). Patients were eligible for the study if they had at least two of the following adverse prognostic factors: high serum LDH levels, mediastinal mass >0.45, more than one extranodal involved site, low hematocrit (<34% for women and <38% for men), and inguinal involvement. Those patients achieving complete or partial remission with four courses of ABVD or ABVD-containing chemotherapy were randomized to receive either HDT plus ASCT or four additional courses of chemotherapy, followed by ASCT in second remission, if appropriate. Between April 1993 and September 1995, 55 patients from 14 different centers have been enrolled into the trial. Twenty patients (45%) were in stage IV, and 37 patients (84%) had systemic symptoms. Twenty-seven patients (61%) had two adverse prognostic factors, and 17 patients (39%) had three or more risk factors. After four cycles of ABVD-containing CT, 44 patients were assessable for response. Overall 12 patients achieved CR (27%), 25 obtained a PR (57%) and seven patients failed to respond (16%). Thirty-six patients were randomized between ASCT (20 patients) or four additional cycles of conventional CT (16 patients). With a median follow-up after ASCT of 13 months (range 1-23 months), no major ASCT-related toxicity has been reported to the trial office. In conclusion, the first 44 patients registered in the HD01 trial and assessable for response, had a very aggressive disease and responded poorly to conventional CT, thus warranting a more aggressive approach, such as HDT followed by ASCT.

Festag, M., C. Sehner, et al. (2007). "An in vitro embryotoxicity assay based on the disturbance of the differentiation of murine embryonic stem cells into endothelial cells. I: Establishment of the differentiation protocol." Toxicol In Vitro **21**(8): 1619-30.

The aim of the present study was to establish an experimental protocol to differentiate murine embryonic stem (ES) cells into endothelial cells in vitro. The spinner flask technique as well as the hanging drop method were used to generate so-called embryoid bodies (EBs). In order to find out the optimal differentiation environment, EBs were cultured under various experimental conditions for up to 14 days. The influence of basic fibroblast growth factor (bFGF) alone, vascular endothelial growth factor (VEGF) alone, bFGF and VEGF together and a cocktail consisting of bFGF, VEGF, interleukin-6 (IL-6) and erythropoietin (Epo) on the induction of differentiation of ES cells into endothelial cells was studied. Different concentrations of growth factors and times of treatment were applied. Endothelial cells were characterized by analyzing the expression of platelet-endothelial cell adhesion molecule (PECAM-1), the endothelial-specific vascular endothelial cadherin (VE-Cadherin), the angiopoietin receptor Tie-2, VEGF receptors 1 and 2 (Flt-1 and Flk-1, respectively) and the soluble form of Flt-1 (sFlt) at the mRNA level. PECAM-1 and VE-Cadherin were also studied at the protein level. The data clearly showed

that EBs generated by the hanging drop method, followed by their transfer into suspension culture on day 3 of differentiation and their subsequent plating on day 5 is the best of the studied methods to differentiate ES cells into endothelial cells. Addition of VEGF alone or a cocktail consisting of VEGF, bFGF, IL-6 and Epo resulted in the strongest gene expression levels of the above mentioned endothelial cell markers in the differentiated ES cells.

Fico, A., G. Manganelli, et al. (2008). "High-throughput screening-compatible single-step protocol to differentiate embryonic stem cells in neurons." Stem Cells Dev **17**(3): 573-84.

Biotechnologies such as high-throughput screening (HTS) enable evaluation of large compound libraries for their biological activity and toxic properties. In the field of drug development, embryonic stem (ES) cells have been instrumental in HTS for testing the effect of new compounds. We report an innovative method in one step to differentiate ES cells in neurons and glial cells. The four different neuronal subtypes, gamma-aminobutyric acid (GABA)-ergic, dopaminergic, serotonergic, and motor neurons, are formed in culture. This protocol is adaptable to small wells and is highly reproducible, as indicated by the Z-factor value. Moreover, by using either leukemia inhibitory factor (LIF) or recombinant Cripto protein in our culture conditions, we provide evidence that this protocol is suitable for testing the effect of different molecules on neuronal differentiation of ES cells. Finally, thanks to the simplicity in carrying out the experiment, this method provides the possibility of following the morphological evolution of the in vitro differentiating neuronal cells by timelapse videomicroscopy. Our experimental system provides a powerful tool for testing the effect of different substances on survival and/or differentiation of neuronal and glial cells in an HTS-based approach. Furthermore, using genetically modified ES cells, it would be possible to screen for drugs that have a therapeutic effect on specific neuronal pathologies or to follow, by time-lapse videomicroscopy, their ability to in vitro differentiate.

Fontelonga, A., A. J. Kelly, et al. (1997). "A novel high-dose chemotherapy protocol with autologous hematopoietic rescue in patients with metastatic breast cancer or recurrent non-Hodgkin's lymphoma." Bone Marrow Transplant **19**(10): 983-8.

In this phase II trial, we used a double dose-intensive chemotherapy and stem cell rescue protocol to treat breast cancer (BCA) patients or non-Hodgkin's lymphoma patients (NHL). The first cycle consisted of high-dose melphalan followed by ABMT. The second cycle used a novel chemotherapy combination; thiotepa, etoposide, carboplatin and cyclophosphamide (TECC) followed by ABMT. We treated 12 patients in total, nine with BCA, three with NHL. All nine BCA patients were treated with the two cycle protocol. The three NHL patients were treated with the second cycle only. Bone marrow (BM, 1 patient), peripheral blood stem cells (PBSC, 10 patients) or both (1 patient) were reinfused 60-72 h after completion of each cycle of chemotherapy. Recovery was rapid; the ANC rose to greater than 500/microl on day +11 (+8 to + 20) and the platelet count to greater than 20000/microl on day +12 (+6 to +20). The toxicities included the expected neutropenic fevers, severe mucositis, diarrhea, and a low incidence of mild renal insufficiency. No patients developed veno-occlusive disease, hemorrhagic cystitis or overt bleeding. With a mean follow-up of 37 months, 83.3% of the patients are alive. Six patients are in complete remission; one patient with BCA relapsed and expired; one patient with NHL is in CR now over 18 months after relapse and subsequent treatment with interferon; one patient is too early to evaluate. Progression-free survival overall is 75%, which is at least equivalent to many other recent studies using similar regimens. In addition, we have also found that delayed addition of G-CSF during the mobilization of PBSC was feasible and resulted in excellent CD34+ cell counts and engraftments, and reduced treatment costs. These results indicate that this chemotherapy is effective with good remission rates and high progression-free survival rates. It is also well tolerated with acceptable toxicities that are manageable. Long-term follow-up of a larger cohort of patients will be needed to ascertain the overall efficacy of this type of therapy.

Freie, B. W., P. Dutt, et al. (1996). "Correction of Fanconi anemia type C phenotypic abnormalities using a clinically suitable retroviral vector infection protocol." Cell Transplant **5**(3): 385-93.

Fanconi anemia (FA) is a complex autosomal recessive disease with hematologic manifestations characterized by a progressive hypoplastic anemia, hypersensitivity to clastogenic agents, and an increased incidence of acute myelogenous leukemia. The cDNA that corrects one of four FA complementation subtypes, named Fanconi anemia Type C (FAC) has recently been identified. We constructed a a simplified recombinant retrovirus (vMFGFAC) encoding only the FAC cDNA, and tested its ability to correct the FAC defect in a lymphocytic cell line and primary mobilized blood progenitor cells. In addition, the gene transfer efficiency using a clinically applicable gene transfer protocol into normal primitive hematopoietic

progenitor cells, high proliferating potential colony forming cells (HPP-CFC), derived from CD34+ purified cord blood cells was examined. The gene transfer efficiency was significantly enhanced when cells were transduced with supernatant while adherent to a 30/35 KD fragment of fibronectin, FN30/35, and was similar to efficiency obtained by coculture with retrovirus packaging cells. Transduction of an FAC deficient lymphoid cell line with vMFGFAC supernatant resulted in an enhanced cell viability, and G-CSF mobilized peripheral blood cells from an FAC-deficient patient transduced with the vMFGFAC virus demonstrated enhanced progenitor cell colony formation. These data indicate that the vMFGFAC virus allows functional complementation of FAC in lymphoblasts and primary hematopoietic progenitors, and that primitive cord blood hematopoietic stem/progenitor cells can be transduced at an efficiency comparable to protocols using cocultivation if adherent to FN 30/35 fragment.

Goebel, W. S., M. C. Yoder, et al. (2002). "Donor chimerism and stem cell function in a murine congenic transplantation model after low-dose radiation conditioning: effects of a retroviral-mediated gene transfer protocol and implications for gene therapy." Exp Hematol **30**(11): 1324-32.

OBJECTIVE: We investigated low-dose radiation conditioning for the transplantation of retrovirus-transduced cells in a C57Bl6/J murine model. MATERIALS AND METHODS: The effect of low-dose radiation on stem cell function was investigated using a competitive repopulation assay. Stem cell function of marrow cells that underwent a retroviral-mediated gene transfer (RMGT) protocol was examined by this assay, and donor chimerism of these cells when transplanted into 160-cGy conditioned syngeneic hosts was compared to fresh marrow. RESULTS: Irradiation with 300 or 160 cGy substantially decreased stem cell function as measured by competitive repopulation. Animals conditioned with 160 cGy and transplanted with 20 x 10(6) fresh marrow cells permitted donor cell engraftment of 53.6% +/- 11.4% 6 months after transplant compared to 100% donor cell engraftment after 1100 cGy irradiation. Lymphoid and myeloid engraftment did not significantly differ from total engraftment in submyeloablated hosts. When transplanted into lethally irradiated hosts, the competitive repopulating activity of marrow treated with a single dose of 5-fluorouracil followed by ex vivo culture according to a standard RMGT protocol was equal to 5-fluorouracil-only treated marrow. However, cells treated with 5-fluorouracil or 5-fluorouracil plus ex vivo culture for RMGT repopulated less well than fresh marrow cells in 160 cGy conditioned hosts. CONCLUSIONS: Low-dose irradiation decreases host stem cell function, allowing engraftment of both fresh and RMGT protocol-treated marrow, although the engraftment of 5-fluorouracil-treated cells was reduced at least two-fold, and 5-fluorouracil plus RMGT protocol-treated cells at least three-fold, compared to fresh marrow. Modification of current RMGT protocols may be important for optimizing engraftment under these conditions.

Gunetti, M., I. Ferrero, et al. (2008). "Refreezing of cord blood hematopoietic stem cells for allogenic transplantation: in vitro and in vivo validation of a clinical phase I/II protocol in European and Italian Good Manufacturing Practice conditions." Exp Hematol **36**(2): 235-43.

OBJECTIVE: Several requirements need to be fulfilled for clinical use of expanded hematopoietic stem cells (HSCs). Because most cord blood (CB) samples are frozen in single bags and only an aliquot (approximately 25%) of the blood can be expanded, the thawing and refreezing of samples must be validated in the current European and Italian Good Manufacturing Practice (eIGMP) conditions. Here, we describe in vitro and in vivo validation of the phase I/II protocol for CD34+ expansion of thawed CB units according to the current Cell Therapy Products (CTPs) Guidelines. MATERIALS AND METHODS: CB units were thawed and 25% of the total volume was processed for CD34+ selection by CliniMACS. The 75% of the unit was immediately refrozen. CD34+ cells were expanded for 3 weeks with stem cell factor, Flt-3/Flk-2 ligand, thrombopoietin, and interleukin-6. RESULTS: In vitro results demonstrated that this culture system induces expansion of thawed CD34+ (median value = 8.3). In vivo data demonstrated that after culture, the final CTPs maintain their repopulating ability in nonobese diabetic severe combined immunodeficient (SCID) mice. Limiting dilution assays performed by injecting decreasing doses of expanded CD34+ cells revealed that the frequency of SCID repopulating cells after ex vivo expansion is 1:8,034. Analyses for sterility, viability, cell senescence, and cytogenetic assessment demonstrated that expansion procedures in eIGMP conditions are safe for clinical protocols. CONCLUSIONS: This offers promising new options for expansion of allogenic HSCs and also for autologous usage in transplantation and other cell therapy protocols.

Hacein-Bey, S., F. Gross, et al. (2001). "Optimization of retroviral gene transfer protocol to maintain the lymphoid potential of progenitor cells." Hum Gene Ther **12**(3): 291-301.

We have attempted to improve retrovirus-mediated gene transfer efficacy into hematopoietic progenitor cells (HPCs) without causing them to lose their lymphoid potential. Highly purified CD34(+) cells on CH-296 fibronectin fragments have been transduced with three different cytokine combinations. Murine CD2 was used as a marker gene. Transgene expression was assayed by FACS analysis shortly after transduction of CD34(+) cells and after long-term culture (LTC) extended by differentiation of various lymphoid lineages: NK cells, B cells, and dendritic cells. Compared with the historical cytokine mix, i.e., SCF (stem cell factor) + IL-3 (interleukin 3) + IL-6, the combination SCF + FL (Flt-3 ligand) + M-GDF (megakaryocyte growth and differentiation factor) + IL-3 significantly improved the total number of viable cells and CD34(+) cells after transduction and the long term-cultured progenitors after 6 weeks. In addition, the combination of SCF + FL + M-GDF + IL-3 maintained more efficiently the lymphoid potential of the progeny of transduced long term-cultured CD34(+) cells, as attested by the significantly higher number of CD56(+), CD19(+), and CD1a(+) cells recovered when FL and M-GDF were added to SCF + IL-3. Thus, even though additional improvements may still be needed in transduction of HPCs, these conditions were adopted for a clinical trial of gene therapy for X-linked severe combined immunodeficiency.

Haioun, C., E. Lepage, et al. (2000). "Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol--a groupe d'Etude des lymphomes de l'Adulte study." J Clin Oncol **18**(16): 3025-30.

PURPOSE: To present the final analysis, with a median follow-up of 8 years, of the LNH87-2 randomized study, which compares consolidative sequential chemotherapy (ifosfamide plus etoposide, asparaginase, and cytarabine) with high-dose therapy (HDT) using cyclophosphamide, carmustine, and etoposide (CBV regimen) followed by stem-cell transplantation in patients with aggressive non-Hodgkin's lymphoma in first complete remission after induction, focusing on high/intermediate- and high-risk patients identified by the age-adjusted international prognostic index. PATIENTS AND METHODS: Among the 916 eligible patients, 451 presented with two (n = 318) or three (n = 133) risk factors. After reaching complete remission to induction therapy, 236 of these higher risk patients were assessable for the consolidation phase, with 125 patients in the HDT arm and 111 in the sequential chemotherapy arm. RESULTS: Among these 451 higher risk patients, 277 (61%) achieved complete remission after induction treatment. In the population of 236 randomized patients, HDT was superior to sequential chemotherapy, with 8-year disease-free survival rates of 55% (95% confidence interval [CI], 46% to 64%) and 39% (95% CI, 30% to 48%), respectively (P =.02; relative risk, 1.56). The 8-year survival rate was significantly superior in the HDT arm (64%; 95% CI, 55% to 73%) compared with the sequential chemotherapy arm (49%; 95% CI, 39% to 59%) (P =.04; relative risk, 1.51). CONCLUSION: On the basis of the final analysis of this prospectively treated series of patients, retrospectively analyzed on the basis of the International Prognostic Index, we hypothesize that HDT benefits patients at higher risk who achieve complete remission after induction treatment.

Harris, E., S. Paneesha, et al. (2002). "Burkitt's lymphoma: single-centre experience with modified BFM protocol." Clin Lab Haematol **24**(2): 111-4.

Burkitt's lymphoma is a rare aggressive lymphoma, which responds poorly to standard chemotherapy regimens used to treat high-grade non-Hodgkin's lymphoma (NHL). The use of intensive chemotherapy protocols using alkylating agents and intensive CNS prophylaxis has dramatically altered prognosis. We have treated eight patients with Burkitt's lymphoma with a modified BFM protocol. The dose of methotrexate was reduced from 5 g/m2 to 1.5 g/m2 with the aim of reducing toxicity. Seven patients received a total of six cycles of chemotherapy each and one patient received five cycles of chemotherapy. Each cycle included high-dose methotrexate, an alkylating agent (ifosphamide or cyclophosphamide) and two triple intrathecal injections of chemotherapy. Two patients with bulky abdominal disease in addition received an autologous stem cell transplant. The regimen was well tolerated with minimal toxicity. At a median follow-up of 16 months (range 10-28), six of the eight patients (75%) were alive and in complete remission. Two patients relapsed, one 24 months post-BFM chemotherapy and the other 1-month post-autologous stem cell transplantation and 2 months post-BFM chemotherapy.

Hennemann, B., E. Conneally, et al. (1999). "Optimization of retroviral-mediated gene transfer to human NOD/SCID mouse repopulating cord blood cells through a systematic analysis of protocol variables." Exp Hematol **27**(5): 817-25.

Retroviral transduction of human hematopoietic stem cells is still limited by lack of information about conditions that will maximize stem cell self-renewal divisions in vitro. To address this, we first compared the kinetics of entry into division of single human CD34+CD38- cord blood (CB) cells

exposed in vitro to three different flt3-ligand (FL)-containing cytokine combinations. Of the three combinations tested, FL + hyperinterleukin 6 (HIL-6) yielded the least clones and these developed at a slow rate. With either FL + Steel factor (SF) + HIL-6 + thrombopoietin (TPO) or FL + SF + interleukin 3 (IL-3) + IL-6 + granulocyte-colony-stimulating factor (G-CSF), >90% of the cells that formed clones within 6 days undertook their first division within 4 days, although not until after 24 hours. These latter two, more stimulatory, cytokine combinations then were used to assess the effect of duration of cytokine exposure on the efficiency of transducing primitive CB cells with a gibbon ape leukemia virus-pseudotyped murine retroviral vector containing the enhanced green fluorescent protein (GFP) cDNA and the neomycin resistance gene. Fresh lin- CB cells exposed once to medium containing this virus plus cytokines on fibronectin-coated dishes yielded 23% GFP+ CD34+ cells and 52-57% G418-resistant CFC when assessed after 2 days. Prestimulation of the target cells (before exposing them to virus) with either the four or five cytokine combination increased their susceptibility. In both cases, the effect of prestimulation assessed using the same infection protocol was maximal with 2 days of prestimulation and resulted in 47-54% GFP+ CD34+ cells and 67-69% G418-resistant CFC. Repeated daily addition of new virus (up to three times), with assessment of the cells 2 days after the last addition of fresh virus, gave only a marginal improvement in the proportion of transduced CD34+ cells and CFC, but greatly increased the proportion of transduced LTC-IC (from 40% to >99%). Transplantation of lin- CB cells transduced using this latter 6-day protocol into NOD/SCID mice yielded readily detectable GFP+ cells in 10 of 11 mice that were engrafted with human cells. The proportion of the regenerated human cells that were GFP+ ranged from 0.2-72% in individual mice and included both human lymphoid and myeloid cells in all cases. High-level reconstitution with transduced human cells was confirmed by Southern blot analysis. These findings demonstrate that transplantable hematopoietic stem cells in human CB can be reproducibly transduced at high efficiency using a 6-day period of culture in a retrovirus-containing medium with either FL + SF + HIL-6 + TPO or FL + SF + IL-3 + IL-6 + G-CSF in which virus is added on the third, fourth, and fifth day.

