

UMBILICAL CORD BLOOD STEM CELL RESEARCH

Cord Blood Stem Cells and Brain Cell Development

<p>This study demonstrated that hematopoietic-derived cells responded to neural growth factors in the subventricular zone in both 6 month old (adult) and 16 month old (elderly) rats. Human umbilical cord cells were implanted into the brain of the rats. Many cord cells retained their hematopoietic identity but a few cells expressed markers (nestin and doublecortin) for endogenous neural progenitors. The cord cells of both the adult rats showed greater migration and survival than those of the elderly rats.</p>	<p>Walczak P, Chen N, Hudson JE, Willing AE, Garbuzova-Davis SN, Song S, Sanberg PR, Sanchez-Ramos J, Bickford PC, Zigova T. Do hematopoietic cells exposed to a neurogenic environment mimic properties of endogenous neural precursors? <i>J Neurosci Res</i> 2004, 76(2): 244-54.</p>
<p>Human umbilical cord blood cells were cultured with brain-derived neurotrophic factor (BDNF) and after ten days, the differentiated cells expressed glial fibrillary acidic protein (GFAP) and neuron-specific nuclear protein. The authors conclude that BDNF (and to a lesser extent, NF-KappaB ligand) can induce human umbilical cord blood cells into neurons and glial cells. It is hopeful that these stem cells might be useful in the treatment of central nervous system injury.</p>	<p>Zhao ZM, Lu SH, Zhang QJ, Liu HY, Yang RC, Cai YL, Han ZC. The preliminary study on in vitro differentiation of human umbilical cord blood cells into neural cells. <i>Zhonghua Xue Ye Xue Za Zhi</i> 2003, 24(9): 484-7.</p>
<p>This articles reviews studies in which cells from bone marrow or umbilical cord blood are induced to proliferate and differentiate into glia and neurons. These stem/progenitor cells open up the possibility of their use to treat degenerative, post-traumatic and hereditary disease of the central nervous system.</p>	<p>Sanchez-Ramos JR. Neural cells derived from adult bone marrow and umbilical cord blood. <i>J Neurosci Res</i> 2002, 69(6): 880-93.</p>
<p>Rare cells are present in human umbilical cord blood that do not produce cells of hematopoietic lineage. These multipotent stem cells behave as multilineage progenitor cells and can be expanded in tissue culture. Exposure to basic fibroblast growth factor and human epidermal growth factor for at least 7 days in culture induced expression of neural (beta-tubulin III) and glial markers (glial fibrillary acidic protein). The oligodendrocyte marker (galactocerebroside) was present in most cells. Many cells showed dual labeling, expressing both neuronal and glial markers.</p>	<p>Bicknese AR, Goodwin HS, Quinn CO, Henderson VC, Chien SN, Wall DA. Human umbilical cord blood cells can be induced to express markers for neurons and glia. <i>Cell Transplant</i> 2002; 11(3) 261-4.</p>
<p>Mononuclear cells from human umbilical cord blood have the ability to respond to nerve growth factor by increased expression of neural markers typical for nervous system-derived stem cells. The DNA microarray showed a downregulation of several genes associated with blood cell lines. One month after these cells were transplanted into 1 day old rat brains, approximately 20% of the cells survived. In addition, some of the cells differentiated into cells with distinct glial or neuronal phenotypes.</p>	<p>Zigova T, Song S, Willing AE, Hudson JE, Newman MB, Saporta S, Sanchez-Ramos J, Sanberg PR. Human umbilical cord blood cells express neural antigens after transplantation into the developing rat brain. <i>Cell Transplant</i>. 2002, 11(3): 265-74.</p>
<p>Cord blood contains a population of cells that can be expanded in culture and are able to express the phenotype of multiple lineages.</p>	<p>Goodwin HS, Bichnese AR, Chien SN, Bogucki BD, Quinn CO, Wall</p>

<p>The cell populations express adhesion molecules CD13+, CD29+ and CD44+. Cord blood may prove to be a new source of cells for cellular therapeutics for stromal, bone and potentially, neural repair.</p>	<p>DA. Multilineage differentiation activity by cells isolated from umbilical cord blood: expression of bone, fat and neural markers. <i>Biol Blood Marrow Transplant</i> 2001; 7(11): 581-8.</p>
<p>There is a neural-stem-cell-like subpopulation from human umbilical cord blood cells that can be selected and expanded in vitro. The cells and resulting clones express nestin, a neurofilament protein that is one of the most specific markers of multipotent neural stem cells. With selected growth factors, the progeny can be oriented towards neurons, astroglia or oligodendroglia. The cells show high commitment (about 30-40%) to neuronal (30%) and astrocytic (40%) fate and about 11% of the total population of cells give rise to oligodendrocytes.</p>	<p>Buzanska L, Machaj EK, Zablocka B, Pojda Z, Domanska-Janik K. Human cord blood-derived cells attain neuronal and glial features in vitro. <i>J Cell Sci</i> 2002, 115(Pt 10): 2131-8.</p>
<p>CD133+ hematopoietic stem cells expressed neuronal and glial phenotypes after being grown in retinoic acid culture. The cells also expressed neuronal (nestin, Musashi1, and RA receptors), glial (GFAP), and oligodendrocyte markers (myelin basic protein, proteolipid protein and cyclic nucleotide phosphodiesterase).</p>	<p>Jang YK, Par JJ, Lee MC, Yoon BH, Yang YS, Yang SE, Kim SU. Retinoic acid-mediated induction of neurons and glial cells from human umbilical cord-derived hematopoietic stem cells. <i>J Neurosci Res</i> 2004; 75(4): 573-84.</p>
<p>CD133(+) hematopoietic stem/progenitor cells in culture expressed different pluripotency markers. Following treatment with retinoic acid, CD133(+) cells exhibited neural morphology associated with expression of beta-III-tubulin. CD133(+) cells may have a greater differentiation potential than previously recognized.</p>	<p>Baal N, Reisinger K, Jahr H, Bohle RM, Liang O, Munstedt K, Rao CV, Preissner KT, Zygmunt MT. Expression of transcription factor Oct-4 and other embryonic genes in CD133 positive cells from human umbilical cord blood. <i>Thromb Haemost</i> 2004, 92(4): 767-75.</p>
<p>This study investigated the dynamics of biological properties of CD133(+) cells from human umbilical cord blood. About 79.62% of CD34+ cells expressed CD133, and more than 97% of CD133(+) cells were CD133(+)CD34(+). CD133(+) cells may be more primitive hematopoietic stem/progenitor cells than CD34(+) cells.</p>	<p>Hao SG, Sun GL, Wu WL, Wu YL. Studies on the dynamics of biological characteristics of CD133+ cells from human umbilical cord blood during short-term culture. <i>Zhongguo Shi Yan Xue Ye Xue Za Zhi</i>. 2003, 11(6): 569-75.</p>
<p>CD 34- Hematopoietic tissue-derived adult stem cells can transdifferentiate into neural progenitor cells. This creates an interesting alternative to central nervous system or embryonic-derived stem cells as a viable source for cellular therapies for brain regeneration. Umbilical cord blood due to its primitive nature and its unproblematic collection appears as a promising candidate for</p>	<p>McGuckin CP, Forraz N, Allouard Q, Pettengell R. Umbilical cord blood stem cells can expand hematopoietic and neuroglial progenitors in vitro. <i>Exp Cell Res</i> 2004, 295(2): 350-9.</p>