Horn, P. A., K. A. Keyser, et al. (2004). "Efficient lentiviral gene transfer to canine repopulating cells using an overnight transduction protocol." Blood **103**(10): 3710-6.

The use of lentiviral vectors for the transduction of hematopoietic stem cells has evoked much interest owing to their ability to stably integrate into the genome of nondividing cells. However, published large animal studies have reported highly variable gene transfer rates of typically less than 1%. Here we report the use of lentiviral vectors for the transduction of canine CD34(+) hematopoietic repopulating cells using a very short, 18-hour transduction protocol. We compared lentiviral transduction of hematopoietic repopulating cells from either stem cell factor (SCF)- and granulocyte-colony stimulating factor (G-CSF)-primed marrow or mobilized peripheral blood in a competitive repopulation assay in 3 dogs. All dogs engrafted rapidly within 9 days. Transgene expression was detected in all lineages (B cells, T cells, granulocytes, and red blood cells as well as platelets) indicating multilineage engraftment of transduced cells, with overall long-term marking levels of up to 12%. Gene transfer levels in mobilized peripheral blood cells were slightly higher than in primed marrow cells. In conclusion, we show efficient lentiviral transduction of canine repopulating cells using an overnight transduction protocol. These results have important implications for the design of stem cell gene therapy protocols, especially for those diseases in which the maintenance of stem cells in culture is a major limitation.

Hristov, M., S. Schmitz, et al. (2009). "An optimized flow cytometry protocol for analysis of angiogenic monocytes and endothelial progenitor cells in peripheral blood." Cytometry A **75**(10): 848-53.

Circulating adult CD34(+)VEGFR2(+) endothelial progenitor cells (EPCs) have been shown to differentiate into endothelial cells, thus contributing to vascular homeostasis. Furthermore, a subset of circulating CD14(+) monocytes coexpresses CD16 together with the angiopoietin receptor Tie2 and has been functionally implicated in tumor angiogenesis. However, clinically applicable protocols for flow cytometric quantification of EPCs and Tie2(+) monocytes in peripheral blood and a consensus on reference values remain elusive. The number of Tie2(+)CD14(+)CD16(mid) angiogenic monocytes and CD34(+)VEGFR2(+)CD45(low/-) EPCs was assessed in the peripheral venous blood of patients with stable coronary artery disease by three-color flow cytometry using specific monoclonal antibodies conjugated to PerCP, PE, PE-Cy7, APC, and APC-Cy7. Scatter multigating with exclusion of dead cells was performed to dissect complex mononuclear cell populations. This analysis was further refined by matching bright fluorochromes (PE-Cy7, PE, APC) with dimly expressed markers (CD34, VEGFR2, Tie2), by automatic compensation for minimizing fluorescence spillover and by using fluorescence-

minus-one (FMO) controls to determine positive/negative boundaries. Presuming a Gaussian distribution, we obtained average values (mean +/- SD) of 1.45 +/- 1.29% for Tie2(+)CD14(+)CD16(mid) monocytes (n = 11, range: 0.12-3.64%) and 0.019 +/- 0.013% for CD34(+)VEGFR2(+)CD45(low/-) EPCs (n = 17, range: 0.003-0.042%). The intra- and inter-assay variability was 1.6% and 4.5%, respectively. We have optimized a fast and sensitive assay for the flow cytometric quantification of circulating angiogenic monocytes and EPCs in cardiovascular medicine. This protocol may represent a basis for standardized analysis and monitoring of these cell subsets to define their normal range and prognostic/diagnostic value in clinical use.

Hurd, D. D. and W. P. Peters (1995). "Randomized, comparative study of high-dose (with autologous bone marrow support) versus low-dose cyclophosphamide, cisplatin, and carmustine as consolidation to adjuvant cyclophosphamide, doxorubicin, and fluorouracil for patients with operable stage II or III breast cancer involving 10 or more axillary lymph nodes (CALGB Protocol 9082). Cancer and Leukemia Group B." J Natl Cancer Inst Monogr(19): 41-4.

The prognosis for patients with primary breast cancer involving multiple axillary lymph nodes is poor. Only about 30% of patients remain disease-free at 5 years from diagnosis despite surgery, conventional-dose chemotherapy, and radiation therapy. In nonrandomized studies, the use of high-dose chemotherapy as consolidation therapy after standard-dose induction chemotherapy has resulted in an apparent improvement in disease-free survival rates to over 70%. These results have prompted the National Cancer Institute to sponsor large-scale, multicenter, randomized comparative trials of this strategy. This Intergroup Study (Cancer and Leukemia Group B 9082, Southwest Oncology Group 9114, and National Cancer Institute of Canada MA13) compares two treatment strategies in women with primary breast cancer involving 10 or more axillary lymph nodes. Arms A and B are identical in the use of four cycles of conventional therapy with cyclophosphamide and doxorubicin and fluorouracil, radiation therapy, and tamoxifen. The only difference between the two arms is the dose intensity of the cyclophosphamide, cisplatin, and carmustine given following conventional adjuvant treatment. Arm A dictates bone marrow, peripheral blood stem cell, and hematopoietic growth factor support and frequently requires a prolonged hospital stay with high resource utilization. Arm B, with its less dose-intensive therapy, requires considerably less support to apply the treatment. Because of the high cost of this therapy and the requirement for technology-intensive support, there has been considerable interest in economic outcome assessments.

Izadyar, F., J. J. Matthijs-Rijsenbilt, et al. (2002). "Development of a cryopreservation protocol for type A spermatogonia." J Androl **23**(4): 537-45.

The aim of this study was to develop a cryopreservation protocol for type A spermatogonia. Testes from 5- to 7-month-old calves were collected, and type A spermatogonia were isolated using two-step enzymatic digestion and Percoll separation. Cells were resuspended in minimum essential medium (MEM) supplemented with 1% bovine serum albumin (BSA) in a final concentration of 6 x 10(6) per mL, and the effects of different cryoprotectants and freezing protocols were tested. Cells frozen/thawed in medium containing 10% fetal calf serum (FCS) and 1.4 M glycerol or dimethyl sulfoxide (DMSO) had a significantly (P <.05) higher percentage of living cells compared to medium with only FCS, whereas DMSO gave a significantly better cell survival rate than glycerol did. An increase in the concentration of FCS in the DMSO-based medium to 20% had no effect on survival after freezing and thawing. Furthermore, inclusion of 0.07, 0.14, or 0.21 M sucrose in DMSO-based medium resulted in a significant improvement of cell survival, cell proliferation in culture, and colonization efficiency in recipient testes. A controlled slow-freezing rate (1 degrees C/min) resulted in significantly (P <.05) more viable cells than fast (5 degrees C/min) freezing. However, noncontrolled-rate freezing, with a comparably low cooling rate, gave even better results than the controlled-rate slow freezing. Cryopreservation in MEM-based medium containing 10% FCS, 10% DMSO, and 0.07 M sucrose using a non-controlled-rate freezing protocol appeared to be a simple and effective way to preserve type A spermatogonia, with a high yield of almost 70% living cells after thawing. Frozen/thawed spermatogonia survived in culture and retained the ability to proliferate as determined by colorimetric and bromodeoxyuridine incorporation assays. To test whether the stem cells among the A spermatogonia retained their ability to colonize the testis of a recipient mouse, bovine spermatogonia were transplanted. This resulted in colonization 2-3 months after transplantation. In conclusion, for the first time, a method specific for cryopreservation of type A spermatogonia, including spermatogonial stem cells was developed, which allows long-term preservation of these cells without apparent harmful effects to their function.

Jantunen, E., T. Kuittinen, et al. (2006). "High-dose melphalan (200 mg/m2) supported by autologous stem cell transplantation is safe and effective in elderly

(>or=65 years) myeloma patients: comparison with younger patients treated on the same protocol." Bone Marrow Transplant **37**(10): 917-22.

Limited information is available on the feasibility and efficacy of autologous stem cell transplantation (ASCT) in multiple myeloma (MM) patients >65 years of age. In 1995-2005, 22 myeloma patients >or=65 years (median 68, eight >or=70) and 79 patients <65 years (median 57) were included in an identical treatment protocol. The first progenitor cell mobilization with cyclophosphamide plus granulocyte-colony stimulating factor (G-CSF) was successful in 95 and 96% of the patients, respectively. To date, 92 patients have received MEL (melphalan) 200 mg/m2 supported by ASCT. No early treatment-related deaths were observed among 22 elderly patients, whereas one younger patient died early. Engraftment and the need for supportive care were comparable between groups. The elderly patients tended to have more WHO grade 3-4 oral or gastrointestinal toxicity when compared to the younger patients (45 vs 23%, P=0.06). After ASCT, a complete response was observed in 44% of the elderly patients and 36% of the younger patients, respectively. No difference was observed between these age groups in progression-free survival (23 vs 21 months) or overall survival (57 vs 66 months) after ASCT. We conclude that MEL200 is a safe and efficacious treatment in selected elderly myeloma patients.

Jantunen, E., E. Mahlamaki, et al. (2000). "Feasibility and toxicity of high-dose chemotherapy supported by peripheral blood stem cell transplantation in elderly patients (>/=60 years) with non-Hodgkin's lymphoma: comparison with patients <60 years treated within the same protocol." Bone Marrow Transplant **26**(7): 737-41.

Limited data are available concerning feasibility and toxicity of progenitor cell mobilization and high-dose therapy (HDT) supported by peripheral blood stem cell transplantation (PBSCT) in elderly patients (>/=60 years) with non-Hodgkin's lymphoma (NHL). From 1995 to 1999, 17 elderly NHL patients (median age 63 years, range 60-70) entered our HDT program and were mobilized with CY (4 g/m2) followed by G-CSF. Mobilization was successful in 13 patients, who then received BEAM or BEAC followed by PBSCT. The feasibility and toxicity of progenitor cell mobilization and HDT in the elderly patients were compared with experiences in 62 NHL patients <60 years (median 46 years, range 16-59), who received the same mobilization protocol and of whom 48 patients received HDT supported by PBSCT. No significant differences were observed between these groups in the success rate of progenitor cell mobilization, in the number of CD34-positive cells collected or in the number of aphereses needed. HDT appeared to be somewhat more toxic in the elderly patients: a higher peak CRP value (P = 0.08) and longer in-hospital stay (P = 0. 05) were observed. No differences were found in transplant-related mortality or severe organ toxicity between these age groups except for oral mucositis grade >2, which tended to be more common in the elderly patients (P = 0.07). We conclude that progenitor cell mobilization and HDT supported by PBSCT is also feasible in selected elderly patients with NHL. Bone Marrow Transplantation (2000) 26, 737-741.

Jones, E. A., A. English, et al. (2006). "Optimization of a flow cytometry-based protocol for detection and phenotypic characterization of multipotent mesenchymal stromal cells from human bone marrow." Cytometry B Clin Cytom **70**(6): 391-9.

BACKGROUND: To study the biology of rare bone marrow (BM) multipotent mesenchymal stromal cells (MSCs), recognized protocols are needed. Colony-forming unit-fibroblast (CFU-F) assays have historically been used for the enumeration of MSCs. However, the need to isolate and further analyze MSCs requires new strategies based on cell surface markers. The purpose of this work was to verify the phenotype of BM MSCs in vivo and to develop flow cytometry-based methods for their evaluation. METHODS: Pre-enrichment with D7-FIB-conjugated microbeads, cell sorting for CD45low D7-FIB+ LNGFR+ cells, and CFU-F assay were used to confirm the phenotype of BM MSCs in vivo. Further phenotypic characterization of MSCs was performed using three-color flow cytometry following pre-enrichment or by direct four-color flow cytometry. The sensitivity of direct flow cytometry/rare event analysis for the accurate enumeration of MSCs was validated using 85 samples from patients with neoplastic BM diseases. RESULTS: In normal BM, a significant correlation was found between the frequencies of CFU-Fs and CD45low D7-FIB+ LNGFR+ cells (n = 19, R = 0.719, P = 0.001). Following cell sorting, 15% of these cells were clonogenic. The same cells were enriched using LNGFR-based positive selection, CD45/Glycophorin A-based depletion, or plastic adherence. CD45low D7-FIB+ LNGFR+ cells expressed classic makers of cultured MSCs CD73/SH3 and CD105/SH2 and markers of stromal reticular cells CD106/VCAM and alkaline phosphatase. Novel markers were identified including leukemia inhibitory factor receptor and gp130. CD45low D7-FIB+ LNGFR+ cells were increased fourfold in the floating fat fraction of normal BM aspirates. Their frequency was decreased in chronic lymphocytic leukemia (threefold, n = 13, P = 0.049) and chronic myelogenous leukemia

(ninefold, n = 11, P = 0.001) compared with that in age-matched controls (n = 26 and n = 31, respectively). CONCLUSIONS: This study demonstrates the usefulness of flow cytometry-based methods for the detection, enumeration and further phenotypic analysis of BM MSCs. These findings have broad applications for the future evaluation of BM MSCs in health and disease.

Kapelushnik, J., H. Mandel, et al. (2000). "Fludarabine-based protocol for haploidentical peripheral blood stem cell transplantation in Hurler syndrome." J Pediatr Hematol Oncol **22**(5): 433-6.

To assess the feasibility of performing a haploidentical peripheral blood stem cell transplantation (PBSCT) in a child with Hurler syndrome after a novel conditioning regimen consisting of fludarabine monophosphate, anti-T-lymphocyte globulin, low-dose busulfan, and single-dose total body irradiation of 750 cGy. A 16-month old boy with Hurler syndrome underwent haploidentical PBSCT from his 3/6 HLA-matched sister. Pretransplant conditioning consisted of fludarabine (30 mg/m2 per day) from day -10 to day -5, busulfan (4 mg/kg per day) on days -7 and -6, rabbit anti-T-lymphocyte globulin (10 mg/kg per day) from day -4 to day -1, and total body irradiation of 750 cGy on day -1. In vitro T-cell depletion was carried out with rat antihuman CDw52 monoclonal antibody (Campath-1G). The fludarabine-based protocol was well-tolerated, with mild toxicity and no major transplant-related complications or graft-versus-host disease. Engraftment was complete and stable. Chimerism was 100% donor origin, as determined by restriction fragment length polymorphism. Cytogenetic and polymerase chain reaction-various number of tandem repeats (PCR-VNTR) analyses of peripheral blood and bone marrow showed 100% reconstitution with female donor cells. The patient underwent the transplant 30 months ago and is in good clinical condition, with normal counts, no signs of graft-versus-host disease, and no infectious episodes; neurologic signs have stabilized. Haploidentical PBSCT, T-cell-depleted by means of Campath-1G, may serve as a therapeutic alternative for patients with Hurler syndrome when a fully matched sibling is not available.

Kitano, M., M. Kakinuma, et al. (2006). "Gene expression profiling of mouse embryonic stem cell progeny differentiated by Lumelsky's protocol." Cells Tissues Organs **183**(1): 24-31.

Successful conversion of embryonic stem (ES) cells into insulin-producing cells has been reported by Lumelsky et al. (Science 2001;292:1389-1394); however, it remains controversial. In this study, we investigated the properties of ES cell progeny-induced differentiation according to Lumelsky's protocol by immunocytochemistry, oligonucleotide microarray and real-time RT-PCR. Insulin-positive cells were observed at stages 3, 4 and 5. Microarray analysis demonstrated upregulation and appearance of some genes involved in pancreatic development but not beta-cell-specific functional genes in cells at stage 5. Similarly, real-time RT-PCR revealed that expression of beta-cell-specific functional genes such as islet amyloid polypeptide, insulin I and II was not increased in cells at stage 5. These results suggest that terminal differentiation of ES cell progeny toward functional pancreatic beta-cell is insufficient. This study also demonstrates the usefulness of multiple time-course expression profiles for validating differentiation fates of ES cell progeny.

Kolb, E. A. and P. G. Steinherz (2003). "A new multidrug reinduction protocol with topotecan, vinorelbine, thiotepa, dexamethasone, and gemcitabine for relapsed or refractory acute leukemia." Leukemia **17**(10): 1967-72.

We report the results of a phase 2 nonrandomized single-arm trial of a combination therapy for relapsed or refractory leukemia. From January 1999 to June 2002, 28 patients with multiple relapsed or refractory acute leukemia received a combination of topotecan, vinorelbine, thiotepa, dexamethasone, and, for patients with an M3 marrow on day 7, gemcitabine. A total of 14 patients had pre-B-ALL (acute lymphoblastic leukemia), three had T-cell leukemia, nine acute myeloblastic leukemia (AML), and two biphenotypic leukemia. In all, 13 patients achieved a significant response (10 complete responses and three partial responses). Among the responders, five had pre-B-ALL, two had T-cell leukemias, five had AML, and one had biphenotypic leukemia. In total, 10 of these patients subsequently underwent hematopoietic stem cell transplantation, and four are alive without disease. One patient died, while in remission, of complications resulting from an episode of sepsis and pneumonia that occurred during topotecan, vinorelbine, thiotepa, dexamethasone, and gemcitabine (TVTG) reinduction. Other toxicities included grade 4 neutropenia in all patients and transient grade 2 hepatotoxicity in 10 patients (36%). In summary, we report that 47% of heavily pretreated pediatric patients with multiply relapsed or refractory leukemia achieved a significant response after therapy on the TVTG protocol. Further studies are warranted to evaluate the role of the TVTG combination in the treatment of leukemia.