<p>multipotent stem cell harvest. Umbilical cord blood negative lineage stem cells could be expanded to produce slow-dividing adherent cells with neuroglial progenitor cell morphology over 8 weeks. Gene expression analysis showed upregulation of primitive neuroglial progenitor cell markers, including GFAP, nestin, musashi-1, and necdin. This study demonstrates that a single cord blood specimen can produce both primitive hematopoietic and neuropoietic progenitors and thus widens its clinical potential for cellular therapies.</p>	
<p>CD 45- , a rare population from human cord blood can be expanded to 10(15) cells without losing pluripotency. They are able to differentiate into osteoblasts, chondroblasts, adipocytes, hematopoietic and neural cells including astrocytes and neurons that express neurofilament, sodium channel protein and various neurotransmitter phenotypes. Implantation of these cells into the intact rat brain showed that they persisted for up to 3 months and showed migratory activity and a typical neuron-like morphology.</p>	<p>Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, feldhahn N, Liektke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Muller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med 2004, 200(2): 123-35.</p>
<p>Mesenchymal cells from umbilical cord are capable of rapid proliferation in vitro. When cultured in neuronal conditioned medium, they started to express neuron-specific proteins such as NeuN and neurofilament by the 3 rd day. By the 9 th -12 th day, 87% of the mesencymal cells were expressing neurofilament and differentiating into mature neurons.</p>	<p>Fu YS, Shih YT, Cheng YC, Min MY. Transformation of human umbilical mesenchymal cells into neurons in vitro. J Biomed Sci 2004, 11(5): 652-60.</p>
<p>Human umbilical cord blood contains hematopoietic stem cells and mesenchymal stem cells, both of which are regarded as valuable sources for cell transplantation and therapy. Under proneurogenic conditions, mesenchymal cells rapidly assume the morphology of multipolar neurons and express neural markers (Tuj1, TrkA, GFAP and CNPases). The neurogenic potential of umbilical cord blood derived cells may facilities stem cell therapeutic approaches to neurodegenerative diseases.</p>	<p>Jeong JA, Gang EJ, Hong SH, Hwang SH, Kim SW, Yang IH, Ahn C, Han H, Kim H. Rapid neural differentiation of human cord blood-derived mesenchymal stem cells. Neuroreport 2004, 15(11): 1731-4.</p>
<p>Mesenchymal stem cells in human umbilical cord blood are capable of differentiating into neurocytes in vitro. Induction resulted in about 70% of the cells exhibiting a typical neuron-like phenotype (with neurofilament and neuron-specific enolase).</p>	<p>Hou L, Cao H, Wang D, Wei G, Bai C, Zhang Y, Pei X. Induction of umbilical cord blood mesenchymal stem cells into neuron-like cells in vitro. Int J Hematol 2003, 778(3): 256-61.</p>

Disorders Treated in Animals Using Umbilical Cord Stem Cells

Stroke	Evidence suggests that delivery of circulating CD34(+)	Peterson DA. Umbilical
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	human umbilical cord blood cells can produce functional recovery in an animal stroke model with concurrent angiogenesis and neurogenesis, leading to some restoration of cortical tissue.	cord blood cells and brain stroke injury: bringing in fresh blood to address an old problem. J Clin Invest 2004, 114(3): 312-4.
Stroke	Systemic administration of human cord blood-derived CD34(+) cells to immunocompromised mice subjected to stroke 48 hours earlier induces neovascularization in the ischemic zone and provides a favorable environment for neuronal regeneration. Endogenous neurogenesis is accelerated as a result of enhanced migration of neuronal progenitor cells to the damaged area, followed by their maturation and functional recovery. The data suggest an essential role for CD34(+) in promoting directly or indirectly an environment conducive to neovascularization of ischemic brain so that neuronal regeneration can proceed.	Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest 2004; 114(3): 330-8.
Stroke	Human umbilical cord stem cells were administered intravenously into the femoral (leg) vein or directly into the striatum (brain) of rat models for stroke. It was found that behavioral recovery was similar with both methods of delivery. However, with the step test, greater improvement was seen with the femoral delivery. The results suggest that therapy with umbilical cord stem cells may be effective for brain injuries and neurodegenerative disorders and that intravenous administration may be more effective than striatal implantation for long-term functional improvement in stroke animal models.	Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S, Hart C, Sanchez-Ramos J, Sanberg PR. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. J Neurosci Res 2003, 73(3): 296-307.
Stroke	Both polypeptide growth factors and stem cell populations from bone marrow and umbilical cord blood hold promise as treatments to enhance neurological recovery after stroke. Growth factors may exert their effects through stimulation of neural sprouting and enhancement of endogenous progenitor cell proliferation, migration, and differentiation in the brain. Exogenous stem cells may exert their effects by acting as miniature "factories" for trophic substances in the poststroke brain. Stroke recovery as opposed to acute stroke, represents an important and underexplored opportunity for the development of new stroke treatments.	Cairns K, Finklestein SP. Growth factors and stem cells as treatments for stroke recovery. Phys Med Rehabil Clin N Am 2003, 14(1 Suppl): S135-42.
Brain Injury	The hematopoietic system offers alternative sources for stem cells compared to those of fetal or embryonic origin. Umbilical cord cells and bone marrow stromal cells have been used in pre-clinical models of brain injury, directed to differentiate into neural phenotypes, and have been related to functional recovery after engraftment in central	Newman MB, Davis CD, Kuzmin-Nichols N, Sanberg PR. Human umbilical cord blood (HUCB) cells for central nervous system repair. Neurotox Res 2003,

	nervous system injury models.	5(5): 355-68.
Brain Injury	The authors review the possible alternative cell sources for repair of the brain and spinal cord, including Sertoli cells, neurotrophics, bone marrow and umbilical cord blood derived stem cells.	Sanberg PR, Willing AE, Cahill DW. Novel cellular approaches to repair of neurodegenerative disease: from Sertoli cells to umbilical cord blood stem cells. Neurotox Res 2002, 4(2): 95-101.