Lalvani, A., T. Dong, et al. (1997). "Optimization of a peptide-based protocol employing IL-7 for in vitro

restimulation of human cytotoxic T lymphocyte precursors." J Immunol Methods **210**(1): 65-77.

A variety of different methods for the in vitro restimulation of human cytotoxic T lymphocyte (CTL) precursors (CTLp) are in use. Our aim was to enhance the detection of circulating human CTLp in peripheral blood. We have developed a standardized and highly efficient method for restimulating CTLp. Synthetic peptides were used to restimulate cognate CTLp from peripheral blood mononuclear cells (PBMC), and effector CTL capable of lysing peptide-pulsed and virus infected targets were generated. The effects of several parameters on CTL specific for influenza A, EBV and HIV-1 were evaluated, and the optimum peptide concentration for CTL generation was established. Supplementation of initial cultures with IL-7 greatly enhanced peptide-specific lytic activity for all peptides tested and the dose-response relationship for IL-7 was delineated. A novel technique using peptide-MHC class I molecule tetramers to stain T cells bearing cognate T cell receptors permitted enumeration of antigen-specific CD8 + CTL during in vitro restimulation; IL-7 supplementation selectively expanded the population of peptide-specific CD8 + CTL. Importantly, this protocol, whilst enhancing the restimulation and lytic activity of secondary CTL, does not induce primary CTL in vitro. The improved efficiency with which CTL are generated in this system substantially enhances the sensitivity of CTL culture and the 51Cr release assay to detect low levels of CTL activity.

Lappalainen, J., K. A. Lindstedt, et al. (2007). "A protocol for generating high numbers of mature and functional human mast cells from peripheral blood." Clin Exp Allergy **37**(9): 1404-14.

BACKGROUND: Mast cells (MCs) are multi-functional effector cells with an essential role in innate immunity and host defence, and under several pathological conditions, such as allergy. Here, we aimed at defining the culture conditions that would allow efficient generation of mature and functional human MCs from their progenitor cells. METHODS: Human peripheral blood-derived CD34(+) progenitor cells were cultured in vitro under serum-free conditions with human stem cell factor for 9 weeks. Growth and differentiation of the cells into MCs were optimized by selected cytokines and a combination of hypoxic and normoxic conditions. MCs were phenotypically characterized by immunocytochemistry, their preformed mediators were quantified, and their functional ability to degranulate and release histamine was tested. RESULTS: On average, 20 x 10(6) mature MCs were generated from 0.5 x 10(6) progenitor cells during 9 weeks of culture, i.e. at least a 40-fold increase in cell number was achieved. The mature MCs had oval-shaped non-lobular nuclei, contained histamine, heparin, tryptase, chymase, and cathepsin G in their secretory granules, and strongly expressed c-kit (CD117) and Fc epsilon receptor I on their surface. Histamine release from the cells could be brought about by IgE-anti-IgE cross-linkage, compound 48/80, substance P, and anaphylatoxin C3a. The MCs remained functional for several weeks after their maturation. CONCLUSION: This study describes an efficient protocol for generating mature MCs from human peripheral blood with a functional phenotype of connective tissue-type MCs. Use of these cultured human MCs will increase our knowledge and understanding about human MC development and biology in human disease.

Lotz, J. P., B. Bui, et al. (2005). "Sequential high-dose chemotherapy protocol for relapsed poor prognosis germ cell tumors combining two mobilization and cytoreductive treatments followed by three high-dose chemotherapy regimens supported by autologous stem cell transplantation. Results of the phase II multicentric TAXIF trial." Ann Oncol **16**(3): 411-8.

BACKGROUND: High-dose chemotherapy (HD-CT) is able to circumvent platinum resistance of resistant/refractory germ-cell tumors (GCTs), but expectancy of cure remains low. New strategies are needed with new drugs and a sequential approach. MATERIALS AND METHODS: Patients with relapsed poor-prognosis GCTs were scheduled to receive two cycles combining epirubicin and paclitaxel (Taxol) followed by three consecutive HD-CT supported by stem cell transplantation [one course combining cyclophosphamide, 3 g/m(2) + thiotepa, 400 mg/m(2), followed by two ICE regimens (ifosfamide, 10 g/m(2), carboplatin, AUC 20, etoposide, 1500 mg/m(2))]. RESULTS: From March 1998 to September 2001 (median follow-up, 31.8 months), 45 patients (median age, 28 years) were enrolled in this phase II study. Twenty-two patients received the complete course. Twenty-five patients died from progression and five from toxicity. The overall response rate was 37.7%, including an 8.9% complete response rate. The median overall survival was 11.8 months. The 3-year survival and progression-free survival rate was 23.5%. The 'Beyer' prognostic score predicted the outcome after HD-CT. CONCLUSION: Although our results warrant further studies on HD-CT in relapsed poor prognosis GCTs, patients with a Beyer score >2 did not benefit from this approach and should not be enrolled in HD-CT trials. Better selection criteria have to be fulfilled in forthcoming studies.

Martino, R., R. Guardia, et al. (1999). "Time sequential chemotherapy for primary refractory or relapsed adult acute myeloid leukemia: results of the phase II Gemia protocol." Haematologica **84**(3): 226-30.

BACKGROUND AND OBJECTIVE: High-dose cytarabine (HDAra-C), mitoxantrone and etoposide are the mainstay of several active regimens against relapsed or refractory acute myelogenous leukemia (AML). We designed a phase II study to assess the efficacy and side effects of a time sequential application of mitoxantrone plus intermediate-dose Ara-C followed by HDAra-C plus etoposide (GEMIA) in adult patients with refractory or relapsed AML. DESIGN AND METHODS: Patients with refractory or relapsed AML were eligible for GEMIA salvage therapy, which comprised mitoxantrone 12 mg/m2/day on days 1-3, Ara-C 500 mg/m2/day as a 24-hour continuous infusion on days 1-3, followed by HDAra-C 2 g/m2/12-hourly on days 6-8 and etoposide 100 mg/m2/12-hourly on days 6-8. Granulocyte colony-stimulating factor was started on day 14. In patients above the age of 55 the dose of Ara-C in the first sequence (days 1-3) was reduced to 250 mg/m2. RESULTS: Twenty patients were included, of whom 12 achieved complete remission after GEMIA (60%, 95% CI 40-80%), one was refractory and five died early from infection. Two additional patients achieved partial remission after GEMIA and complete remission after consolidation chemotherapy, for a final CR rate of 70% (95% CI 48-88%). Neutrophils recovered at a median of 27 days (range, 22-43) and platelets 46 days (range, 25-59) after the start of treatment. The median duration of remission was 133 days (range, 36-417+) whereas overall survival time lasted for a median of 153 days (range, 13-554+). Treatment-associated toxicity was comprised predominantly of infection, mucositis and diarrhea that reached World Health Organization grades III-V in 40%, 40% and 30% of patients, respectively. Despite the intention to rapidly proceed to a hematopoietic stem cell transplant in patients in remission, only five patients reached the transplant. INTERPRETATION AND CONCLUSIONS: The GEMIA time sequential chemotherapy regimen appears effective in obtaining remissions in refractory and relapsed adult AML. The high toxicity seen, however, suggests that its design is amenable to further improvements, especially in more elderly patients. Since remissions are short-lived, more innovative post-remission strategies are needed.

McBride, S. H. and M. L. Knothe Tate (2008). "Modulation of stem cell shape and fate A: the role of density and seeding protocol on nucleus shape and gene expression." Tissue Eng Part A **14**(9): 1561-72.

Mesenchymal stem cell shape and fate are intrinsic manifestations of form and function at the cellular level. We hypothesize that cell seeding density and initial seeding protocol influence stem cell shape and fate. Nucleus shape and early (within days of seeding) expression of genes typical for pre-, peri-, and postcondensation events were compared between groups of cells after seeding at or proliferating to target density (low density [LD], 16,500 cells/cm2; high density [HD], 35,000 cells/cm2; very high density [VHD], 86,500 cells/cm2). Significant differences in nuclear shape could be attributed to seeding protocol in the VHD group, where nuclei from cells that proliferated to VHD were significantly rounder than nuclei from cells seeded at target VHD. Furthermore, cells that proliferated to VHD exhibited significantly rounder nuclei than nuclei from all other cell density and seeding protocol groups. In contrast, nuclei from cells that were seeded at the VHD were flatter than nuclei from cells of all other groups. Furthermore, the significant rounding of nuclei in the cells that proliferated to VHD was accompanied by a two-, six-, and ninefold increase from baseline in Runx2, Sox9, and Aggrecan (AGC) expression, markers indicative of precondensation, peri-, and post-condensation events, respectively. None of the other groups showed significant changes in gene expression over baseline. Finally, seeding at target density results in greater overlap of cells compared to groups in which cells proliferate to target density, conferring increased thickness to multicellular culture aggregates seeded at target density. These data suggest that seeding protocols can be exploited to modulate mesenchymal stem cell shape and early gene expression typical for condensation events in development, which occur over an approximately 12-h period at E11.5 in the mouse limb bud. Follow-on studies will delineate longer-term effects of density and seeding protocol on modulation of stem cell fate and cell assembly to form tissues.

Menichella, G., M. Lai, et al. (1997). "Evaluation of a new protocol for peripheral blood stem cell collection with the Fresenius AS 104 cell separator." J Clin Apher **12**(2): 82-6.

In this report we analyzed sixty leukapheresis procedures on 35 patients with a new protocol for the Fresenius AS 104. Yields and efficiencies for MNC, CD 34+ cells, and CFU-GM indicate that the new protocol is able to collect large quantities of hemopoietic progenitors. Procedures were performed processing 8.69 +/- 2.8 liters of whole blood per apheresis and modifying 3 parameters: spillover-volume 7 ml, buffy-coat volume 11.5 ml, centrifuge speed 1,500 rpm; blood flow rate was 50 ml/min and the anticoagulant ratio was 1:12. No side effects were

observed during apheresis procedures except for transient paresthesia episodes promptly resolved with the administration of calcium gluconate. Yields show a high capacity of the new program to collect on average MNC 17.28 +/- 10.85 x 10(9), CD 34+ 471 +/- 553.5 x 10(6) and CFU-GM 1278.7 +/- 1346.3 x 10(4) per procedure. Separator collection efficiency on average was 49.91 +/- 23.28% for MNC, 55.1 +/- 35.66% for CFU-GM, and 62.97 +/- 23.09% for CD 34+ cells. Particularly interesting are results for MNC yields and CD 34+ efficiency; these results make the new program advantageous or similar to the most progressive blood cell separators and capable to collect a sufficient number of progenitor cells for a graft with a mean of 1.80 +/- 0.98 procedures per patient.

Micallef, I. N., P. J. Stiff, et al. (2009). "Successful stem cell remobilization using plerixafor (mozobil) plus granulocyte colony-stimulating factor in patients with non-hodgkin lymphoma: results from the plerixafor NHL phase 3 study rescue protocol." Biol Blood Marrow Transplant **15**(12): 1578-86.

In a phase 3 multicenter, randomized, double-blinded, placebo-controlled study of 298 patients with non-Hodgkin lymphoma (NHL), granulocyte colony-stimulating factor (G-CSF) plus plerixafor increased the proportion of patients who mobilized >or=5 x 10(6) CD34(+) hematopoietic stem cells (HSCs)/kg compared with placebo plus G-CSF (P < .001). Patients in either study arm who failed mobilization (< 0.8 x 10(6) CD34(+) cells/kg in 2 collections or <2 x 10(6) CD34(+) cells/kg in 4 collections) were eligible to enter the opened-label rescue protocol. Following a 7-day minimum rest period, these patients received G-CSF (10 microg/kg/day) for 4 days, followed by daily plerixafor (0.24 mg/kg) plus G-CSF and apheresis for up to 4 days. Of the 68 patients failing initial mobilization (plerixafor, n = 11; placebo, n = 57), 62 patients (91%) entered the rescue procedure (plerixafor, n = 10; placebo, n = 52). Four of 10 patients (40%) from the plerixafor group and 33 of 52 (63%) from the placebo group mobilized sufficient CD34(+) cells (>or= 2 x 10(6) cells/kg) for transplantation from the rescue mobilization alone (P = .11). Engraftment of neutrophils (11 days) and platelets (20 days) was similar to that in patients who did not fail initial mobilization, and all patients had durable grafts at the 12-month follow-up. Common plerixafor-related adverse events (AEs) included mild gastrointestinal (GI) effects and injection site reactions. There were no drug-related serious AEs. These data support that plerixafor plus G-CSF can safely and effectively remobilize patients with NHL who have failed previous mobilization.

Millington, M., A. Arndt, et al. (2009). "Towards a clinically relevant lentiviral transduction protocol for primary human CD34 hematopoietic stem/progenitor cells." PLoS One **4**(7): e6461.

BACKGROUND: Hematopoietic stem cells (HSC), in particular mobilized peripheral blood stem cells, represent an attractive target for cell and gene therapy. Efficient gene delivery into these target cells without compromising self-renewal and multi-potency is crucial for the success of gene therapy. We investigated factors involved in the ex vivo transduction of CD34(+) HSCs in order to develop a clinically relevant transduction protocol for gene delivery. Specifically sought was a protocol that allows for efficient transduction with minimal ex vivo manipulation without serum or other reagents of animal origin. METHODOLOGY/PRINCIPAL FINDINGS: Using commercially available G-CSF mobilized peripheral blood (PB) CD34(+) cells as the most clinically relevant target, we systematically examined factors including the use of serum, cytokine combinations, pre-stimulation time, multiplicity of infection (MOI), transduction duration and the use of spinoculation and/or retronectin. A self-inactivating lentiviral vector (SIN-LV) carrying enhanced green fluorescent protein (GFP) was used as the gene delivery vehicle. HSCs were monitored for transduction efficiency, surface marker expression and cellular function. We were able to demonstrate that efficient gene transduction can be achieved with minimal ex vivo manipulation while maintaining the cellular function of transduced HSCs without serum or other reagents of animal origin. CONCLUSIONS/SIGNIFICANCE: This study helps to better define factors relevant towards developing a standard clinical protocol for the delivery of SIN-LV into CD34(+) cells.

Moreau, P., C. Hullin, et al. (2006). "Tandem autologous stem cell transplantation in high-risk de novo multiple myeloma: final results of the prospective and randomized IFM 99-04 protocol." Blood **107**(1): 397-403.

The combination of high levels of beta2-microglobulin (beta2-m) and chromosome 13 deletion allows identification of a high-risk subgroup of patients with de novo multiple myeloma (MM). In this population of patients, we have evaluated the impact of a murine anti-interleukin 6 (anti-IL-6) monoclonal antibody (BE-8) as part of the second conditioning regimen in a multicenter prospective randomized trial of tandem autologous stem cell transplantation (ASCT). Conditioning for the first ASCT was accomplished with melphalan 200 mg/m2 and for the second one with melphalan 220 mg/m2 plus

dexamethasone with or without BE-8 infusion. This trial included 219 patients, of whom 166 were randomized, 85 without BE-8 (arm A) and 81 with BE-8 (arm B). The median overall survival (OS) and event-free survival (EFS) times of the whole group of patients were 41 and 30 months, respectively. Response rates, OS, and EFS were not different between the 2 arms of the trial. OS at 54 months was 46% in arm A and 51% in arm B (P = .90); median EFS was 35 months in arm A and 31 in arm B (P = .39). In high-risk patients the dose intensity of melphalan at 420 mg/m2 led to encouraging results, but the addition of anti-IL-6 monoclonal antibody to the second conditioning regimen did not improve either OS nor EFS.

Morita, E., Y. Watanabe, et al. (2008). "A novel cell transplantation protocol and its application to an ALS mouse model." Exp Neurol **213**(2): 431-8.

Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease, which selectively affects motor neurons throughout the central nervous system. The extensive distribution of motor neurons is an obstacle to applying cell transplantation therapy for the treatment of ALS. To overcome this problem, we developed a cell transplantation method via the fourth cerebral ventricle in mice. We used mouse olfactory ensheathing cells (OECs) and rat mesenchymal stem cells (MSCs) as donor cells. OECs are reported to promote regeneration and remyelination in the spinal cord, while MSCs have a capability to differentiate into several types of specific cells including neural cells. Furthermore both types of cells can be relatively easily obtained by biopsy in human. Initially, we confirmed the safety of the operative procedure and broad distribution of grafted cells in the spinal cord using wild-type mice. After transplantation, OECs distributed widely and survived as long as 100 days after transplantation, with a time-dependent depletion of cell number. In ALS model mice, OEC transplantation revealed no adverse effects but no significant differences in clinical evaluation were found between OEC-treated and non-transplanted animals. After MSC transplantation into the ALS model mice, females, but not males, showed a statistically longer disease duration than the non-transplanted controls. We conclude that intrathecal transplantation could be a promising way to deliver donor cells to the central nervous system. Further experiments to elucidate relevant conditions for optimal outcomes are required.

Movassagh, M., C. Desmyter, et al. (1998). "High-level gene transfer to cord blood progenitors using gibbon ape leukemia virus pseudotype retroviral vectors and an improved clinically applicable protocol." Hum Gene Ther **9**(2): 225-34.

The best methods for transducing hematopoietic progenitor cells usually involve either direct co-cultivation with virus-producing cells or human stromal supportive cells. However, these methods cannot be safely or easily applied to clinical use. Therefore, we aimed at improving retrovirus-mediated gene transfer into hematopoietic progenitors derived from cord blood CD34+ cells using viral supernatant to levels achieved at least with direct co-cultivation and under conditions that are suitable for clinical applications. In a first set of experiments, CD34+ cells were infected with supernatant containing amphotropic retroviral particles carrying the nls-lacZ reporter gene and the effects of centrifugation, cell adhesion to fibronectin, and Polybrene on the transduction of both clonogenic progenitors (CFC) and long-term culture initiating cells (LTC-IC) were studied. Transduction efficiency was evaluated on the percentage and total number of progenitors expressing the beta-galactosidase activity. Results show that a 48-hr infection of CD34+ cells with viral supernatant combining centrifugation at 1000 x g for 3 hr followed by adhesion to fibronectin allows transduction levels for both CFC and LTC-IC to be reached that are as good as using direct co-cultivation. In a second set of experiments, CD34+ cells were infected using this optimized protocol with pseudotyped retroviral particles carrying the gibbon ape leukemia virus (GALV) envelope protein. Under these conditions, between 50 and 100% of CFC and LTC-IC were transduced. Thus, we have developed a protocol capable of highly transducing cord blood progenitors under conditions suitable for a therapeutical use.