Disorders Treated in Animals Using Umbilical Cord Blood (which contains stem cells)

Stroke	<p>Human umbilical cord blood cells (HUCBC) are rich in stem and progenitor cells. In this study the authors tested whether intravenously infused HUCBC enter brain, survive, differentiate, and improve neurological functional recovery after stroke in rats. In addition, they tested whether ischemic brain tissue extract selectively induces chemotaxis of HUCBC in vitro in adult male Wistar rats.</p> <p>Treatment at 24 hours after MCAO with HUCBC significantly improved functional recovery. In vitro, significant HUCBC migration activity was present at 24 hours after MCAO compared with normal brain tissue. Intravenously administered HUCBC enter brain, survive, migrate, and improve functional recovery after stroke. HUCBC transplantation may provide a cell source to treat stroke.</p>	Chen J, Sanberg P, Li Y, Wang L, Lu M, Willing A, Sanchez-Ramos J, Chopp M. "intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats." Stroke, 2001; 32: 2682-2688.
Traumatic Brain Injury	Human umbilical cord blood was administered intravenously in the tail vein of rats 24 hours after TBI. The cord blood cells significantly reduced motor and neurological deficits compared with the control animals by the 28 th day. The cells preferentially entered the brain, migrated into the injured area and expressed markers for neurons (NeuN and MAP-2) and markers for astrocytes (GFAP). Some cord blood cells also integrated into the vascular walls within the injured area. The results suggest that human umbilical cord blood may be useful for treating TBI.	Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transplant 2002, 11(3): 275-81.

ALS	<p>The authors studied the long term effects of the intravenous administration of umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis. The treatment in presymptomatic G93A mice resulted in a delay of the disease progression by 2-3 weeks and increased lifespan in the diseased mice. In addition, the transplanted cells survived 10-12 weeks after administration and entered regions of motor neuron degeneration in the brain and spinal cord. There, the cells expressed neural markers (nestin, III Beta-Tubulin, and glial fibrillary acidic protein). The transplanted cells were also widely distributed in the peripheral circulation and organs, mainly the spleen.</p>	<p>Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration and differentiation. <i>J Hematother Stem Cell Res</i> 2003, 12(3): 255-70.</p>
Huntington's Disease in transgenic mice.	<p>Human umbilical cord blood mononuclear cells given in megadose quantity were able to increase the life span of B6CBA-TgN 62 Gpb mice (Huntington disease) from an average of 88 days to 97.8 and 103.4 days respectively. The rate of weight loss, which begins in these mice before the onset of symptoms of chorea, was far less in the animals receiving human cord blood mononuclear cells than the weight loss in untreated control mice.</p>	<p>Ende N, Chen R. "Human umbilical cord blood cells ameliorate Huntington's disease in transgenic mice." <i>J Med</i>, 2001, 32(3-4): 231-40.</p>
Alzheimer's Disease	<p>Having had success in extending the life of mice with a transgene for amyotropic lateral sclerosis mice and Huntington's disease, we administered megadoses of human umbilical cord blood mononuclear cells to mice with Alzheimer's disease. These mice have an over-expression of human Alzheimer amyloid precursor protein, die early and develop a CNS disorder that include neophobia. When given 110×10^6 human umbilical cord blood mononuclear cells, these mice had considerable extension of life with a p value of 0.001 when compared to control animals.</p>	<p>Ende N, Chen R, Ende-Harris D. "human umbilical cord blood cells ameliorate Alzheimer's disease in transgenic mice." <i>J Med</i>, 2001; 32(3-4): 241-7.</p>
Myocardial Infarction caused by mesenchymal stromal cells	<p>Preliminary experiments examined if isolated Mscs could be safely administered into the coronary circulation of the dog. Mononuclear cells were isolated from fresh canine bone marrow samples by Ficoll-Paque density gradients and grown in selective media to confluence. The cells were labeled with CM-</p>	<p>R. Vulliet, M. Greeley, M. Halloran, K. MacDonald, M.D. Kittleson. "Injection of mesenchymal stromal cells (MSC's) into the coronary circulation produces microscopic myocardial infarction in the dog." Oral Abstract, 9 th Annual Meeting of the International Society for</p>

	<p>DiI and BrdU. Five approximately 20 kg mixed-breed dogs were anesthetized by isoflurane and the left circumflex artery was catheterized. Catheter placement and coronary artery patency were verified using fluoroscopy and angiography and were repeated after each MSC injection. Two ml of freshly-dispersed MSCs (1 million cells.ml) in 50% canine serum and 50% growth media were injected repeatedly every 15 minutes into the catheter at a flow rate of 1 ml per minute.</p> <p>During administration of the MSCs, ECG changes characteristic of acute myocardial ischemia were observed (S-T segment and T wave changes) in all dogs. Following the injection of 10 to 15 million MSCs, one dog experienced acute cardiac arrest, two were sacrificed after one day, while two dogs were allowed to recover and observed daily. From physical appearance and activity, these two dogs were judged to be normal. Following sacrifice at 7 days, macro- and microscopic evidence of myocardial infarction were evident. Pale areas were noted grossly in the myocardium of the left ventricular posterior free wall. H&E sections revealed numerous and scattered regions of dense fibroplasia, accompanied by mononuclear cell infiltrates. The presence of macrophages and absence of T and B cells and PMNs were confirmed by immunocytochemical staining. Collagen fibrils were demonstrated with Masson's trichrome stain. These findings are consistent with subacute myocardial infarction. The authors conclude that injection of MSCs into the coronary circulation of dogs causes acute obstruction of coronary arterioles that leads to myocardial infarction under these conditions.</p>	<p>Cellular Therapy, May 29-June 1, 2003, Phoenix, Arizona.</p>
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Diseases Already Treated in Humans

<p>First cord blood transplant</p>	<p>Since 1988, when the first cord blood (CB) transplantation was successfully performed in a Fanconi aplasia, placental hematopoietic stem cells have emerged as an alternative</p>	<p>Dal Cortivo L, Robert I, Mangin C, Sameshima T, Kora S, Gluckman E, Benbunan M, Marolleau JP. "Cord blood banking: volume reduction using</p>
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	<p>source of stem and progenitor cells. Currently, about 1000 of CB have been transplanted worldwide, with encouraging results.</p>	<p>Procord Terumo Filter." J Hematotherapy & Stem Cell Research, 2000; 9: 885-890.</p>
<p>Related Transplantation</p>	<p>Eurocord reported the results of 78 related UCB transplants performed in Europe between October 1988 and December 1996 with a median follow up of 29 months. The patients were mainly children; median age was 5 years (range 2-20 yrs) and median weight was 19 kg (range 5 to 50). Thirty eight patients had leukemia. In 60 cases the donor was an HLA identical sibling and in 18 there was a degree of HLA disparity.</p> <p>Overall 53 patients survived one year; 44 in the HLA matched group and six in the HLA mismatched group. In 10 patients receiving UCB mismatched at three or more HLA antigens only one survived for 12 months. Neutrophil engraftment was better in patients younger than 6 years of age and weighing less than 20 kg. 35 of 48 patients receiving greater than 3.7×10^7 cells/kg had neutrophil engraftment compared with 34 of 40 who received less.</p>	<p>Will AM, "Umbilical cord blood transplantation." Arch Dis Child, 1999, 80: 3-6.</p>
<p>Related transplantation (continued)</p>	<p>Platelet engraftment was also favored by age under 6 years and weight more than 20 kg. HLA disparity was significantly associated with poor platelet engraftment. Acute GVHD developed to grade II or more in 9% of the fully matched recipients and in 9 of the mismatched transplants. 14.3% of evaluable patients went on to develop chronic GVHD. 10 of the 38 patients with leukemia relapsed during the median follow up of 29 months. Survival was also significantly affected by recipient cytomegalovirus status: 31 of 40 cytomegalovirus negative recipients but only 16 of 36 cytomegalovirus positive recipients survived for 12 months.</p> <p>Results for related UCB grafts from the US are broadly similar but with less acute GVHD. This may be related to the use of more intensive GVHD prophylaxis.</p>	<p>Will AM, "Umbilical cord blood transplantation." Arch Dis Child, 1999, 80: 3-6.</p>

<p>Unrelated cord blood transplantation</p>	<p>The results for 65 unrelated donor UCB transplantation from the Eurocord registry have also recently been reported. The patients had a median age of 9 years (range 0.3 to 45) and a median weight of 30 kg (range 4 to 90). 41 patients had acute or chronic leukemia. The median follow up was 10 months. 7 patients had an HLA identical donor at HLA-A,-B and -DRB1; 43 had a one antigen mismatch; 11 had two mismatches; and two had three HLA differences.</p> <p>19 patients survived for one year. Patients receiving $> 3.7 \times 10^7$ nucleated cells/kg fared better with a survival of 41% at one year compared with 22% in those infused with fewer cells. Cytomegalovirus serology was important to outcome: 42% of cytomegalovirus negative recipients survived 12 months compared with 20% of cytomegalovirus positive patients.</p> <p>HLA differences did not affect survival. Engraftment was slow and related to the number of nucleated cells/kg infused. The probability by day 60 of a neutrophil count $> 0.5 \times 10^9$ per litre was 87% and for a platelet count of $> 20 \times 10^9$ per litre the probability was 39%. Acute GVHD of grade II or more was seen in 21 patients: eight grade II, nine grade III, and four grade IV. The incidence of acute GVHD was significantly less in cytomegalovirus negative recipients. Seven of the 41 patients with leukemia relapsed.</p> <p>Results from the US are better with two year survival of approximately 45% and more rapid engraftment probably because of the greater use of growth factors post-transplant. There was a significant incidence of acute GVHD. In the largest study reported so far involving 272 cases, Rubinstein reported an incidence of acute GVHD of grade III or IV of 23%. The incidence of GVHD did not appear to be related to the apparent degree of</p>	<p>Will AM, "Umbilical cord blood transplantation." Arch Dis Child, 1999, 80: 3-6.</p>
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	<p>HLA mismatch.</p> <p>The results of unrelated UCB transplantation compare favorably with results of unmanipulated unrelated BMT.</p> <p>The author concludes that UCB transplantation is feasible.</p> <p>There are apparent limitations owing to the small numbers of progenitor cells present in the harvest. Immunological naivete of cord blood has been demonstrated by the relatively reduced incidence of GVHD in umbilical cord transplants compared with unmanipulated unrelated donor BMT and by poor outcome in patients who are cytomegalovirus positive before transplantation.</p> <p>However, despite routine prophylaxis, clinically significant acute GVHD was reported in all studies.</p> <p>Initial results are favorable for matched related UCB transplantation in patients younger than 6 years of age weighing less than 20 kg who are cytomegalovirus negative. In addition, patients from non-white European populations for whom bone marrow donors are difficult to find on the existing donor panels may benefit from the greater proportion of ethnic minority donors represented in the cord blood banks.</p> <p>New alternatives are on the horizon. In particular the initial results of mismatched CD34 selected stem cell transplantation are encouraging.</p>	
Fanconi anemia	A potential therapeutic option for patients with Fanconi anemia is collection of peripheral blood stem cells prior to the development of severe pancytopenia. These hematopoietic cells potentially could be infused when symptomatic bone marrow failure develops, as autologous rescue after chemotherapy in the event of leukemic	Croop J, Cooper R, Fernandez C, Graves V, Kreissman S, Hanenberg H, Smith F, Williams D. "Mobilization and collection of peripheral blood CD34+ cells from patients with Fanconi anemia." Blood, 2001; 98: 2917-2921.