Moviglia, G. A., R. Fernandez Vina, et al. (2006). "Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients." Cytotherapy **8**(3): 202-9.

BACKGROUND: This is a preliminary report on successful results obtained during treatment of two patients with chronic spinal cord injury. The therapeutic approach was based on the generation of controlled inflammatory activity at the injury site that induced a microenvironment for the subsequent administration of autologous, BM-driven transdifferentiated neural stem cells (NSC). METHODS: BM mesenchymal stem cells (MSC) were cocultured with the patient's autoimmune T (AT) cells to be transdifferentiated into NSC. Forty-eight hours prior to NSC implant, patients received an i.v. infusion of 5 x 10(8) to 1 x 10(9) AT cells. NSC were infused via a feeding artery of the lesion site. Safety evaluations were performed everyday, from the day of

the first infusion until 96 h after the second infusion. After treatment, patients started a Vojta and Bobath neurorehabilitation program. RESULTS: At present two patients have been treated. Patient 1 was a 19-year-old man who presented paraplegia at the eight thoracic vertebra (T8) with his sensitive level corresponding to his sixth thoracic metamere (T6). He received two AT-NSC treatments and neurorehabilitation for 6 months. At present his motor level corresponds to his first sacral metamere (S1) and his sensitive level to the fourth sacral metamere (S4). Patient 2 was a 21-year-old woman who had a lesion that extended from her third to her fifth cervical vertebrae (C3-C5). Prior to her first therapeutic cycle she had severe quadriplegia and her sensitive level corresponded to her second cervical metamere (C2). After 3 months of treatment her motor and sensitive levels reached her first and second thoracic metameres (T1-T2). No adverse events were detected in either patient. DISCUSSION: The preliminary results lead us to think that this minimally invasive approach, which has minor adverse events, is effective for the repair of chronic spinal cord lesions.

Naujok, O., F. Francini, et al. (2008). "A new experimental protocol for preferential differentiation of mouse embryonic stem cells into insulin-producing cells." Cell Transplant **17**(10-11): 1231-42.

Mouse embryonic stem (ES) cells have the potential to differentiate into insulin-producing cells, but efficient protocols for in vitro differentiation have not been established. Here we have developed a new optimized four-stage differentiation protocol and compared this with an established reference protocol. The new protocol minimized differentiation towards neuronal progeny, resulting in a population of insulin-producing cells with beta-cell characteristics but lacking neuronal features. The yield of glucagon and somatostatin cells was negligible. Crucial for this improved yield was the removal of a nestin selection step as well as removal of culture supplements that promote differentiation towards the neuronal lineage. Supplementation of the differentiation medium with insulin and fetal calf serum was beneficial for differentiation towards monohormonal insulin-positive cells. After implantation into diabetic mice these insulin-producing cells produced a time-dependent improvement of the diabetic metabolic state, in contrast to cells differentiated according to the reference protocol. Using a spinner culture instead of an adherent culture of ES cells prevented the differentiation towards insulin-producing cells. Thus, prevention of cell attachment in a spinner culture represents a means to keep ES cells in an undifferentiated state and to inhibit differentiation. In conclusion, this study describes a new optimized four-stage protocol for differentiating ES cells to insulin-producing cells with minimal neuronal cell formation.

Olivero, S., T. Alario, et al. (1999). "CD34+ cell enumeration in peripheral blood and apheresis samples, using two laboratory diagnostic kits or an institutional protocol." Bone Marrow Transplant **23**(4): 387-94.

In order to prepare the substitution of a commercially available diagnostic kit, ProCOUNT (Becton Dickinson) or Stem-Kit (Coulter Immunotech), for our institutional protocol, we compared the three techniques for the numeration of CD34+ progenitor cells in 50 peripheral blood and 51 apheresis samples, obtained from cancer patients or healthy donors. We show here that the three techniques yield results of the same order of magnitude. Although statistical analyses demonstrate significant differences between the three methods, these differences turned out to be clinically insignificant in most situations. Observed differences mostly affect samples with the highest content of CD34+ cells, while the three assays provide equivalent results for values that are close to clinically relevant thresholds (20 x 10(3) CD34+ cells/ml in peripheral blood to start apheresis, and accumulated number above 3 x 10(6) CD34+ cells/kg to stop apheresis). This study also supports the view that institutional protocols can provide a highly reliable determination of CD34+ cells counts and percentages. However, because institutional protocols often use research reagents and vary from institution to institution, the use of diagnostic kits may be prefered as one way to improve quality assurance in the practice of cell therapy.

Omori, Y., O. Honmou, et al. (2008). "Optimization of a therapeutic protocol for intravenous injection of human mesenchymal stem cells after cerebral ischemia in adult rats." Brain Res **1236**: 30-8.

The systemic injection of human mesenchymal stem cells (hMSCs) prepared from adult bone marrow has therapeutic benefits after cerebral artery occlusion in rats, and may have multiple therapeutic effects at various sites and times within the lesion as the cells respond to a particular pathological microenvironment. However, the comparative therapeutic benefits of multiple injections of hMSCs at different time points after cerebral artery occlusion in rats remain unclear. In this study, we induced middle cerebral artery occlusion (MCAO) in rats using intra-luminal vascular occlusion, and infused hMSCs intravenously at a single 6 h time point (low and high cell doses) and various multiple time points after MCAO. From MRI analyses lesion volume was reduced in all hMSC cell injection groups as

compared to serum alone injections. However, the greatest therapeutic benefit was achieved following a single high cell dose injection at 6 h post-MCAO, rather than multiple lower cell infusions over multiple time points. Three-dimensional analysis of capillary vessels in the lesion indicated that the capillary volume was equally increased in all of the cell-injected groups. Thus, differences in functional outcome in the hMSC transplantation subgroups are not likely the result of differences in angiogenesis, but rather from differences in neuroprotective effects.

O'Neill, L. P., M. D. VerMilyea, et al. (2006). "Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations." Nat Genet **38**(7): 835-41.

Chromatin immunoprecipitation (ChIP) defines the genomic distribution of proteins and their modifications but is limited by the cell numbers required (ideally >10(7)). Here we describe a protocol that uses carrier chromatin and PCR, 'carrier' ChIP (CChIP), to permit analysis of as few as 100 cells. We assayed histone modifications at key regulator genes (such as Nanog, Pou5f1 (also known as Oct4) and Cdx2) by CChIP in mouse embryonic stem (ES) cells and in inner cell mass (ICM) and trophectoderm of cultured blastocysts. Activating and silencing modifications (H4 acetylation and H3K9 methylation) mark active and silent promoters as predicted, and we find close correlation between values derived from CChIP (1,000 ES cells) and conventional ChIP (5 x 10(7) ES cells). Studies on genes silenced in both ICM and ES cells (Cdx2, Cfc1, Hhex and Nkx2-2, also known as Nkx) show that the intensity of silencing marks is relatively diminished in ES cells, indicating a possible relaxation of some components of silencing on adaptation to culture.

Oriol, A., J. M. Ribera, et al. (2004). "Feasibility and results of autologous stem cell transplantation in de novo acute myeloid leukemia in patients over 60 years old. Results of the CETLAM AML-99 protocol." Haematologica **89**(7): 791-800.

BACKGROUND AND OBJECTIVES: The benefits of high-dose cytarabine, anthracyclines and hematopoietic stem cell transplantation in the treatment of acute myeloid leukemia (AML) are greater in younger rather than in older patients. We assessed the proportion of patients over 60 years with de novo AML who qualified for intensive therapy and determined the feasibility and results of autologous stem cell transplantation (ASCT) in first complete remission (CR). DESIGN AND METHODS: Induction therapy included idarubicin, cytarabine and etoposide. Patients who achieved CR received one cycle of mitoxantrone and cytarabine and ASCT as consolidation therapies. RESULTS: Over a 4-year period, 258 patients were registered of whom 135 (52%) were enrolled for intensive treatment. The CR rate was 61%, advanced age (p=0.033) and unfavorable cytogenetics (p=0.015) emerged as independent negative prognostic factors for CR. The 2-year overall survival (OS) was 23 % (CI 14%-30%) and was poorer in patients with unfavorable cytogenetics (p=0.035), age over 70 years (p=0.019) or leukocytosis (p=0.006). Only 27% of the potential candidates underwent ASCT. The probability of 2-year leukemia-free survival after consolidation was 39% (CI 6%-71%) for these patients and 22% (CI 6% - 39%) for candidate patients not undergoing ASCT (p=0.07). INTERPRETATION AND CONCLUSIONS: Over 25% of the patients 60 to 70 years with de novo AML benefit from standard intensive treatment. In these patients, ASCT has a tolerable toxicity and may have a positive impact on leukemia-free survival.

Patel, R. D., A. V. Vanikar, et al. (2009). "Ahmedabad tolerance induction protocol and chronic renal allograft dysfunction: pathologic observations and clinical implications." Diagn Pathol **4**: 4.

BACKGROUND: Chronic Renal Allograft Dysfunction (CRAD) is responsible for a large number of graft failures. We have abrogated acute T-cell rejections using Ahmedabad Tolerance Induction Protocol (ATIP) with hematopoietic stem cell transplantation (HSCT) under non-myeloablative conditioning pre-transplant. However B-cell mediated rejections and CRAD continue to haunt us. We carried out retrospective analysis of renal allograft biopsies performed in the last 4 years to evaluate the effect of ATIP on CRAD. MATERIALS AND METHODS: Biopsies diagnosed as per modified Banff criteria belonged to 2 groups: ATIP under low dose immunosuppression of cyclosporine/Azathioprine/Mycofenolate mofetil+ Prednisolone, subjected to donor leucocyte transfusion, anti-T/B cell antibodies, low dose target specific irradiation, cyclophosphamide, cyclosporin followed by HSCT pre-transplant; controls who opted out of ATIP were transplanted under standard triple drug immunosuppression. Demographics of both groups were comparable. RESULTS: Incidence of chronic changes was higher in controls (17.5%) vs. 10.98% in ATIP over a mean follow up of 151.9 months in the former and 130.9 months in the latter. Proteinuria and hypertension were higher in controls (48.4%) vs. ATIP (32.7%) with chronic transplant glomerulopathy, focal global sclerosis in 67.7% in controls vs. 46.7% in ATIP, acute on chronic T/B cell rejection in 51.6% controls vs. 28.1% ATIP, with peritubular capillary C4d deposits in 19.4% controls

vs. 1.9% ATIP biopsies. Acute on chronic calcineurin inhibitor toxicity was higher in ATIP (71.9%) vs. 48.4% in controls. CONCLUSION: Chronic immune injury was less with ATIP vs controls as compared to a higher incidence of chronic calcineurin inhibitor toxicity in the former.

Petsa, A., S. Gargani, et al. (2009). "Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications." In Vitro Cell Dev Biol Anim **45**(10): 573-6.

The Wharton's Jelly (WJ) of the umbilical cord (UC) is an excellent source of mesenchymal stem cells (MSCs) with a range of potential therapeutic applications. The present study was conducted to demonstrate the efficiency of the protocols used by Biogenea-Cellgenea Ltd. for isolation and expansion of WJ MSCs from donors across Greece. Umbilical cord samples were collected from 599 females following childbirth and processed for WJ MSC isolation. Stem cells were expanded using DMEM-based media and cell counts and overall viability figures derived using Trypan blue exclusion. To investigate the application of isolation and expansion protocols on samples received 1, 2, 3, 4 and 5 d after their collection, ten fresh samples were processed at these time intervals and evaluated. The cellular yield of most WJ samples was 1.1-5.0x10(6) cells at 21-30 d after processing. As culture time increased, cell counts decreased. Statistical analysis of mean cell counts showed a significant reduction after 21 d. Finally, we demonstrate for the first time that it is possible to obtain satisfactory cell numbers from samples processed 1, 2, 3, 4 and even 5 d after collection. We have derived favourable data on the protocols used at Biogenea-Cellgenea Ltd. to isolate and culture MSCs from the WJ. Protocol choice is crucial when handling large numbers of samples on a daily basis and should be made to ensure the best possible outcome.

Pierelli, L., G. Menichella, et al. (1993). "Evaluation of a novel automated protocol for the collection of peripheral blood stem cells mobilized with chemotherapy or chemotherapy plus G-CSF using the Fresenius AS104 cell separator." J Hematother **2**(2): 145-53.

Forty-seven peripheral blood stem cell (PBSC) collections were carried out on patients mobilized with chemotherapy and 63 on patients mobilized with chemotherapy plus G-CSF (Filgrastim), using the Fresenius AS104 cell separator and a novel automated PBSC collection protocol. As expected, cell yields were significantly higher in the series mobilized using chemotherapy plus G-CSF. The low platelet and red blood cell contamination permitted freezing of the apheresis product without further manipulation, other than plasma removal in both series. In patients mobilized with chemotherapy we obtained a MNC and a hemopoietic progenitor (CFU-GM, BFU-e, and CD34+ cells) collection efficiency comparable or superior to those reported by Bender (1992) with the Baxter CS3000 Plus after mobilization with cyclophosphamide. A significant decrease in MNC, BFU-e, and CD34+ cell collection efficiency was found in patients mobilized with chemotherapy plus G-CSF compared to those obtained in patients mobilized with chemotherapy alone. Ten patients achieved a prompt and stable engraftment after high dose chemotherapy and the infusion of cryopreserved PBSC collected using this protocol. Studies are in progress in order to improve MNC and hemopoietic progenitor collection efficiency in patients mobilized with G-CSF to obtain a graft in no more than one or two procedures.

Pirruccello, S. J., C. J. Page, et al. (1999). "Comparison of ISHAGE protocol CD34 cell enumeration with a lineage negative backgating technique." Cytotherapy **1**(4): 279-86.

BACKGROUND: CD34(+) cell enumeration in PBSC apheresis products has become the standard for assessing graft hematopoietic potential. METHODS: An in-house, three color, lineage negative-gating technique [University of Nebraska Medical Center (UNMC) protocol] for CD34 cell enumeration was compared with the ISHAGE protocol over 100 apheresis products. Cell doses determined by both methods were compared with each other and to colony-forming units-granulocyte/macrophage (CFU-GM) assay results. RESULTS: Overall, the assays compared well with each other for samples with CD34 cell doses > 0.2 2 10(6)/kg (r values > 0.8). The ISHAGE method showed a constant negative bias, with a mean of 38% in comparison to the UNMC protocol, which was more linear at lower cell doses. Both assays showed similar correlation with CFU-GM doses after log conversion (UNMC, r = 0.915; ISHAGE, r = 0.917). When comparing integer values, however, the ISHAGE method correlated with CFU-GM only in the high dose range (CFU-GM > 2 2 10(4)/kg), while the UNMC method correlated across the entire measured range of CFU-GM doses. Finally, an inter-technologist gating reproducibility study (n = 6) yielded a 23% coefficient of variation (CV) for the ISHAGE method and a 7% CV for the UNMC method, when the same two sets of CD34 histograms were analyzed to calculate cell dose. DISCUSSION: In this study the lineage negative protocol (UNMC) had a larger dynamic range, correlated better with

CFU-GM results and showed better inter-technologist reproducibility than the ISHAGE method.

Polikov, V. S., E. C. Su, et al. (2009). "Control protocol for robust in vitro glial scar formation around microwires: essential roles of bFGF and serum in gliosis." J Neurosci Methods **181**(2): 170-7.

Previously, we reported an in vitro cell culture model that recreates many of the hallmarks of glial scarring around electrodes used for recording in the brain; however, the model lacked the reproducibility necessary to establish a useful characterization tool. This methods paper describes a protocol, modeled on protocols typically used to culture neural stem/precursor cells, that generates a predictable positive control of an intense scarring reaction. Six independent cell culture variables (growth media, seeding density, bFGF addition day, serum concentration in treatment media, treatment day, and duration of culture) were varied systematically and the resulting scars were quantified. The following conditions were found to give the highest level of scarring: Neurobasal medium supplemented with B27, 10% fetal bovine serum at treatment, 10 ng/ml b-FGF addition at seeding and at treatment, treatment at least 6 days after seeding and scar growth of at least 5 days. Seeding density did not affect scarring as long as at least 500,000 cells were seeded per well, but appropriate media, bFGF, and serum were essential for significant scar formation-insights that help validate the in vitro-based approach to understanding glial scarring. With the control protocol developed in this study producing a strong, reproducible glial scarring positive control with every dissection, this culture model is suitable for the in vitro study of the mechanisms behind glial scarring and neuroelectrode failure.

Ratei, R., G. Basso, et al. (2009). "Monitoring treatment response of childhood precursor B-cell acute lymphoblastic leukemia in the AIEOP-BFM-ALL 2000 protocol with multiparameter flow cytometry: predictive impact of early blast reduction on the remission status after induction." Leukemia **23**(3): 528-34.

Treatment response is a strong outcome predictor for childhood acute lymphoblastic leukemia (ALL). Here, we evaluated the predictive impact of flow cytometric blast quantification assays (absolute blast count, BC, and blast reduction rate, BRR) in peripheral blood (pB) and/or bone marrow (BM) at early time points of induction therapy (days 0, 8 and 15) on the remission status in the AIEOP-BFM-ALL 2000 protocol. At the single parameter level (905 patients), the strongest predictive parameter for the remission status as a dichotomous minimal residual disease (MRD) parameter (positive/negative) has been provided by the BC at day 15 in BM (cutoff: 17 blasts/microl; 50 vs 15%; odds ratio: 5.6; 95% confidence interval: 4.1-7.6, P<0.001), followed by the BRR at day 15 in BM and by the BC at day 8 in pB (odds ratios: 3.8 and 2.6, respectively). In the multiple regression analysis (440 patients), BC in pB (d0 and d8) and in BM (d15) as well as BRR at day 8 in pB provided significantly contributing variables with an overall correct prediction rate of 74.8%. These data show that the quantitative assessment of early response parameters, especially absolute BCs at day 15 in BM, has a predictive impact on the remission status after induction therapy.