	<p>transformation, or as targets for gene therapy. Eight patients with Fanconi anemia were mobilized with 10 ug/kg per day of granulocyte colony-stimulating factor to determine the feasibility of collecting peripheral blood stem cells for future use. Six patients achieved a peripheral blood CD34+ count of equal or greater 6 uL and underwent apheresis. The collection goal was 2 x 10 to the 6 th CD34+ cells/kg based on a predicted weight 5 years from the date of collection. The collections required a mean of 4 days of apheresis. Six of the 8 subjects had 1X106th CD34+ cells/kg cryopreserved based on both actual and target weights, and 4 subjects had 2 x 106 th CD34+ cells/kg cryopreserved based on the target weight. These results suggest that some patients with Fanconi anemia can have adequate numbers of CD34+ cells mobilized and collected from the peripheral blood prior to the onset of severe bone marrow failure, but they may require an extended mobilization and multiple days of collection.</p>	
<p>Chronic Myelogenous Leukemia</p>	<p>Transplantation with unrelated umbilical cord blood is marked by delayed hematologic recovery. This report summarizes two adults with chronic myelogenous leukemia who received myeloablative conditioning followed by infusion of a non-expanded single UCB graft. These patients were enrolled in a clinical trial incorporating concomitant in vivo administration of stem cell factor (R-MetHuSCF) and filgrastim from day of UCB infusion until attained hematopoietic recovery. Each patient engrafted fully with donor UCB, with days to absolute neutrophil count being 13 and 29 days, respectively. Both patients remain in cytogenetic remission at 28 months follow up. In vivo UCB expansion with administration of concomitant R-MetHuSCF and filgrastim may facilitate prompt hematologic engraftment.</p>	<p>Wadhwa P, Lazarus H, Koc O, Jaroscak J, Woo D, Stevens C, Rubinstein P, Laughlin M. "Hematopoietic recovery after unrelated umbilical cord-blood allogeneic transplantation in adults treated with in vivo stem cell factor (R-MetHuSCF) and filgrastim administration." Leukemia Research, 2002, Article in Press.</p>
<p>Results from</p>	<p>The transplantation of allogeneic hematopoietic stem cells to treat</p>	<p>Laughlin M, Barker J, Bambach B, Koe O, Rizzieri D, Wagner J, Gerson S,</p>

<p>unrelated donors</p> <p>Hemopoietic Cancers</p>	<p>hematological cancers is limited by availability of a human leukocyte antigen (HLA)-matched donor. Using marrow from an unrelated or partially mismatched donor entails a risk of severe graft-versus-host disease (GVHD). Because umbilical cord blood from unrelated, non-HLA-identical donors has restored hematopoiesis to children after myeloablative treatment, this procedure was evaluated in 68 adults with life-threatening hematological conditions who received total-body radiation or intensive chemotherapy. Antithymocyte globulin was administered before cryopreserved cord blood. Cyclosporin, alone or with methylprednisolone, was used to prevent GVHD. Just over two-thirds of patients received cord blood grafts mismatched for two or more HLA antigens.</p> <p>All but 5 of 60 patients who lived at least 28 days after transplantation had neutrophil engraftment, confirmed after a median of 27 days. The overall estimated probability of neutrophil recovery was 0.9. When many nucleated cells were present in the cord blood sample, neutrophils recovered more rapidly. The closeness of HLA matching did not related to the speed of neutrophil recovery. Seven of 55 engrafted patients who lived at least 1 month had grade III GVHD, and 4 had grade IV GVHD. Acute GVHD also could not be related to the degree of HLA mismatching. Chronic GVHD developed in 12 of 33 patients living longer than 100 days after the transplant, but only 1 had more than limited-stage disease. After a median follow-up of 22 months, 19 of the 68 patients were living and 18 of them were free of disease. Event-free survival improved when there were a high number of CD34+ cells in the cord blood graft. HLA-mismatched umbilical cord blood from an unrelated donor is a promising source of hematopoietic stem cells in adult patients treated for serious hematological disorders. TI should be considered whenever an HLA-matched unrelated donor is not readily</p>	<p>Lazarus H, Cairo M, Stevens C, Rubinstein P, Kurtzber J. "Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood form unrelated donors." N Engl J Med, 2001; 344: 1815-1822.</p>
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	<p>available. All but 10% of the present patients were reconstituted hematopoietically, and severe GVHD was infrequent.</p>	
Breast Cancer	<p>The safety and efficacy of administering ex vivo expanded peripheral blood progenitor cells (PBPC) to patients with breast cancer who undergo high-dose chemotherapy and PBPC transplantation was investigated. Unselected PBPC were cultured in gas-permeable bags containing 1-L serum-free media, granulocyte colony-stimulating factor, stem cell factor, and pegylated megakaryocyte growth and development factor for 9 days.</p> <p>No toxicities resulted from infusions of the ex vivo expanded PBPC. The study patients had shorter times to neutrophil and platelet recovery and fewer red cell transfusions than 48 historical controls who received the same conditioning regimen and posttransplantation care and at least 5×10^6 CD34+ PBPC per kilogram. Improvements in all these endpoints were significantly correlated with the expanded cell dose. 9 of 24 (38%) patients recovered neutrophil counts above 500/uL by day 5 or 6 after transplantation, whereas none of the controls had neutrophil recovery before the 8 th day. Seven (29%) patients had neutropenia for 3 or fewer days, and 9 (38%) patients did not experience neutropenic fevers or require broad-spectrum antibiotics.</p> <p>Therefore, ex vivo expanded PBPC are capable of ameliorating posttransplantation neutropenia, thrombocytopenia, and anemia in patients receiving high-dose chemotherapy.</p>	<p>Paquette, R, Dergham S, Karpf E, Wang H, Slamon D, Souza L, Glaspy J. "Ex vivo expanded unselected peripheral blood: progenitor cells reduce posttransplantation neutropenia, thrombocytopenia, and anemia in patients with breast cancer." Blood, 2000; 96: 2385-2390.</p>
Leukemia	<p>Cord blood, a potent source of hematopoietic stem cells has been shown to successfully reconstitute hematopoiesis following allogeneic transplantation in a variety of disorders. A major drawback of cord blood has been the risk of transfusion reactions in ABO blood group incompatibility and drastic reduction in the stem cell pool if the</p>	<p>Pahwa R, Fleischer A, Than S, Good R. "Successful hematopoietic reconstitution with transplantation of erythrocyte-depleted allogeneic human umbilical cord blood cells in a child with leukemia." Proc Natl Acad Sci, 1994, 91(4485-4488)</p>

	<p>cord blood is manipulated to remove red cells prior to cryopreservation or after thawing. This report describes an erythrocyte depletion method employing 3% gelatin-induced erythrocyte sedimentation for the selective removal of red cells from cord blood. The red cell-depleted fraction was shown to be enriched in progenitor cells and in cells secreting hematopoietic cytokines interleukin 3, granulocyte/macrophage colony-stimulating factor, and interleukin 6; a major source for cytokines was from cord T cells. This preparative technique was employed to separate out red cells from cord blood of an infant delivered by cesarean section who had an 8 year old sibling with leukemia. Histocompatibility testing of cord cells revealed complete matching with the patient. A cord cell transplant of cryopreserved and thawed cells consisting of 4×10^7 to the 7^{th} nucleated cells per kg was administered to the patient following myeloablative chemotherapy. The patient's quick hematologic recovery and 9 month disease-free period to date suggest that 3% gelatin separation of erythrocytes is a simple method that can be successfully used for transplanting cord cells from malignant/nonmalignant diseases.</p>	
<p>Cord blood in malignant and nonmalignant diseases</p>	<p>The potential benefits of unrelated donor marrow transplantation are offset by the immunologic complications of graft-versus-host disease (GVHD) and infection. Therefore, we used cryopreserved umbilical cord blood (UCB) as a strategy to reduce the risks of GVHD and treatment-related mortality and improve survival. Data on 102 patients (median age 7.4 years) who received transplants between 1994 and 2001 for the treatment of malignant (n=65; 68% were high-risk patients) and nonmalignant (n=37) diseases were evaluated. Incidences of neutrophil and platelet engraftment were 0.88 and 0.65 respectively. Incidences of severe acute and chronic GVHD were 0.11 and 0.10 respectively. At 1 year after transplantation, proportions of treatment</p>	<p>Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, Goldman A, Kersey J, Krivit W, MacMillan ML, Orchard PJ, Peters C, Weisdorf DJ, Ramsay NK, Davies SM. "Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival." <i>Blood</i>, 2002, 100(5): 1611-8.</p>

	<p>related mortality (TRM) and survival were 0.30 and 0.58 respectively. CD34 cell dose was the one factor consistently identified as significantly associated with rate of engraftment, TRM, and survival. Despite the low incidence of GVHD, the proportion of patients with leukemia relapse at 2 years was 0.17 and 0.45 for patients with standard and high-risk disease respectively. There is a high probability of survival in recipients of UCB grafts that are disparate in no more than 2 human leukocyte antigens when the grafts contain at least 1.7×10^5 CD34+ cells per kilogram of recipient's body weight. Therefore, graft selection should be based principally on CD34 cell dose when multiple UCB units exist with an HLA disparity of 2 or less.</p>	
<p>Registry study for hematopoietic stem cells transplants</p>	<p>The number of umbilical cord blood transplants is increasing worldwide. At this time, it is important to evaluate their results and to compare the outcome of umbilical cord blood transplants with allogeneic bone marrow transplants.</p> <p>Data have been reported in the Eurocord Registry by multiple transplant centers. Close links have been established with the cord blood banks through Netcord. Bone marrow transplant data have been provided by transplant centers and through the European Blood and Marrow Transplant (EBMT) and International Bone Marrow Transplant Registries.</p> <p>Eurocord has analyzed the outcomes of 527 umbilical cord blood transplants from 121 transplant centers and 29 countries. The donor was related in 138 cases and unrelated in 399 cases. The results showed that survival with umbilical cord transplants was comparable to that with related or unrelated bone marrow transplants. Engraftment with cord blood was delayed resulting in an increased incidence of early transplant complications. The incidence of acute and chronic graft-vs-host disease was reduced</p>	<p>Gluckman, Eliane. "Current status of umbilical cord blood hematopoietic stem cell transplantation." <i>Exp Hematology</i>, 2000; 28: 1197-1205.</p>

	<p>with cord blood grafts even in HLA-mismatched transplants and in adults. In patients with leukemia, the rate of relapse was similar to the rate of relapse after bone marrow transplant. The overall event-free survival with umbilical cord blood transplantation was not statistically different compared to bone marrow transplants. This large registry study confirms the potential benefit of using umbilical cord blood hematopoietic stem cells for allogeneic transplants.</p>	
<p>Leukemia and lymphoma</p>	<p>Placenta/umbilical cord blood (PCB) has been used for allogeneic bone marrow replacement since 1988. The Placental Blood Program of the New York Blood Center has developed techniques for collecting, testing, freezing and searching units on behalf of unrelated patients in need of hematopoietic stem cell replacement since 1993 and provided analysis of the outcomes of these transplants identified variables associated with clinical outcomes. In this review, after considering practical and conceptual aspects of the technology, the authors update information on the clinical outcomes of these transplants.</p> <p>All 861 patients transplanted through 1999 with PCB from their Program are included in this report. Two thirds were diagnosed with leukemia or lymphoma, 25% with inherited conditions and 7% with acquired diseases. Outcome data were provided by the respective Transplant Center and analyses included both univariate and multivariate regression tests and actuarial techniques.</p> <p>Engraftment was achieved by over 90% of recipients. Multivariate analysis confirmed the influence of cell dose, HLA matching, disease diagnosis and transplant center location. Patient age and HLA match grade independently affected the frequency and severity of acute graft vs host disease. Leukemic relapse was associated with the stage of disease at transplantation and the</p>	<p>Rubinstein P., Stevens C. "Placental blood for bone marrow replacement: the New York Blood Center's program and clinical results." <i>Bailliere's Clinical Haematology</i>, 2000, 13(4): 565-584.</p>

	<p>prior existence of acute graft vs host disease. The probability of transplant-related events was independently associated with disease diagnosis, cell dose, number of HLA mismatches and transplant center, while the cell dose failed to associate significantly with the relative risk of reaching this endpoint in the subset of patients who achieved engraftment. Overall, event-free survival rates at one year post-transplant were 49 and 30%, respectively for genetic disease and haematologic malignancies and 35% for patients with acquired diseases, respectively.</p> <p>The results confirm and extend earlier data, particularly establishing the significant association of transplant success with histocompatibility matching grades, and indicating the urgency of improving the transplant match levels.</p>	
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Diseases Potentially Treatable