Rippon, H. J., J. M. Polak, et al. (2006). "Derivation of distal lung epithelial progenitors from murine embryonic stem cells using a novel three-step differentiation protocol." Stem Cells **24**(5): 1389-98.

Embryonic stem cells (ESCs) are a potential source for the cell-based therapy of a wide variety of lung diseases for which the only current treatment is transplantation. However, distal lung epithelium, like many other endodermally derived somatic cell lineages, is proving difficult to obtain from both murine and human ESCs. We have previously obtained alveolar epithelium from ESCs, although final cell yield remained extremely low. Here, we present an optimized three-step protocol for the derivation of distal lung epithelial cells from murine ESCs. This protocol incorporates (a) treatment of early differentiating embryoid bodies with activin A to enhance the specification of the endodermal germ layer, followed by (b) adherent culture in serum-free medium and (c) the final application of a commercial, lung-specific medium. As well as enhancing the specification of distal lung epithelium, this protocol was found to yield cells with a phenotype most closely resembling that of lung-committed progenitor cells present in the foregut endoderm and the early lung buds during embryonic development. This is in contrast to our previous differentiation method, which drives differentiation through to mature type II alveolar epithelial cells. The derivation of a committed lung progenitor cell type from ESCs is particularly significant for regenerative medicine because the therapeutic implantation of progenitor cells has several clear advantages over the transplantation of mature, terminally differentiated somatic cells.

Rivadeneyra-Espinoza, L., B. Perez-Romano, et al. (2006). "Instrument- and protocol-dependent variation in the enumeration of CD34+ cells by flow cytometry." Transfusion **46**(4): 530-6.

BACKGROUND: The information regarding the minimum number of CD34+ cells that are

necessary to reconstitute hematopoiesis in patients undergoing peripheral blood progenitor cell transplantation is quite controversial. Some of the differences in these figures might be due to the selection of antibodies, staining protocols, and acquisition strategies for the flow cytometric enumeration of these cells. STUDY DESIGN AND METHODS: Twenty-seven human umbilical cord blood samples and 33 leukapheresis products were consecutively collected for this study. Cells were stained following two different protocols, both using monoclonal antibodies to CD45 and CD34, and analyzed by the same operator in two different flow cytometers to enumerate the percentage of CD34+ mononuclear cells. RESULTS: Relevant differences in the proportion of cells were encountered, and the correlation between the results yielded by both instruments and protocols, although statistically valid, was suboptimal. CONCLUSIONS: Both interinstrument and interprotocol variation can provide additional explanation for the redundantly reported discrepancies concerning the numbers of CD34 cells that suffice to secure hemopoietic grafting. These results point to the need for new and different standardization approaches in this clinically relevant field.

Schipper, L. F., Y. van Hensbergen, et al. (2007). "A sensitive quantitative single-platform flow cytometry protocol to measure human platelets in mouse peripheral blood." Transfusion **47**(12): 2305-14.

BACKGROUND: The NOD/SCID mouse is a widely used model for human cord blood (CB) transplantation. Engraftment is generally estimated with semiquantitative methods, measuring the percentage of human cells among mouse cells. To compare protocols aiming to improve hematopoietic recovery, quantitative methods to enumerate human cells would be preferred. This study describes a single-platform protocol to count human platelets (hPLTs) after transfusion and CB transplantation in the peripheral blood (PB) of the mouse. METHODS: With an anti-human CD41 antibody against hPLTs and counting beads, the sensitivity to detect hPLTs in mouse blood by flow cytometry was validated. PLT recovery after hPLT transfusions and PLT kinetics after transplantation with CB CD34+ cells was followed in time in NOD/SCID mice. RESULTS: hPLTs could be reliably detected to a level as low as 1 PLT per microL with this single-platform protocol, what appeared to be at least 10 times more sensitive than detection with the dual-platform protocol. To verify the applicability for mouse studies, hPLTs were measured serially in transfusion and transplantation studies in NOD/SCID mice. The results showed that earlier detection of PLT recovery was feasible with the single-platform protocol. CONCLUSION: A single-platform flow cytometry method can repeatedly measure low numbers of circulating hPLTs in the PB of the same mouse. This method may be helpful in search of new protocols aiming at accelerating PLT recovery after CB transplantation, but also in a number of clinical settings, such as monitoring PLT reconstitution after hematopoietic stem cell transplantation.

Schmidt, M., T. Simon, et al. (2008). "The prognostic impact of functional imaging with (123)I-mIBG in patients with stage 4 neuroblastoma >1 year of age on a high-risk treatment protocol: results of the German Neuroblastoma Trial NB97." Eur J Cancer **44**(11): 1552-8.

AIM/PURPOSE: (123)I-meta-iodobenzylguanidine ((123)I-mIBG) scintigraphy is well established for staging and evaluation of response in children with high-risk neuroblastoma but its prognostic value in highly intensive first-line treatment protocols is uncertain. The presence of any (123)I-mIBG positive tumour tissue was correlated with event-free survival (EFS) and overall survival (OS). PATIENTS AND METHODS: The prognostic impact of residual (123)I-mIBG uptake into the primary tumour and metastases for predicting outcome in 113 stage 4 neuroblastoma patients >1 year of the German Neuroblastoma Trial NB97 was assessed using a univariate log-rank test and multivariate Cox regression analysis. RESULTS: All patients had (123)I-mIBG positive disease at initial staging. After four courses of induction chemotherapy, 71% of patients were still (123)I-mIBG positive for the primary tumour and 61% for metastases. After six courses, 39% of patients had (123)I-mIBG uptake by the primary tumour and 45% residual (123)I-mIBG positive metastatic disease. The (123)I-mIBG status of the primary tumour site had no bearing on outcome. Residual (123)I-mIBG positive metastatic disease after four (3-y-EFS 25.7+/-5.3% versus 55.9+/-7.6%, p=0.009; 3-y-OS 49.8+/-6.1% versus 65.0+/-7.3%; p=0.021) and after six chemotherapy cycles (3-y-EFS 27.5+/-6.2% versus 47.4+/-6.4%, p=0.011; 3-y-OS 50.5+/-7.1% vs 60.0+/-6.4%, p=0.031) was associated with poor outcome. CONCLUSION: Functional imaging with (123)I-mIBG scintigraphy can identify poor responders with any persistent metastatic (123)I-mIBG uptake who are at a high risk of disease relapse. (123)I-mIBG response of the primary tumour site had no bearing on outcome.

Sen, A., M. S. Kallos, et al. (2004). "New tissue dissociation protocol for scaled-up production of neural stem cells in suspension bioreactors." Tissue Eng **10**(5-6): 904-13.

The successful dissociation of mammalian neural stem cell (NSC) aggregates (neurospheres) into a single-cell suspension is an important procedure when expanding NSCs for clinical use, or when performing important assays such as clonal analyses. Until now, researchers have had to rely primarily on destructive mechanical methods such as trituration with a pipette tip to break apart the aggregates. In this study we report on a new chemical dissociation procedure that is efficient, cost effective, reproducible, and much less harmful to murine NSCs than both mechanical and enzymatic techniques. This method, involving the manipulation of environmental pH levels, resulted in 40% higher measured cell densities and 15-20% higher viabilities compared with mechanical dissociation. Moreover, chemical dissociation resulted in the production of significantly less cellular debris. Chemical dissociation was found to have no adverse effects on the long-term proliferation of the NSCs, which retained the ability to proliferate, form neurospheres, self-renew, and exhibit multipotentiality. This chemical method represents a new approach for the dissociation of tissues.

Serke, S. and H. E. Johnsen (2001). "A European reference protocol for quality assessment and clinical validation of autologous haematopoietic blood progenitor and stem cell grafts." Bone Marrow Transplant **27**(5): 463-70.

Recently, the regulatory authorities have begun to show interest in haematopoietic stem cell products. On a professional rather than a regulatory basis, the International Society for Hematotherapy and Graft Engineering (ISHAGE) has established the Foundation for the Accreditation of Haematopoietic Cell Therapy (FACHT), which has drawn up guidelines for standards and accreditation of such activity. In Europe, the regulatory environment with regard to haematopoietic stem cell grafts, processing and storage are currently less stringent. However, in 1998 the European Joint Accreditation Committee Euro-ISHAGE/EBMT (JACIE) prepared a regulatory document 'Standards for Blood and Marrow Progenitor Cell Collection, Processing and Transplantation' which was approved by the EBMT General Assembly. The major objectives were to promote quality of medical and laboratory practice in haematopoietic progenitor cell transplantation. The standards extend and detail the pre-existing activity of EBMT centres including all phases of collection, processing and administration of these cells. This is the platform for the proposed reference protocol for CD34(+) cell enumeration and clinical validation of quality assessment to ensure that appropriate standards of work and product quality are established and will be maintained.

Soleimani, M. and S. Nadri (2009). "A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow." Nat Protoc **4**(1): 102-6.

We explain a protocol for straightforward isolation and culture of mesenchymal stem cells (MSCs) from mouse bone marrow (BM) to supply researchers with a method that can be applied in cell biology and tissue engineering with minimal requirements. Our protocol is mainly on the basis of the frequent medium change in primary culture and diminishing the trypsinization time. Mouse mesenchymal stem cells are generally isolated from an aspirate of BM harvested from the tibia and femoral marrow compartments, then cultured in a medium with Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) for 3 h in a 37 degrees C-5% CO(2) incubator. Nonadherent cells are removed carefully after 3 h and fresh medium is replaced. When primary cultures become almost confluent, the culture is treated with 0.5 ml of 0.25% trypsin containing 0.02% ethylenediaminetetraacetic acid for 2 min at room temperature (25 degrees C). A purified population of MSCs can be obtained 3 weeks after the initiation of culture.

Steigman, S. A., M. Armant, et al. (2008). "Preclinical regulatory validation of a 3-stage amniotic mesenchymal stem cell manufacturing protocol." J Pediatr Surg **43**(6): 1164-9.

PURPOSE: Because of the 4 to 6-month interval between a diagnostic amniocentesis and birth, clinical application of amniotic mesenchymal stem cell (AMSC)-based therapies demands a 3-stage cell manufacturing process, including isolation/primary expansion, cryopreservation, and thawing/secondary expansion. We sought to determine the feasibility and cell yield of such a staged cell manufacturing process, within regulatory guidelines. METHODS: Human AMSCs isolated from diagnostic amniocentesis samples (n = 11) were processed under Food and Drug Administration-accredited good manufacturing practice. Expanded cells were characterized by flow cytometry and cryopreserved for 3 to 5 months. Cell release criteria included more than 90% CD29+, CD73+, and CD44+; less than 5% CD34+ and CD45+; negative mycoplasma quantitative polymerase chain reaction (QPCR) and endotoxin assay; and at least 70% viability. RESULTS: Isolation and ample expansion of AMSCs was achieved in 54.5% (6/11) of the samples. Early processing and at least a 2-mL sample were necessary for reliable cell manufacturing. Cell yield before cryopreservation was 223.2 +/- 65.4 x 10(6) cells (44.6-fold expansion), plus a 14.7 x 10(6)-cell backup, after 36.3 +/- 7.8 days. Cell viability postthaw was 88%. Expanded cells

maintained a multipotent mesenchymal progenitor profile. CONCLUSIONS: Human amniotic mesenchymal stem cells can be manufactured in large numbers from diagnostic amniocentesis, by an accredited staged processing, under definite procurement guidelines. These data further support the viability of clinical trials of amniotic mesenchymal stem cell-based therapies.

Suthar, K., A. V. Vanikar, et al. (2008). "Renal transplantation in primary focal segmental glomerulosclerosis using a tolerance induction protocol." Transplant Proc **40**(4): 1108-10.

We report our experience of transplantation using Ahmedabad Tolerance Induction Protocol (ATIP) in primary focal segmental glomerulosclerosis (FSGS) known to recur leading to graft loss. METHODS: Twenty-four primary FSGS patients were transplanted at our centre from 1997 to 2007. The 15 ATIP patients using hematopoietic stem cell transplantation (HSCT) under minimum nonmyeloablative conditioning was performed using living donors. Recipient mean age was 28 years and mean follow-up is 4.4 years. In contrast, nine control subjects of mean age 36 years had 4.8-year follow-up and received standard immunosuppression. Our Institutional Ethics Committee approved ATIP. Donor-recipient HLA match was similar. RESULTS: Mean serum creatinine (SCr) values of the 12 ATIP patients who were doing well at 1, 3, 5 years and at present were: 1.62, 1.48, 1.6, and 1.92 mg%, respectively, on cyclosporine (1.5 +/- 1 mg/kg per day) and prednisone (7.5 mg/day). No recurrence was observed. One patient died at 19 months posttransplant following relentless acute rejection; two patients with unrelated grafts were lost due to acute rejection at 11 weeks and 2 years posttransplant. Two graft biopsies performed at 1 and 2 years posttransplant revealed unremarkable histology, seven showed acute CsA toxicity at 1 year posttransplant. No chronic changes were noted. Among control group B, with 5 of 9 functioning grafts, two were obtained from deceased and three from unrelated donors with mean SCr values at 1, 3, and 4 years as well as present, were 1.77, 1.65, 1.6, and 1.53 mg%, respectively. Four patients displayed recurrences at 8 days, 1 month, and 1 and 2 years posttransplant. One patient died at 1 year posttransplant; one patient received a subsequent cadaver donor graft and two are on maintenance dialysis. CONCLUSION: Transplantation under ATIP seemed to mitigate recurrence of primary FSGS.

Suzue, T., Y. Kawano, et al. (1994). "Cell processing protocol for allogeneic peripheral blood stem cells mobilized by granulocyte colony-stimulating factor." Exp Hematol **22**(9): 888-92.

Although there is a growing body of information available regarding restoration of hematopoiesis with peripheral blood stem cell (PBSC) autografts, few studies have explored this procedure using allografts. In this study with healthy donors, we investigated the feasibility of a protocol for mobilizing PBSC using recombinant human granulocyte colony-stimulating factor (G-CSF) and subsequent bulk depletion of T cells from apheresis-harvested cells. Nine informed healthy donors were given G-CSF subcutaneously at two different dosing schedules (5 micrograms/kg/d in five donors and 2 micrograms/kg/d in four) for 5 consecutive days, and serial changes in blood components, including hematopoietic progenitor cells, were monitored. After 5 days of stimulation with G-CSF, PBSCs were collected by apheresis, and yields were compared. The number of white blood cells (WBC) reached a plateau level on either day 2 (5 micrograms) or 3 (2 micrograms), but the numbers of red blood cells and platelets were not affected. Circulating colony-forming unit-granulocyte/macrophage (CFU-GM) levels started to increase 1 or 2 days after the increase in the WBC count. By performing a 3L apheresis, the number of CFU-GM harvested was 4.6 +/- 3.3 x 10(6) (mean +/- standard error of the mean [SEM]) in the 5-micrograms group and 1.8 +/- 0.7 x 10(6) in the 2-micrograms group. Different procedures for depleting T cells, including the use of L-phenylalanine methyl ester (PME) and flasks coated with anti-CD5/CD8 monoclonal antibodies or neuraminidase-treated sheep red blood cells (SRBC), were also tested on the harvested cells. We found that cell lysis with PME before selective removal of T cells was very effective in reducing the number of cells that required further processing and was suitable for routine use. However, our current procedure resulted in unsatisfactory depletion of T cells (99.5% removal) while retaining hematopoietic progenitor cells (7.5% recovery). Further research is required in this area.

Tewogbade, A., K. FitzGerald, et al. (2008). "Attitudes and practices of nurses on a pediatric cancer and stem cell transplant ward: adaptation of an oral care protocol." Spec Care Dentist **28**(1): 12-8.

This study evaluated nurses' current practices and understanding of oral health for hematology and oncology patients. A written questionnaire administered to 33 nurses on the pediatric cancer and blood disorders unit of Children's Medical Center of Dallas included questions on oral evaluation, oral hygiene, and case-based questions. Information gathered was used to develop an oral care protocol. The study found that nurses were proficient in

diagnosing obvious conditions including mucositis and pseudomembranous candidiasis, but they were less than proficient when diagnosing less easily recognizable conditions such as xerostomia. The nurses were found to have inadequate knowledge of the treatment and oral hygiene protocols for conditions that they could and could not diagnose. The protocol based on the questionnaire results included information gathering, oral hygiene protocol, evaluation of viral infection, evaluation of fungal infection, oral assessment guide, and treatment protocol. Through information gathering, an oral health care policy may be developed and implemented to aid in the treatment of children undergoing care for malignancies.

Thompson, J. S., C. Pomeroy, et al. (2004). "Use of a T cell-specific monoclonal antibody, T10B9, in a novel allogeneic stem cell transplantation protocol for hematologic malignancy high-risk patients." Biol Blood Marrow Transplant **10**(12): 858-66.

To reduce the toxicity of traditional conditioning regimens for allogeneic stem cell transplantation (allo-SCT), we used single-agent chemotherapy conditioning with either busulfan (total cumulative dose, 16 mg/kg) or melphalan (200 to 240 mg/m 2), followed by the anti-T cell-specific monoclonal antibody T10B9 (MEDI-500) daily for 3 days. T cell-replete SCT was performed from HLA-identical sibling donors. Acute graft-versus-host disease (aGVHD) prophylaxis consisted of 7 additional days of T10B9 and delayed onset of cyclosporine (ie, on day +4 or +5). Twenty-six high-risk hematologic malignancy patients were entered onto this study. All 24 patients who survived longer than 8 days engrafted, although 1 patient experienced late graft failure. Deaths occurred in 21 of 26 patients because of infection (n = 7), progression/recurrence of primary disease (n = 6), aGVHD (n = 4), regimen-related toxicity (n = 1), and other causes (n = 3). Five of these patients are enjoying disease-free survival with a median survival of 1193 days after allo-SCT. The conditioning regimen induced modulation of surface expression of CD3 (but not CD4 or CD8) and was associated with decreasing tumor necrosis factor-alpha (but not interleukin-6) serum levels. In conclusion, single-agent chemotherapy conditioning with T10B9 produced durable engraftment and long-term survival in some patients who would not have qualified for a traditional allo-SCT.

Tonks, A., A. J. Tonks, et al. (2005). "Optimized retroviral transduction protocol which preserves the primitive subpopulation of human hematopoietic cells." Biotechnol Prog **21**(3): 953-8.