Transplantation review	<p>More than 500 umbilical cord blood stem cell transplants have been performed. Umbilical cord blood banks have been set up in the USA and Europe to store these cells, which in the past have been considered a waste product of reproduction.</p> <p>Allogeneic bone marrow transplantation can potentially be used to cure a variety of diseases including haematological malignancies, bone marrow failure syndrome, haemoglobinopathies, immunodeficiencies, and some inborn errors of metabolism. The use of allogeneic bone marrow transplantation (BMT) is limited by the need for adequate tissue matching of host and donor cells to reduce the risks of rejection and the severity of graft versus host disease in the short term, while allowing immune reconstitution in the longer term. There is a need for a source of haematopoietic</p>	Will AM, "Umbilical cord blood transplantation." Arch Dis Child, 1999, 80: 3-6.
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	<p>stem cells with a less rigorous requirement for tissue matching that could be used for transplantation into patients who do not have a conventional donor. UCB cells are possible candidates for this clinical role.</p>	
Review (continued)	<p>UCB is a rich source of haemopoietic stem cells. UCB contains an increased proportion of the earliest progenitors, and per nucleated cell UCB has approximately 10 times the repopulation potential of bone marrow. Naïve UCB lymphocytes are also potentially less immunologically active than those usually found in the blood or marrow and may therefore produce fewer problems with GVHD than functionally mature lymphocytes harvested from live donors.</p> <p>When UCB is being donated for unrelated use postplacental delivery harvesting is more appropriate as it is less intrusive and reduces the need for donor counseling. Only mothers who produce adequate UCB harvests need to be approached for permission to store and test the UCB and to have a medical history taken.</p>	<p>Will AM, "Umbilical cord blood transplantation." Arch Dis Child, 1999, 80: 3-6.</p>
Diabetes and Zinc-chelating Dithizone	<p>Embryonic stem cells have a pluripotent ability to differentiate into a variety of cell lineages in vitro. The authors recently indentified the emergence of cellular clusters within differentiated embryonic cell cultures by staining with dithizone (DTZ). DTZ is a zinc-chelating agent known to selectively stain pancreatic beta cells because of their high zinc content. The aim of the present study was to investigate the characteristics of DTZ-stained cellular clusters originating from ES cells. Embryoid bodies, formed by a 5-day hanging drop culture of ES cells, were allowed to form outgrowths in the culture. The outgrowths were incubated in DTZ solution (final concentration, 100 ug/ml) for 15 minutes before being examined microscopically. The gene expression of endocrine pancreatic markers was also</p>	<p>Shiroi A, Yoshikawa M, Yokota H, Fukui H, Ishizaka S, Tatsumi K, Takahasi Y. "Identification of insulin-producing cells derived from embryonic stem cells by zinc-chelating Dithizone." Stem Cells, 2002; 20: 284-292.</p>

	<p>analyzed by reverse transcriptase-polymerase chain reaction. In addition, insulin production was examined immunohistochemically, and its secretion was examined using enzyme-linked immunosorbent assay.</p> <p>DTZ-stained cellular clusters appeared after approximately 16 days in the EB culture and became more apparent by day 23. They were found to be immunoreactive to insulin and expressed pancreatic-duodenal homeobox 1 (PDX1), proinsulin 1, proinsulin 2, glucagons, pancreatic polypeptide, glucose transporter-2 (GLUT2), and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) mRNA. They were also able to secrete detectable amounts of insulin.</p> <p>ES cell-derived DTZ-positive cellular clusters possess characteristics of the endocrine pancreas, including insulin secretion. Further, DTZ staining is a useful method for the identification of differentiated pancreatic islets developed from EBs in vitro.</p>	
<p>Vision and Bone Marrow Stem Cells</p>	<p>It has recently been shown that bone marrow cells can differentiate into various lineage cells including neural cells in vitro and in vivo The authors therefore examined whether bone marrow stem cells can differentiate into retinal neural cells in adult rats. PKH-67-labeled stem cell-enriched bone marrow cells were injected into the vitreous space of eyes in which the retinas had been mechanically injured. Two weeks after the injection of these cells, immunohistochemical examinations were carried out. The stem cell-enriched BMCs had been incorporated and had differentiated into retinal neural cells in the injured retina. The stem cell-enriched BMCs had accumulated mainly in the outer nuclear layer around the injured sites. The</p>	<p>Tomita M, Adachi, Y, Yamada H, Takahashi K, Kiuchi K, Ovaizu H, Ikebukuro K, Kaneda H, Matsumura M, Ikehara S. "Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina." <i>Stem Cells</i>, 2002; 20: 279-283.</p>

	<p>incorporated cells expressed glial fibrillary acidic protein, calbindin, rhodopsin, and vimentin. These results raise the possibility that stem cell-enriched BMCs have the ability to differentiate into retinal neural cells, and that the injection of stem cell-enriched BMCs into the retina would help repair damaged retinal cells.</p>	
<p>Antitumor Therapeutic potential against Leukemia and breast cancer</p>	<p>There was a significant increase in the survival of IL-2 activated UCBCs or PBC iv on day 3 and day 5 after tumor transplantation compared with untreated mice. Similar antitumor cytotoxicity of UCBCs and PBCs was also observed against MDA-231 human breast cancer grown in severe combined immunodeficient mice. These studies suggest that IL-2 activated UCBCs may be a useful source of cellular therapy for patients with hematological malignancies and breast cancer.</p>	<p>Joshi S, Tarantolo S, Kuszynski C, Kessinger A. "Antitumor Therapeutic potential of activated human umbilical cord blood cells against leukemia and breast cancer." <i>Clinical Cancer Research</i>, 2000.</p>
<p>Augmenting cytotoxic T lymphocyte-mediated cytotoxicity for potential therapy for leukemia and breast cancer.</p>	<p>Dendritic cells (DC) are important accessory cells that are capable of initiating an immune response. Generation of functional DC has potential clinical use in treating diseases such as cancer. In this report, we have demonstrated the generation of functional DC from mononuclear cells isolated from human umbilical cord blood cells and peripheral blood cells using a defined medium Prime Complete Growth Medium (PCGM) (Gene Prime LLC, Gaithersburg, MD). DC generated using PCGM showed the typical phenotype of DC as determined by flow cytometry and electron microscopy. Further analysis of the DC using confocal microscopy showed localization of the antigen and major histocompatibility complex (MHC) molecules in the cytoplasm 3-5 days following tumor antigen loading into DC. Subsequently, the tumor antigen-MHC complex was localized on the surface of DC. DC generated from UCBC or PBC also increased the allogeneic mixed</p>	<p>Joshi, S, Vu, U, Lovgren T, Lorkovic M, Patel W, Todd G, Kuszynski C, Joshi B, Dave H. "Comparison of phenotypic and functional dendritic cells derived from human umbilical cord blood and peripheral blood mononuclear cells." <i>J Hematotherapy & Stem Cell Research</i>, 2002; 11: 337-347.</p>