Though both low-speed centrifugation and the use of fibronectin (Retronectin) fragments increase gene transduction efficiency, they still do not overcome the adverse effects of the presence of virus-containing medium (VCM). In this study, we improved transduction efficiency of primitive human hematopoietic cells by optimizing the conditions for preadsorbing culture dishes with retrovirus using a centrifugation protocol allowing subsequent infection to be carried out in the absence of VCM. We also demonstrate that preadsorbing tissue culture plates with retrovirus is dependent on the volume of VCM used for preadsorption and the length of centrifugation and the type of plasticware used but not on the temperature of centrifugation (4-33 degrees C). Direct exposure of CD34+ target cells to VCM depletes the primitive CD34+CD38neg subpopulation by more than 30%, whereas the optimized VCM-free infection protocol targets this population with equivalent efficiency but had no detrimental effects on CD34+CD38neg frequency. In summary, we demonstrate a high-frequency transduction protocol which preserves the therapeutically relevant primitive subpopulation of human hematopoietic cells.

Trarbach, T., S. Greifenberg, et al. (2000). "Optimized retroviral transduction protocol for human progenitor cells utilizing fibronectin fragments." Cytotherapy **2**(6): 429-38.

BACKGROUND: Retroviral transduction in the presence of fibronectin (FN) fragments has proven an efficient and clinically-applicable procedure for gene transfer into hematopoietic cells. So far, FN-based transduction protocols have been optimized primarily for transduction of stem cells, whereas for several therapeutic applications transduction of clonogenic progenitors (CFU) may be sufficient. METHODS: Transduction protocols for CFU were optimized by evaluating the effect of growth factors, timing of retroviral transduction, CD34-selection and heparin, using a neomycin-phosphotransferase (neo(R))-expressing retroviral vector. RESULTS: The presence of multiple growth factors during prestimulation and transduction, including the differentiating cytokines G-CSF or GM-CSF, substantially enhanced transduction of CFU. Best results were achieved when 24 h of prestimulation were followed by a 24-48 h transduction period in the presence of the CH-296 FN-fragment and IL-3, IL-11, SCF, erythropoietin (EPO), and GM-CSF. With this proto-col we observed highly efficient transduction of BM-derived CFU (90.7 +/- 8.8 % G 418-resistant colonies), even with retrovirus preparations of moderate infectious titer (5 x 10(4) - 2 x 10(5) CFU/mL). The number of CFU increased on average 2.6-fold (range 1.5-3.8) during the transduction

procedure. Selection of CD34(+) cells prior to transduction did not improve transduction efficiency. Heparin, even in concentrations as low as 2.0 microg/mL, significantly inhibited transduction of CFU on FN-fragments. DISCUSSION: An optimized protocol for retroviral gene transfer into human clonogenic progenitor cells that allows highly efficient transduction, even with moderate titer retroviral vectors, is presented.

Tsai, M. S., J. L. Lee, et al. (2004). "Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol." Hum Reprod **19**(6): 1450-6.

BACKGROUND: The aim of this study was to isolate mesenchymal stem cells (MSCs) from amniotic fluid obtained by second-trimester amniocentesis. METHODS: A novel two-stage culture protocol for culturing MSCs was developed. Flow cytometry, RT-PCR and immunocytochemistry were used to analyse the phenotypic characteristics of the cultured MSCs. Von Kossa, Oil Red O and TuJ-1 stainings were used to assess the differentiation potentials of MSCs. RESULTS: Amniotic fluid-derived MSCs (AFMSCs) were successfully isolated, cultured and enriched without interfering with the routine process of fetal karyotyping. Flow cytometry analyses showed that they were positive for SH2, SH3, SH4, CD29, CD44 and HLA-ABC (MHC class I), low positive for CD90 and CD105, but negative for CD10, CD11b, CD14, CD34, CD117, HLA-DR, DP, DQ (MHC class II) and EMA. Importantly, a subpopulation of Oct-4-positive cells was detectable in our cultured AFMSCs. Under specific culture conditions, AFMSCs could be induced to differentiate into adipocytes, osteocytes and neuronal cells. CONCLUSIONS: We demonstrate that human multipotent MSCs are present in second-trimester amniotic fluid. Considering the great potential of cellular therapy using fetal stem cells and the feasibility of intrauterine fetal tissue engineering, amniotic fluid may provide an excellent alternative source for investigation of human MSCs.

Tsyrulnyk, A. and R. Moriggl (2008). "A detailed protocol for bacterial artificial chromosome recombineering to study essential genes in stem cells." Methods Mol Biol **430**: 269-93.

Bacterial artificial chromosome (BAC) recombineering is a novel technique for DNA manipulation. It starts from an original chromosomal gene locus that is modified to introduce a transgene under the expression control of the original gene locus. In most cases a cell type specific promoter is chosen and the transgene is placed in a way that the exon containing the start codon is replaced. Alternatively, BACs such as the Rosa26 BAC are chosen because of their known open chromatin and ubiquitous promoter activity that allows a broad expression profile of the transgene in the whole body. Thus, transgenes can be overexpressed within their natural transcriptional regulatory circuit. BAC transgenes have a high tendency to maintain their appropriate chromatin status because the endogenous locus was expressed in different cell types. Here, we give a detailed protocol based on the original idea to choose a BAC approach until the injection of the modified BAC DNA that leads to the generation of novel transgenic mouse lines. As an example for a BAC mouse model suitable for the analysis of stem cell or hematopoietic stem cell functions, we chose modification of the locus for the transcription factor Stat3. Stat3 variants replace the wild-type Stat3 gene to study their function in particular in the earliest cell types of the body.

Twardowski, P. W., L. Smith-Powell, et al. (2008). "Biologic markers of angiogenesis: circulating endothelial cells in patients with advanced malignancies treated on phase I protocol with metronomic chemotherapy and celecoxib." Cancer Invest **26**(1): 53-9.

Preclinical studies demonstrate anti-angiogenic activity of low doses of chemotherapy; selective cox-2 inhibitors are also inhibitors of angiogenesis. Animal data indicates the presence of circulating endothelial cells (CEC), tumor-derived activated endothelial cells (AEC) and endothelial cell progenitors (ECP). Bone marrow-derived ECP have been shown to be incorporated into tumor vasculature. We conducted two combination Phase I studies of celecoxib with either cyclophosphamide or etoposide. Exploratory correlative studies were performed to evaluate the detectability of CEC, AEC and ECP in patients treated with these anti-angiogenic combinations. Patients were treated with oral cyclophosphamide at 50 mg daily or etoposide at 50 mg daily. Celecoxib was given at 400 mg twice daily. Blood samples were collected on days 0, 7, 28 and monthly until disease progression. Blood from healthy volunteers was collected on days 0 and 28. Peripheral mononuclear cells (PMNC) were isolated and stained with fluorescent antibodies and analyzed utilizing 5-color flow cytometry. Forty-four heavily pretreated patients (20 F; 24 M) with various solid tumors were enrolled. Median age was 65 (23-72). Therapy was well tolerated. No responses were seen. Six patients had stable disease for at least 16 weeks. The longest duration on therapy is 420 days in a patient with metastatic thymoma. Pre-therapy CEC were detected in cancer patients and normal controls with mean concentrations of 0.47 cells/uL and 0.14 cells/uL,

respectively. Mean ECP in patients and controls were 0.09 cells/uL and 0.03 cells/uL, respectively. No AEC were detected. No consistent changes were seen in CEC or ECP during therapy. The combinations of oral cyclophosphamide or etoposide at 50 mg daily with celecoxib at 400 mg twice daily are well tolerated with occasional prolonged disease stabilizations observed. CEC and ECP are detectable in cancer patients but their levels did not change significantly during therapy with our regimen. Further evaluation of CEC and ECP in patients treated with clinically more active anti-angiogenic therapies would be of interest.

Usui, N., N. Dobashi, et al. (2002). "Intensified daunorubicin in induction therapy and autologous peripheral blood stem cell transplantation in postremission therapy (Double-7 protocol) for adult acute myeloid leukemia." Int J Hematol **76**(5): 436-45.

To investigate whether an intensified dose of daunorubicin (DNR) in induction therapy and autologous peripheral blood stem cell transplantation (PBSCT) in the postremission period are effective treatments, we used a Double-7 protocol to treat adult patients with de novo acute myeloid leukemia (excluding M0 and M3). Induction therapy consisted of 40 mg/m2 of DNR intravenous drip infusion for 7 days and 200 mg/m2 of ara-C by continuous infusion for 7 days (7 + 7 DC regimen). Patients who achieved complete remission (CR) were given high-dose chemotherapy with autologous PBSCT in postremission therapy. Of the 22 assessable patients, 16 attained CR (73%). Disease-free survival (DFS) and overall survival (OS) at 3 years were 61.2% and 48.1%, respectively. Nine of the CR patients underwent PBSCT without therapy-related mortality. Patients in a favorable cytogenetic group (n = 7) attained 100% CR and long-term survival (71.4% DFS and 85.7% OS at 3 years). Thus, intensified DNR administration of 280 mg/m2 (40 mg/m2 per day for 7 days) in induction therapy for adult patients younger than 60 years of age might be optimal or at least comparable with the new anthracyclines such as idarubicin. In addition, autologous PBSCT in postremission therapy might improve DFS and OS, at least for patients in a favorable cytogenetic group, such as those with a t(8;21) abnormality.

van Dartel, D. A., N. J. Zeijen, et al. (2009). "Disentangling cellular proliferation and differentiation in the embryonic stem cell test, and its impact on the experimental protocol." Reprod Toxicol **28**(2): 254-61.

The mouse embryonic stem cell test (EST) was designed to predict embryotoxicity based on the inhibition of the differentiation of embryonic stem cells (ESC) into beating cardiomyocytes in combination with cytotoxicity data in monolayer ESC cultures and 3T3 cells. In the present study, we have tested a diverse group of chemicals in the EST, applying different exposure durations, in an attempt to discriminate between effects on proliferation and differentiation within the EST protocol. Chemicals tested were monobutyl phthalate (MBP), 6-aminonicotinamide (6-AN), 5-fluorouracil (5-FU) and 5-bromo-2'-deoxyuridine (BrdU). We showed that 5-FU and BrdU behaved principally different from MBP and 6-AN. 5-FU and BrdU specifically affected cell proliferation during the first three days of the EST protocol, as shown by EB size, protein concentration and cell cycle stage analysis. In addition, we studied the differentiation state of cells in the EST protocol with time to elucidate the transition of pluripotent ESC to more differentiated cell types. Analysis by flow cytometry of the pluripotency marker SSEA-1 in EST showed that although total SSEA-1 positive cells remained unchanged up to and including day 5, the signal intensity already decreased from day 3 onwards. Furthermore, RT-PCR data showed an upregulation of the mesodermal marker T at day 3, whereas the cardiac muscle marker Myh6 was upregulated from day 5 onwards. These findings confirm that proliferation and differentiation of ESC in the EST are highly intertwined processes. Based on these findings we suggest an amended EST protocol which could more clearly discriminate between proliferation and differentiation effects of chemicals within the same EST differentiation protocol. This proposal includes a cytotoxicity assessment in EB at day 3 of the EST after day 0-3 exposure, and cardiac muscle foci counts after exposure from day 3-10 in the EST.

Vieira Pinheiro, J. P., E. Ahlke, et al. (1999). "Pharmacokinetic dose adjustment of Erwinia asparaginase in protocol II of the paediatric ALL/NHL-BFM treatment protocols." Br J Haematol **104**(2): 313-20.

Native forms of asparaginase stem from different biological sources. Previously reported data from children treated with Erwinase showed significantly lower trough levels and pharmacokinetic dose intensity than after E. coli-derived preparations. Hence, schedule optimization was initiated to achieve relevant serum activities. 21 children on reinduction therapy received Erwinase on Mondays, Wednesdays and Fridays for 3 weeks (9 x 20000 IU/m2 i.v.) instead of 4x 10 000IU/m2 of E. coli asparaginase (twice weekly for 2 weeks). Asparaginase trough activities were measured as the primary parameter, targeting 100-200 IU/l after 2 d and >50 IU/l after 3 d. Concurrently, asparagine trough concentrations were monitored. The mean trough activity was 156+/-99

IU/l, with 2/108 samples showing no detectable activity. Regarding trough levels per individual (three or more measurements/patient), means ranged from 52+/-29 to 276+/-114 IU/l (20 patients, 106 samples), with nine, six, and five children inside, below, and above the target range, respectively. The mean 3 d trough activity was 50+/-39 IU/l (20 patients, 51 samples). In 11 of these samples no activity was measurable. Mean trough activities calculated per individual ranged from < 20-84+/-30 IU/l (14 patients, 42 samples) with seven children below the target limit of 50 IU/l and asparagine concentrations <0.2 - 1.5microM. We concluded that an increased dose of 9x20000 IU/m2 of Erwinia asparaginase within 3 weeks resulted in a pharmacokinetic dose intensity comparable to former observations made with 4 x 10 000IU/m2 of the E. coli product Crasnitin which is no longer marketed. High interindividual variability and the phenomenon of 'silent' inactivation necessitate monitoring wherever possible.

Ware, C. B. and S. W. Baran (2007). "A controlled-cooling protocol for cryopreservation of human and non-human primate embryonic stem cells." Methods Mol Biol **407**: 43-9.

Freeze storage of human embryonic stem (hES) cells has not proven effective using the methods employed for mouse ES (mES) cells, while rhesus ES (rhES) cells are only modestly effectively frozen using common mES cell methods. Because human and rhES cells are passaged and frozen in clusters that approximate the size of embryos, we employed a mammalian embryo freezing method to cryopreserve primate ES cells. This protocol involves freezing in a dimethylsulfoxide cryoprotectant using straws. An ice crystal seed is induced at -10 degrees C followed by controlled cooling at -1 degrees C per minute down to -33 degrees C with a plunge from there directly into liquid nitrogen (LN2) at -196 degrees C. Thaw is effected rapidly by moving the frozen cells directly from LN2 into a water bath and placing directly into culture medium without step-wise cryoprotectant removal. Using this protocol, we have increased the survival of human ES cells from < or = 1 to approximately 80% and rhES cells from approximately 30 to > or = 90%. Thus, this protocol describes a technically simple but effective means of long-term storage of primate ES cells.

Wierenga, P. K., J. H. Dillingh, et al. (1997). "Reduction of heat-induced haemotoxicity in a hyperthermic purging protocol of murine acute myeloid leukaemic stem cells by AcSDKP." Br J Haematol **99**(3): 692-8.

The tetrapeptide AcSDKP (Goralatide) is a cytokine with known inhibitory effects on cell proliferation. Many purging agents used in autologous bone marrow transplantation protocols, including hyperthermia, preferentially kill cycling cells. A pretreatment with Goralatide offers a possibility to reduce the haemotoxicity in many purging settings. The impact of Goralatide on the hyperthermic purging protocol was investigated in normal and myeloid leukaemic (SA8) murine cells. The median survival time after transplantation (i.e. leukaemia incidences) was used as an in vivo parameter to determine the effects on leukaemic cells. The hyperthermic effect on normal and leukaemic cells was also investigated in vitro using the cobblestone area-forming cell (CAFC) assay. A heat treatment of 90 min at 43 degrees C resulted in a 4-log depletion of leukaemic stem cells. For normal progenitor cells (CFU-GM) a 2-log cell kill was shown. The reduction in proliferative activity of the CFU-GM after an 8 h incubation with 10(-9) M Goralatide resulted in a decrease in the heat sensitivity of the progenitor subset to approximately a 1-log cell kill. The leukaemic precursor cells seem insensitive to Goralatide inhibition, implicating an increase in the therapeutic window of the hyperthermic purging protocol. Finally, simulated remission bone marrow (5% leukaemic blasts) was incubated with Goralatide followed by a heat treatment of 90 min at 43 degrees C. Lethally irradiated (10 Gy) mice transplanted with heat-treated remission bone marrow (10(6) normal bone marrow cells versus 5 x 10(4) leukaemic cells) died of aplasia while Goralatide-pretreated remission bone marrow could rescue the irradiated mice without revealing leukaemic engraftment. These findings confirmed the enhanced protection against hyperthermia of the normal haemopoietic subsets by Goralatide and thus increased the success of the hyperthermic purging protocol.

Williams, C. B., S. D. Day, et al. (2004). "Dose modification protocol using intravenous busulfan (Busulfex) and cyclophosphamide followed by autologous or allogeneic peripheral blood stem cell transplantation in patients with hematologic malignancies." Biol Blood Marrow Transplant **10**(9): 614-23.

We evaluated the safety and toxicity through a 5-cohort dose-modification model of once-daily administration of IV busulfan (Bu) in combination with high-dose cyclophosphamide (Cy) as preparative therapy for stem cell transplantation. Twenty-one adult patients with hematologic malignancies were evaluated. Eleven patients underwent autologous and 10 patients underwent HLA-matched sibling allogeneic transplantation. Patients were sequentially enrolled into 5 cohorts. Cohort 1 received intravenous (IV) Bu 1.6 mg/kg every 12 hours for 2 doses and then 0.8 mg/kg every 6 hours for 12 doses; cohort 2

received IV Bu 1.6 mg/kg every 12 hours for 4 doses and then 0.8 mg/kg every 6 hours for 8 doses; cohort 3 received IV Bu 3.2 mg/kg for 1 dose and then 1.6 mg/kg every 12 hours for 2 doses and 0.8 mg/kg every 6 hours for 8 doses; cohort 4 received IV Bu 3.2 mg/kg every 24 hours for 2 doses and then 0.8 mg/kg every 6 hours for 8 doses; and cohort 5 received IV Bu 3.2 mg/kg every 24 hours for 4 doses. In all groups, Bu was administered on day -7 through day -4 and was followed at least 6 hours after the last Bu dose by Cy 60 mg/kg daily for 2 doses on days -3 and -2. Blood samples were collected for pharmacokinetic analysis on the first and last day of IV Bu administration. All patients were alive and had engrafted at day 30. Five patients developed grade 3 or 4 toxicities. Four patients developed hepatic abnormalities, and 3 exhibited evidence of veno-occlusive disease. Two of 3 patients in cohort 5 with a Bu area under the curve >6000 micromol/min developed autopsy-confirmed veno-occlusive disease. Interpatient variability in AUCs was observed in patients within and between cohorts, but no statistically significant interpatient differences were observed in Bu half-life, volume of distribution, clearance, or dose-adjusted area under the curve. Further, minimal variability in Bu pharmacokinetics was observed between the 2 evaluations performed in each patient, thus reflecting the stability of Bu disposition within individual patients. On the basis of the dosing guidelines and schedule outlined in this study, our data suggest that administration of IV Bu 3.2 mg/kg IV every 24 hours for 4 doses in combination with Cy may result in excessive toxicity.

Xu, L., S. K. Stahl, et al. (1994). "Correction of the enzyme deficiency in hematopoietic cells of Gaucher patients using a clinically acceptable retroviral supernatant transduction protocol." Exp Hematol **22**(2): 223-30.

Gaucher disease is a lysosomal storage disorder caused by a deficiency of the enzyme glucocerebrosidase (GC), and is an excellent candidate for gene replacement therapy. To develop a clinically acceptable protocol for this purpose, we created two amplified (A) high-titer retroviral vector-producer cell lines to efficiently transduce hematopoietic stem and progenitor cells. GP+envAm12/A-LGSN (A-LGSN), contained the GC cDNA driven by the retroviral long terminal repeat (LTR) and the neomycin phosphotransferase gene expressed from the simian virus 40 early promoter. GP+envAm12/A-LG4 (A-LG4) contained only the GC gene driven by the LTR. Both A-LGSN and A-LG4 contained multiple proviral copies and gave approximately 10-fold higher titers on 3T3 cells compared to their unamplified counterparts. These vectors were packaged in GP+envAm12 cells because vectors produced in this cell line transduced hematopoietic cells more efficiently than other packaging cells tested. Bone marrow mononuclear cells and purified CD34+ cells were infected with virus supernatants four times in the presence of interleukin-3 (IL-3), IL-6, and stem cell factor (SCF) over 96 hours in culture. Cells were then plated in semisolid cultures and colony-forming unit-granulocyte/macrophage (CFU-GM) colonies were scored for vector presence by polymerase chain reaction (PCR). Transduction efficiency of CFU-GM colonies derived from CD34+ cells was improved considerably using the amplified vectors in the GP+envAm12 packaging line. For A-LGSN, A-LG4, and unamplified LGSN, transduction efficiencies were 41, 42, and 25%, respectively. Therefore, multiple proviral copies resulting in higher titer improves retroviral transduction of human hematopoietic progenitor cells. Hematopoietic cells from Gaucher patients were transduced and placed into long-term bone marrow culture (LTBMC). Viral supernatant from the amplified producer lines transduced long-term culture initiating cells (LTCIC) efficiently (30 to 50%) using this clinically acceptable protocol. Both sustained mRNA expression and GC enzyme production are achieved in the long-term culture of LTCIC and lead to correction of the GC deficiency in their progeny cells.

Yamagata, K., K. Onizawa, et al. (2006). "A prospective study to evaluate a new dental management protocol before hematopoietic stem cell transplantation." Bone Marrow Transplant **38**(3): 237-42.

Pre-hematopoietic stem cell transplantation (HSCT) dental treatment is essential to prevent serious infections from oral sources during immunosuppression, in patients who undergo HSCT therapy. This study was planned to establish a dental management protocol for such patients. Forty-one patients scheduled for HSCT to treat hematological malignancies were consecutively enrolled in the prospective trial. The dental status of all patients was evaluated by clinical and radiographic examination at a median of 47 days before the commencement of HSCT therapy. Thirty-six patients had one or more dental diseases; the remaining five had none. Caries was found in 26 patients, apical periodontitis in 19, marginal periodontitis in 24 and a partially erupted third molar in 11. Our policy is to preserve patients' teeth whenever possible, and therefore minimal dental intervention was planned. Treatment was completed for all 36 patients with dental pathologies, before the conditioning regimen began. All patients received the scheduled HSCT therapy without alteration,

interruption or delay, and did not show any signs or symptoms associated with odontogenic infection while they were immunosuppressed. This protocol, therefore, appears to be appropriate for the pre-HSCT dental treatment of patients with hematological diseases.

Yang, P. F., T. C. Hua, et al. (2006). "Cryopreservation of human embryonic stem cells: a protocol by programmed cooling." Cryo Letters **27**(6): 361-8.

Human embryonic stem (ES) cells have far-reaching applications in the areas of tissue engineering, regenerative medicine, pharmacology and basic scientific research. Although the culture conditions can maintain the human ES cells in an undifferentiated state for a transient period, spontaneous differentiation has also been observed during the routine culturing of ES cells. However, the maintenance of ES cells in the undifferentiated, pluripotent state for extended periods of time will be required in many areas of scientific research. Cryopreservation is a technology with potentially far reaching implication for the development and widespread use of such cell lines. This study was undertaken to develop and optimize a protocol for cryopreservation of human ES cells through programmed cooling. The effects of the seeding temperature, the cooling rate and the sub-zero temperature to which the samples were cooled before plunging into liquid nitrogen(the terminal temperature), all significantly affected the recovery of cryopreserved ES cells. After studying these factors, an improved protocol was obtained: the sample was cooled from 0 degree C to -35 degree C at a cooling rate of 0.5 degree per min, with seeding was set at -10 degree C, before being plunged immediately into the liquid nitrogen. Using this protocol, 9 of 11 colony fragments survived freezing and thawing and could be cultured for prolonged periods. They retained the properties of pluripotent cells, had a normal karyotype and showed histochemical staining for alkaline phosphatase.

Zhao, Y., A. Raouf, et al. (2007). "A modified polymerase chain reaction-long serial analysis of gene expression protocol identifies novel transcripts in human CD34+ bone marrow cells." Stem Cells **25**(7): 1681-9.

Transcriptome profiling offers a powerful approach to investigating developmental processes. Long serial analysis of gene expression (LongSAGE) is particularly attractive for this purpose because of its inherent quantitative features and independence of both hybridization variables and prior knowledge of transcript identity. Here, we describe the validation and initial application of a modified protocol for amplifying cDNA preparations from <10 ng of RNA (<10(3) cells) to allow representative LongSAGE libraries to be constructed from rare stem cell-enriched populations. Quantitative real-time polymerase chain reaction (Q-RT-PCR) analyses and comparison of tag frequencies in replicate LongSAGE libraries produced from amplified and nonamplified cDNA preparations demonstrated preservation of the relative levels of different transcripts originally present at widely varying levels. This PCR-LongSAGE protocol was then used to obtain a 200,000-tag library from the CD34+ subset of normal adult human bone marrow cells. Analysis of this library revealed many anticipated transcripts, as well as transcripts not previously known to be present in CD34+ hematopoietic cells. The latter included numerous novel tags that mapped to unique and conserved sites in the human genome but not previously identified as transcribed elements in human cells. Q-RT-PCR was used to demonstrate that 10 of these novel tags were expressed in cDNA pools and present in extracts of other sources of normal human CD34+ hematopoietic cells. These findings illustrate the power of LongSAGE to identify new transcripts in stem cell-enriched populations and indicate the potential of this approach to be extended to other sources of rare cells. Disclosure of potential conflicts of interest is found at the end of this article.

Zhou, J. M., J. X. Chu, et al. (2008). "An improved protocol that induces human embryonic stem cells to differentiate into neural cells in vitro." Cell Biol Int **32**(1): 80-5.

Human embryonic stem (ES) cells have the capacity for self-renewal and are able to differentiate into any cell type. However, obtaining high-efficient neural differentiation from human ES cells remains a challenge. This study describes an improved 4-stage protocol to induce a human ES cell line derived from a Chinese population to differentiate into neural cells. At the first stage, embryonic bodies (EBs) were formed in a chemically-defined neural inducing medium rather than in traditional serum or serum-replacement medium. At the second stage, rosette-like structures were formed. At the third stage, the rosette-like structures were manually selected rather than enzymatically digested to form floating neurospheres. At the fourth stage, the neurospheres were further differentiated into neurons. The results show that, at the second stage, the rate of the formation of rosette-like structures from EBs induced by noggin was 88+/-6.32%, higher than that of retinoic acid 55+/-5.27%. Immunocytochemistry staining was used to confirm the neural identity of the cells. These results show a

major improvement in obtaining efficient neural differentiation of human ES cells.

11. Selected Protocols for Stem Cell Researches

11.1 INFT2 Protocol

Hematologic malignancies (blood cell cancers) in very young children are hard to treat with standard doses of chemotherapy (anti-cancer drugs). Stem cell transplantation (infusion of healthy blood forming cells) has been used but has not always been successful. The best donor of stem cells is a sibling (brother or sister) who is a match (the sibling's cells match the subject's immune type, or HLA type). But few very young children with leukemia have a matched sibling donor. This research study is for those children who do not have a matched sibling donor. In this study, a parent will be the stem cell donor. Using a parent donor (a parent donor is a partial match for the subject's HLA type) increases the risk of graft-versus-host disease (GVHD). GVHD occurs when the donor cells (the graft) recognize that the body tissues of the child (the host) are different. Because severe GVHD can be life-threatening, the parent's stem cells will be filtered using a machine called the CliniMACS system, which removes the cells that cause GVHD. This system has not been approved by the Food and Drug Administration (FDA) and is considered experimental. In addition to the stem cell transplant, parent donor natural killer (NK) cells will be given. NK cells are special cells in the immune system (the body organs and cells that defend the body against infection and disease) that target cancer cells. NK cells may help donor cells to grow and may reduce the chance of GVHD. In this experimental treatment, chemotherapy will be used in addition to the stem cell and NK cell transplants. It is unknown if these treatments will work better than the treatments now being used to treat very young children with hematologic malignancies. **(**Leung, 2007**)**

11.2 OPBMT2 Protocol

Malignant osteopetrosis **is a** genetic disease in which cells in the bone tissue (osteoclasts) do not function properly. These cells are unable to perform their biological job of breaking down old bone tissue as new bone tissue is being made. This causes the bone tissue to build up, producing thick bones that do not work properly and causing the child to lose his/her bone marrow space, where red cells, platelets, and white cells are made.

Stem cell transplantation from an allogeneic donor is the only known cure for this disease. Stem cells are immature cells found in the bone marrow that can grow into other kinds of cells. An allogeneic donor is another person who provides the stem cells. There are three types of donors:

(1) A matched sibling donor (brother or sister) is the ideal treatment, but is not possible for the majority of patients.

(2) A matched unrelated donor may also be used, but finding such a match may take several months. During this time the disease may get worse; the child may need red cell or platelet transfusions as the child may be unable to make these cells and permanent damage to vision and hearing may occur.

(3) A haploidentical parental donor (a mother or father), has not been studied previously as a treatment for malignant osteopetrosis.

This study is designed to use a haploidentical parental donor in the event that a matched sibling donor is unavailable. Using a parental donor would enable transplantation earlier in the disease process than waiting for a matched unrelated donor. This might reduce the chance of the disease getting worse before the transplant is done. With a parental donor, the risk of graft rejection (the patient's body will not accept and allow donated cells to grow) may be greater than the risk of rejection using a matched sibling donor.

The purpose of this study is to learn more about the cause and treatment of malignant osteopetrosis. It is designed to determine if children with malignant osteopetrosis can properly accept a parental donor transplant and to study the genetic (characteristics carried by genes) factors which cause the disease (Kasow, 2007).

11.3 SCDHAP Protocol

Sickle cell disease is a lifelong blood condition that can cause damage to the brain and other organs of the body. Children may develop severe clinical states with recurrent vaso-occlusive crises (VOC) which can cause severe pain, acute chest syndrome (ACS) and/or stroke. Treatment may include blood transfusions which may be required to prevent some of the conditions caused by this disease. Unfortunately, blood transfusions can cause iron overload, which can lead to severe and sometimes fatal complications.

Stem cells are young blood cells that can grow to make new blood cells such as white blood cells that help fight infections, platelets that help the blood to clot, and red blood cells that carry oxygen to the vital organs of the body. These cells may be taken from one individual (donor) and given to another (recipient). These stem cells, when placed in the body of the recipient, travel through the body to the bone

marrow space and begin to grow and make new blood cells.

A stem cell transplant has been shown to help, and possibly cure, patients with sickle cell disease. Stem cells taken from a brother or sister may provide bone marrow that is a perfect match (same tissue type) for the recipient. Unfortunately, only about 10-20% of sickle cell patients have a matched sibling donor. Stem cells from partially matched (partial tissue match) family members have been tried with a few children with sickle cell disease. The risk and benefits of these types of transplants are not as well known as transplants using a matched donor. When children with sickle cell disease have no matched brother or sister donor, allogeneic transplants are a possible treatment available for these patients (Paul Woodard, 2007)

11.4 SCT521 (COG # ASCT0521) Protocol

Idiopathic pneumonia syndrome is a complication that may occur in children who have had a stem cell transplant. Often patients with pneumonia have a cough and chest pain, are short of breath, or require oxygen to help them breathe. In some transplant patients, pneumonia is caused by a bacteria or virus. However, with idiopathic pneumonia syndrome, pneumonia occurs in the absence of infection. Despite corticosteroids and supportive care, this condition may be fatal.

This research study will use a drug named etanercept. The drug has been approved by the Food and Drug Administration (FDA) for the treatment of certain joint or skin conditions in children over 4 years of age. Etanercept works by blocking the effects of a protein known as Tumor Necrosis Factor (TNF). TNF has been found in lung fluid from patients with idiopathic pneumonia syndrome. TNF may be involved in the development of lung injury in idiopathic pneumonia syndrome. An earlier study has determined the largest amount of etanercept that can be given without causing bad effects. A small research study has been done with adults and children with idiopathic pneumonia syndrome. Etanercept was found to be safe, and several patients had improvement in their breathing (Madden, 2007).

References

1. Abe, Y., K. Kouyama, et al. (2003). "Analysis of neurons created from wild-type and Alzheimer's mutation knock-in embryonic stem cells by a highly efficient differentiation protocol." J Neurosci **23**(24): 8513-25.
2. Aker, M., G. Varadi, et al. (1999). "Fludarabine-based protocol for human umbilical cord blood transplantation in children with Fanconi anemia." J Pediatr Hematol Oncol **21**(3): 237-9.
3. Alvarez, I., A. Sureda, et al. (2006). "Nonmyeloablative stem cell transplantation is an effective therapy for refractory or relapsed hodgkin lymphoma: results of a spanish prospective cooperative protocol." Biol Blood Marrow Transplant **12**(2): 172-83.
4. Antonucci, I., I. Iezzi, et al. (2009). "Isolation of osteogenic progenitors from human amniotic fluid using a single step culture protocol." BMC Biotechnol **9**: 9.
5. Astori, G., W. Malangone, et al. (2001). "A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use." Blood Cells Mol Dis **27**(4): 715-24; discussion 725-7.
6. Ayash, L. J., M. Clarke, et al. (2001). "Clinical protocol. Purging of autologous stem cell sources with bcl-x(s) adenovirus for women undergoing high-dose chemotherapy for stage IV breast carcinoma." Hum Gene Ther **12**(16): 2023-5.
7. Balint, B., M. Ljubenov, et al. (2008). "Stem cell harvesting protocol research in autologous transplantation setting: large volume vs. conventional cytapheresis." Vojnosanit Pregl **65**(7): 545-51.
8. Balint, B., Z. Ivanovic, et al. (1999). "The cryopreservation protocol optimal for progenitor recovery is not optimal for preservation of marrow repopulating ability." Bone Marrow Transplant **23**(6): 613-9.
9. Brice, P., D. Simon, et al. (2000). "High-dose therapy with autologous stem-cell transplantation (ASCT) after first progression prolonged survival of follicular lymphoma patients included in the prospective GELF 86 protocol." Ann Oncol **11**(12): 1585-90.
10. Budak-Alpdogan, T., M. Przybylowski, et al. (2006). "Functional assessment of the engraftment potential of gammaretrovirus-modified CD34+ cells, using a short serum-free transduction protocol." Hum Gene Ther **17**(7): 780-94.
11. Cavaleri, F., L. Gentile, et al. (2006). "Recombinant human albumin supports development of somatic cell nuclear transfer embryos in mice: toward the establishment of a chemically defined cloning protocol." Cloning Stem Cells **8**(1): 24-40.
12. Chapple, P., H. M. Prince, et al. (2000). "Comparison of three methods of CD34+ cell enumeration in peripheral blood: dual-platform ISHAGE protocol versus single-platform, versus microvolume fluorimetry." Cytotherapy **2**(5): 371-6.
13. Coleman, B., N. A. Rickard, et al. (2009). "A protocol for cryoembedding the adult guinea pig cochlea for fluorescence immunohistology." J Neurosci Methods **176**(2): 144-51.
14. Corso, A., M. G. Hogeweg-Platenburg, et al. (1993). "A protocol for the enrichment of different types of CFU-S from fetal mouse liver." Haematologica **78**(1): 5-11.
15. Dal Negro, G., L. Vandin, et al. (2006). "A new experimental protocol as an alternative to the colony-forming unit-granulocyte/macrophage (CFU-GM) clonogenic assay to assess the haematotoxic potential of new drugs." Toxicol In Vitro **20**(5): 750-6.
16. Dalbagni, G., W. Han, et al. (1997). "Evaluation of the telomeric repeat amplification protocol (TRAP) assay for telomerase as a diagnostic modality in recurrent bladder cancer." Clin Cancer Res **3**(9): 1593-8.
17. Davis, R. P., M. Costa, et al. (2008). "A protocol for removal of antibiotic resistance cassettes from human embryonic stem cells genetically modified by homologous recombination or transgenesis." Nat Protoc **3**(10): 1550-8.
18. de la Fuente, J., S. Reiss, et al. (2003). "Non-TBI stem cell transplantation protocol for Fanconi anaemia using HLA-compatible sibling and unrelated donors." Bone Marrow Transplant **32**(7): 653-6.
19. Deola, S., S. Scaramuzza, et al. (2004). "Mobilized blood CD34+ cells transduced and selected with a clinically applicable protocol reconstitute lymphopoiesis in SCID-Hu mice." Hum Gene Ther **15**(3): 305-11.

20. Duda, D. G., K. S. Cohen, et al. (2007). "A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood." Nat Protoc **2**(4): 805-10.
21. Eduardo, F. P., L. Bezinelli, et al. (2009). "Severity of oral mucositis in patients undergoing hematopoietic cell transplantation and an oral laser phototherapy protocol: a survey of 30 patients." Photomed Laser Surg **27**(1): 137-44.
22. Federico, M., V. Clo, et al. (1996). "High-dose therapy autologous stem cell transplantation vs conventional therapy for patients with advanced Hodgkin's disease responding to first-line therapy: analysis of clinical characteristics of 51 patients enrolled in the HD01 protocol. EBMT/ANZLG/Intergroup HD01 Trial." Leukemia **10 Suppl 2**: s69-71.
23. Festag, M., C. Sehner, et al. (2007). "An in vitro embryotoxicity assay based on the disturbance of the differentiation of murine embryonic stem cells into endothelial cells. I: Establishment of the differentiation protocol." Toxicol In Vitro **21**(8): 1619-30.
24. Fico, A., G. Manganelli, et al. (2008). "High-throughput screening-compatible single-step protocol to differentiate embryonic stem cells in neurons." Stem Cells Dev **17**(3): 573-84.
25. Fontelonga, A., A. J. Kelly, et al. (1997). "A novel high-dose chemotherapy protocol with autologous hematopoietic rescue in patients with metastatic breast cancer or recurrent non-Hodgkin's lymphoma." Bone Marrow Transplant **19**(10): 983-8.
26. Freie, B. W., P. Dutt, et al. (1996). "Correction of Fanconi anemia type C phenotypic abnormalities using a clinically suitable retroviral vector infection protocol." Cell Transplant **5**(3): 385-93.
27. Goebel, W. S., M. C. Yoder, et al. (2002). "Donor chimerism and stem cell function in a murine congenic transplantation model after low-dose radiation conditioning: effects of a retroviral-mediated gene transfer protocol and implications for gene therapy." Exp Hematol **30**(11): 1324-32.
28. Gunetti, M., I. Ferrero, et al. (2008). "Refreezing of cord blood hematopoietic stem cells for allogenic transplantation: in vitro and in vivo validation of a clinical phase I/II protocol in European and Italian Good Manufacturing Practice conditions." Exp Hematol **36**(2): 235-43.
29. Hacein-Bey, S., F. Gross, et al. (2001). "Optimization of retroviral gene transfer protocol to maintain the lymphoid potential of progenitor cells." Hum Gene Ther **12**(3): 291-301.
30. Haioun, C., E. Lepage, et al. (2000). "Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol--a groupe d'Etude des lymphomes de l'Adulte study." J Clin Oncol **18**(16): 3025-30.
31. Harris, E., S. Paneesha, et al. (2002). "Burkitt's lymphoma: single-centre experience with modified BFM protocol." Clin Lab Haematol **24**(2): 111-4.
32. Hennemann, B., E. Conneally, et al. (1999). "Optimization of retroviral-mediated gene transfer to human NOD/SCID mouse repopulating cord blood cells through a systematic analysis of protocol variables." Exp Hematol **27**(5): 817-25.
33. Horn, P. A., K. A. Keyser, et al. (2004). "Efficient lentiviral gene transfer to canine repopulating cells using an overnight transduction protocol." Blood **103**(10): 3710-6.
34. Hristov, M., S. Schmitz, et al. (2009). "An optimized flow cytometry protocol for analysis of angiogenic monocytes and endothelial progenitor cells in peripheral blood." Cytometry A **75**(10): 848-53.
35. Hurd, D. D. and W. P. Peters (1995). "Randomized, comparative study of high-dose (with autologous bone marrow support) versus low-dose cyclophosphamide, cisplatin, and carmustine as consolidation to adjuvant cyclophosphamide, doxorubicin, and fluorouracil for patients with operable stage II or III breast cancer involving 10 or more axillary lymph nodes (CALGB Protocol 9082). Cancer and Leukemia Group B." J Natl Cancer Inst Monogr(19): 41-4.
36. Izadyar, F., J. J. Matthijs-Rijsenbilt, et al. (2002). "Development of a cryopreservation protocol for type A spermatogonia." J Androl **23**(4): 537-45.
37. Jantunen, E., E. Mahlamaki, et al. (2000). "Feasibility and toxicity of high-dose chemotherapy supported by peripheral blood stem cell transplantation in elderly patients (>/=60 years) with non-Hodgkin's lymphoma: comparison with patients <60 years treated within the same protocol." Bone Marrow Transplant **26**(7): 737-41.
38. Jantunen, E., T. Kuittinen, et al. (2006). "High-dose melphalan (200 mg/m2) supported by autologous stem cell transplantation is safe and effective in elderly (>or=65 years) myeloma patients: comparison with younger patients treated on the same protocol." Bone Marrow Transplant **37**(10): 917-22.
39. Jones, E. A., A. English, et al. (2006). "Optimization of a flow cytometry-based protocol for detection and phenotypic characterization of multipotent mesenchymal stromal cells from human bone marrow." Cytometry B Clin Cytom **70**(6): 391-9.
40. Kapelushnik, J., H. Mandel, et al. (2000). "Fludarabine-based protocol for haploidentical peripheral blood stem cell transplantation in Hurler syndrome." J Pediatr Hematol Oncol **22**(5): 433-6.
41. Kitano, M., M. Kakinuma, et al. (2006). "Gene expression profiling of mouse embryonic stem cell progeny differentiated by Lumelsky's protocol." Cells Tissues Organs **183**(1): 24-31.
42. Kolb, E. A. and P. G. Steinherz (2003). "A new multidrug reinduction protocol with topotecan, vinorelbine, thiotepa, dexamethasone, and gemcitabine for relapsed or refractory acute leukemia." Leukemia **17**(10): 1967-72.
43. Lalvani, A., T. Dong, et al. (1997). "Optimization of a peptide-based protocol employing IL-7 for in vitro restimulation of human cytotoxic T lymphocyte precursors." J Immunol Methods **210**(1): 65-77.
44. Lappalainen, J., K. A. Lindstedt, et al. (2007). "A protocol for generating high numbers of mature and functional human mast cells from peripheral blood." Clin Exp Allergy **37**(9): 1404-14.
45. Lotz, J. P., B. Bui, et al. (2005). "Sequential high-dose chemotherapy protocol for relapsed poor prognosis germ cell tumors combining two mobilization and cytoreductive treatments followed by three high-dose chemotherapy regimens supported by autologous stem cell transplantation. Results of the phase II multicentric TAXIF trial." Ann Oncol **16**(3): 411-8.
46. Martino, R., R. Guardia, et al. (1999). "Time sequential chemotherapy for primary refractory or relapsed adult acute myeloid leukemia: results of the phase II Gemia protocol." Haematologica **84**(3): 226-30.
47. McBride, S. H. and M. L. Knothe Tate (2008). "Modulation of stem cell shape and fate A: the role of density and seeding protocol on nucleus shape and gene expression." Tissue Eng Part A **14**(9): 1561-72.
48. Menichella, G., M. Lai, et al. (1997). "Evaluation of a new protocol for peripheral blood stem cell collection with the Fresenius AS 104 cell separator." J Clin Apher **12**(2): 82-6.
49. Micallef, I. N., P. J. Stiff, et al. (2009). "Successful stem cell remobilization using plerixafor (mozobil) plus granulocyte colony-stimulating factor in patients with non-hodgkin lymphoma: results from the plerixafor NHL phase 3 study rescue protocol." Biol Blood Marrow Transplant **15**(12): 1578-86.

50. Millington, M., A. Arndt, et al. (2009). "Towards a clinically relevant lentiviral transduction protocol for primary human CD34 hematopoietic stem/progenitor cells." PLoS One **4**(7): e6461.
51. Moreau, P., C. Hullin, et al. (2006). "Tandem autologous stem cell transplantation in high-risk de novo multiple myeloma: final results of the prospective and randomized IFM 99-04 protocol." Blood **107**(1): 397-403.
52. Morita, E., Y. Watanabe, et al. (2008). "A novel cell transplantation protocol and its application to an ALS mouse model." Exp Neurol **213**(2): 431-8.
53. Movassagh, M., C. Desmyter, et al. (1998). "High-level gene transfer to cord blood progenitors using gibbon ape leukemia virus pseudotype retroviral vectors and an improved clinically applicable protocol." Hum Gene Ther **9**(2): 225-34.
54. Moviglia, G. A., R. Fernandez Vina, et al. (2006). "Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients." Cytotherapy **8**(3): 202-9.
55. Naujok, O., F. Francini, et al. (2008). "A new experimental protocol for preferential differentiation of mouse embryonic stem cells into insulin-producing cells." Cell Transplant **17**(10-11): 1231-42.
56. Olivero, S., T. Alario, et al. (1999). "CD34+ cell enumeration in peripheral blood and apheresis samples, using two laboratory diagnostic kits or an institutional protocol." Bone Marrow Transplant **23**(4): 387-94.
57. Omori, Y., O. Honmou, et al. (2008). "Optimization of a therapeutic protocol for intravenous injection of human mesenchymal stem cells after cerebral ischemia in adult rats." Brain Res **1236**: 30-8.
58. O'Neill, L. P., M. D. VerMilyea, et al. (2006). "Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations." Nat Genet **38**(7): 835-41.
59. Oriol, A., J. M. Ribera, et al. (2004). "Feasibility and results of autologous stem cell transplantation in de novo acute myeloid leukemia in patients over 60 years old. Results of the CETLAM AML-99 protocol." Haematologica **89**(7): 791-800.
60. Patel, R. D., A. V. Vanikar, et al. (2009). "Ahmedabad tolerance induction protocol and chronic renal allograft dysfunction: pathologic observations and clinical implications." Diagn Pathol **4**: 4.
61. Petsa, A., S. Gargani, et al. (2009). "Effectiveness of protocol for the isolation of Wharton's Jelly stem cells in large-scale applications." In Vitro Cell Dev Biol Anim **45**(10): 573-6.
62. Pierelli, L., G. Menichella, et al. (1993). "Evaluation of a novel automated protocol for the collection of peripheral blood stem cells mobilized with chemotherapy or chemotherapy plus G-CSF using the Fresenius AS104 cell separator." J Hematother **2**(2): 145-53.
63. Pirruccello, S. J., C. J. Page, et al. (1999). "Comparison of ISHAGE protocol CD34 cell enumeration with a lineage negative backgating technique." Cytotherapy **1**(4): 279-86.
64. Polikov, V. S., E. C. Su, et al. (2009). "Control protocol for robust in vitro glial scar formation around microwires: essential roles of bFGF and serum in gliosis." J Neurosci Methods **181**(2): 170-7.
65. Ratei, R., G. Basso, et al. (2009). "Monitoring treatment response of childhood precursor B-cell acute lymphoblastic leukemia in the AIEOP-BFM-ALL 2000 protocol with multiparameter flow cytometry: predictive impact of early blast reduction on the remission status after induction." Leukemia **23**(3): 528-34.
66. Rippon, H. J., J. M. Polak, et al. (2006). "Derivation of distal lung epithelial progenitors from murine embryonic stem cells using a novel three-step differentiation protocol." Stem Cells **24**(5): 1389-98.
67. Rivadeneyra-Espinoza, L., B. Perez-Romano, et al. (2006). "Instrument- and protocol-dependent variation in the enumeration of CD34+ cells by flow cytometry." Transfusion **46**(4): 530-6.
68. Schipper, L. F., Y. van Hensbergen, et al. (2007). "A sensitive quantitative single-platform flow cytometry protocol to measure human platelets in mouse peripheral blood." Transfusion **47**(12): 2305-14.
69. Schmidt, M., T. Simon, et al. (2008). "The prognostic impact of functional imaging with (123)I-mIBG in patients with stage 4 neuroblastoma >1 year of age on a high-risk treatment protocol: results of the German Neuroblastoma Trial NB97." Eur J Cancer **44**(11): 1552-8.
70. Sen, A., M. S. Kallos, et al. (2004). "New tissue dissociation protocol for scaled-up production of neural stem cells in suspension bioreactors." Tissue Eng **10**(5-6): 904-13.
71. Serke, S. and H. E. Johnsen (2001). "A European reference protocol for quality assessment and clinical validation of autologous haematopoietic blood progenitor and stem cell grafts." Bone Marrow Transplant **27**(5): 463-70.
72. Soleimani, M. and S. Nadri (2009). "A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow." Nat Protoc **4**(1): 102-6.
73. Steigman, S. A., M. Armant, et al. (2008). "Preclinical regulatory validation of a 3-stage amniotic mesenchymal stem cell manufacturing protocol." J Pediatr Surg **43**(6): 1164-9.
74. Suthar, K., A. V. Vanikar, et al. (2008). "Renal transplantation in primary focal segmental glomerulosclerosis using a tolerance induction protocol." Transplant Proc **40**(4): 1108-10.
75. Suzue, T., Y. Kawano, et al. (1994). "Cell processing protocol for allogeneic peripheral blood stem cells mobilized by granulocyte colony-stimulating factor." Exp Hematol **22**(9): 888-92.
76. Tewogbade, A., K. FitzGerald, et al. (2008). "Attitudes and practices of nurses on a pediatric cancer and stem cell transplant ward: adaptation of an oral care protocol." Spec Care Dentist **28**(1): 12-8.
77. Thompson, J. S., C. Pomeroy, et al. (2004). "Use of a T cell-specific monoclonal antibody, T10B9, in a novel allogeneic stem cell transplantation protocol for hematologic malignancy high-risk patients." Biol Blood Marrow Transplant **10**(12): 858-66.
78. Tonks, A., A. J. Tonks, et al. (2005). "Optimized retroviral transduction protocol which preserves the primitive subpopulation of human hematopoietic cells." Biotechnol Prog **21**(3): 953-8.
79. Trarbach, T., S. Greifenberg, et al. (2000). "Optimized retroviral transduction protocol for human progenitor cells utilizing fibronectin fragments." Cytotherapy **2**(6): 429-38.
80. Tsai, M. S., J. L. Lee, et al. (2004). "Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol." Hum Reprod **19**(6): 1450-6.
81. Tsyrulnyk, A. and R. Moriggl (2008). "A detailed protocol for bacterial artificial chromosome recombineering to study essential genes in stem cells." Methods Mol Biol **430**: 269-93.
82. Twardowski, P. W., L. Smith-Powell, et al. (2008). "Biologic markers of angiogenesis: circulating endothelial cells in patients with advanced malignancies treated on phase I protocol with metronomic chemotherapy and celecoxib." Cancer Invest **26**(1): 53-9.
83. Usui, N., N. Dobashi, et al. (2002). "Intensified daunorubicin in induction therapy and autologous peripheral blood stem cell transplantation in postremission therapy (Double-7 protocol) for adult acute myeloid leukemia." Int J Hematol **76**(5): 436-45.

84. van Dartel, D. A., N. J. Zeijen, et al. (2009). "Disentangling cellular proliferation and differentiation in the embryonic stem cell test, and its impact on the experimental protocol." Reprod Toxicol **28**(2): 254-61.
85. Vieira Pinheiro, J. P., E. Ahlke, et al. (1999). "Pharmacokinetic dose adjustment of Erwinia asparaginase in protocol II of the paediatric ALL/NHL-BFM treatment protocols." Br J Haematol **104**(2): 313-20.
86. Ware, C. B. and S. W. Baran (2007). "A controlled-cooling protocol for cryopreservation of human and non-human primate embryonic stem cells." Methods Mol Biol **407**: 43-9.
87. Wierenga, P. K., J. H. Dillingh, et al. (1997). "Reduction of heat-induced haemotoxicity in a hyperthermic purging protocol of murine acute myeloid leukaemic stem cells by AcSDKP." Br J Haematol **99**(3): 692-8.
88. Williams, C. B., S. D. Day, et al. (2004). "Dose modification protocol using intravenous busulfan (Busulfex) and cyclophosphamide followed by autologous or allogeneic peripheral blood stem cell transplantation in patients with hematologic malignancies." Biol Blood Marrow Transplant **10**(9): 614-23.
89. Xu, L., S. K. Stahl, et al. (1994). "Correction of the enzyme deficiency in hematopoietic cells of Gaucher patients using a clinically acceptable retroviral supernatant transduction protocol." Exp Hematol **22**(2): 223-30.
90. Yamagata, K., K. Onizawa, et al. (2006). "A prospective study to evaluate a new dental management protocol before hematopoietic stem cell transplantation." Bone Marrow Transplant **38**(3): 237-42.
91. Yang, P. F., T. C. Hua, et al. (2006). "Cryopreservation of human embryonic stem cells: a protocol by programmed cooling." Cryo Letters **27**(6): 361-8.
92. Zhao, Y., A. Raouf, et al. (2007). "A modified polymerase chain reaction-long serial analysis of gene expression protocol identifies novel transcripts in human CD34+ bone marrow cells." Stem Cells **25**(7): 1681-9.
93. Zhou, J. M., J. X. Chu, et al. (2008). "An improved protocol that induces human embryonic stem cells to differentiate into neural cells in vitro." Cell Biol Int 32(1): 80-5.
94. Paul Woodard. SCDHAP Protocol: ematopoietic Stem Cell Transplantation (HSCT) for Patients with Sickle Cell Disease and Prior Stroke or Abnormal Transcranial Doppler Ultrasound (TCD) using Reduced Conditioning and T-Cell-Depleted Hematopoietic Stem Cells from Partially Matched Family Donors - Phase I Study. http://www.stjude.org/protocols/0,2081,450_2327_18472,00.html. 2007.
95. Renee Madden. SCT521 (COG # ASCT0521) Protocol: Soluble Tumor Necrosis Factor Receptor: Enbrel (Etanercept) for the Treatment of Acute Non-Infectious Pulmonary Dysfunction (Idiopathic Pneumonia Syndrome) Following Allogeneic Stem Cell Transplantation. http://www.stjude.org/protocols/0,2881,450_2333_5873,00.html. 2007.
96. Kimberly Kasow. OPBMT2 Protocol: Allogeneic Hematopoietic Stem Cell Transplantation for Children Affected with Malignant Osteopetrosis - A Pilot Study. http://www.stjude.org/protocols/0,2881,450_2331_17072,00.html. 2007.
97. http://stemcells.nih.gov/staticresources/research/protocols/BresaGen_hESC_manual_2.1.pdf.
98. Mitalipova et al. Stem Cells. 2003;21(5):521-6.
99. Ma H, Chen G (2005). Stem Cell. J Am. Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
100. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci. 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
101. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci. 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
102. Yang Y, Ma H (2010**).** Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
103. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
104. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

8/7/2012