	<p>lymphocyte reaction, confirming their immune accessory functions compared to a control mixed lymphocyte reaction (MLR) without DC added. Interestingly, DC generated using PCGM medium also significantly enhanced the hematopoietic colony (CFU-C)-forming ability. Furthermore, addition of 5% DC derived from cord blood loaded with tumor antigen also significantly increased peripheral and cord blood-derived antigen-specific cytotoxic T lymphocyte (CTL)-mediated killing of human leukemic cells (K562) and breast cancer cells. Thus these results show that functional DC generated from cord blood using a defined medium are a useful source of accessory cells for augmenting CTL-mediated cytotoxicity and have potential use in cellular therapy for human leukemia and breast cancer.</p>	
<p>UCB for unrelated donor bone marrow reconstitution</p>	<p>Both white blood cell and nucleated red blood cell dose were independently predictive of myeloid engraftment speed. Because nucleated red blood cell dose (NRBC) predicted engraftment speed, inclusion of NRBCs in the total nucleated cell count does not reduce the effectiveness of the prefreezing total nuclear cell (TNC) count as an index of the quality of a PCB unit as a graft. The correlation between the number of NRBCs and the number of hematopoietic progenitor cells probably reflects the involvement of early stem cells in erythroid responses.</p>	<p>Stevens C, Gladstone J, Taylor P, Scaradavon A, Migliaccio A, Visser J, Dobrila N, Carrier C, Cabbad M, Wernet P, Kurtzberg J, Rubinstein P. "Placenta/umbilical cored blood for unrelated-donor bone marrow reconstitution: relevance of nucleated red blood cells." <i>Blood</i>, 2002, 100(7): 2662-2664.</p>
<p>Thymic T-cell development and angiogenesis</p>	<p>There is growing interest in using human umbilical cord blood for allogeneic bone marrow transplantation, particularly in children. CB has been identified as a rich source of hematopoietic progenitors of the erythroid, myloid and B-cell lineages. Whether CB blood cells engrafting into BM space also comprise T cell progenitors capable of trafficking to the thymus and reconstituting a functional thymopoiesis show that CB progenitors,</p>	<p>Crisa L, Cirulli V, Smith K, Ellisman M, Torbett B, Salomon D. "Human Cored Blood Progenitors Sustain Thymic T-cell development and a novel form of angiogenesis." <i>Blood</i>, 1999, 94(11): 3928-3940.</p>

	<p>engrafted in the BM of immunodeficient mice, sustain human thymopoiesis by generating circulating T-cell progenitors capable of homing to and developing within a human thymic graft. Surprisingly, development of CB stem cells in this in vivo model extended to elements of the endothelial cell lineage, which contributed to the revascularization of transplants and wound healing. These results demonstrate that human CB stem cell transplantation can reconstitute thymic-dependent T-cell lymphopoiesis and show a novel role of CB-derived hematopoietic stem cells in angiogenesis.</p>	
Cancer and apoptosis	<p>Anticancer treatment using cytotoxic drugs is considered to mediate cell death by activating key elements of the apoptosis program and the cellular stress response. While proteolytic enzymes (caspases) serve as main effectors of apoptosis, the mechanisms involved in activation of the caspase system are less clear. Two distinct pathways upstream of the caspase cascade have been identified. Death receptors, eg. CD95 (APO1/Fas), trigger caspase-8, and mitochondria release apoptogenic factors (cytochrome C, apaf-1, AIF), leading to the activation of caspase-9. The stressed endoplasmic reticulum contributes to apoptosis by the unfolded protein response pathway, which induces ER chaperones, and by the ER overload response pathway, which produces cytokines via nuclear factor-κB. Multiple other stress-inducible molecules, such as p53, JNK, AP-1, NF-κB, PKC/MAPK/ERK, and members of the sphingomyelin pathway have a profound influence on apoptosis. Understanding the complex interaction between different cellular programs provides insights into sensitivity or resistance of tumor cells and identifies molecular targets for rational therapeutic intervention strategies.</p>	Herr I and Debatin K.M. "Cellular stress response and apoptosis in cancer therapy." <i>Blood</i> , 2001; 98(9): 2603.
Paclitaxel, Etoposide and Cyclophosphamide	The dose-intense combination of Etoposide and cyclophosphamide and	Bilgrami S, Feingold J, Kapur D, Bona R, Edwards R, Khan A,

<p>for stem cell mobilization and tumor cyto-reduction in ovarian cancer</p>	<p>paclitaxel with growth factor support described in this report induced a PR in 9 of 10 evaluable patients, and was safe and well tolerated, and proved to be successful in mobilizing and procuring circulating hematopoietic stem cells in 81% of patients. These preliminary findings need to be confirmed in a larger and more homogeneous group of patients in an ongoing trial.</p>	<p>Rodriguez-Pinero F, Tutschka P. "A novel combination of Paclitaxel, Etoposide, and Cyclophosphamide for Stem cell mobilization and tumor cyto-reduction in Ovarian Cancer." J Hematotherapy & Stem Cell Research, 2000, 9: 133-135.</p>
<p>First Trimester fetal blood</p>	<p>MSCs similar to those derived from adult bone marrow, fetal liver and fetal bone marrow circulate in first trimester human blood and may provide novel targets for in utero cellular and gene therapy.</p>	<p>Campagnoli C, Roberts I, Kumar S, Bennett P, Bellantuono I, Fisk N. "Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver and bone marrow." Blood, 2001; 98: 2396-2402.</p>
<p>Heart Disease</p>	<p>It is estimated that 12 million American alive today have a history of heart attack and more than one million people will suffer a heart attack in a given year. Myocardium does not have myogenic stem cells and an injured heart is normally repaired by scar formation, hypertrophy of surviving myocytes, and hyperplasia of nonmuscle cells. Restoring blood flow, improving perfusion, reducing clinical symptoms, and augmenting ventricular functions are the common treatments after acute myocardial infarction. In congestive heart failure other than replacing the whole heart, no clinical procedure is available to restore the lost or dysfunctional myocardium.</p> <p>Cardiomyocytes maintain the ability to proliferate as they proceed through their initial stages of differentiation during embryonic development. Shortly after birth, cardiac myocytes lose their ability to proliferate and heart growth is produced mainly by hypertrophy of reexisting cardiomyocytes.</p> <p>Satellite cells are spindle-shaped, mononucleated myogenic precursor cells located under the basal lamina but outside</p>	<p>Kao, Race "Autologous Satellite cells for myocardial regeneration." E-biomed, 2001, 2: 1-8.</p>

	<p>the sarcolemma of skeletal muscle. Satellite cells can be considered as adult myogenic stem cells. The author has been using autologous satellite cells from skeletal muscle for myocardial regeneration in dogs prior to 1989. The satellite cells have been successfully isolated, labeled, and implanted into injured heart for neomyocardial formation and functional improvement. Significant improvement in contractile function was observed only in the animals having successful cell transplantation.</p> <p>Recently, cellular cardiomyoplasty has been applied in clinical cases by Dr. Philip Menasche (Paris, France) and Drs. Eisen and Furukawa (Philadelphia, PA). Autologous skeletal myoblasts were transplanted into and around the heart scar of a 72 year old man who suffered severe heart failure from heart attack. Dr. Menasche performed the world's first clinical case on June 15, 2000 and striking improvement in the man's condition with new tissue in his heart contracting rhythmically were observed. Autologous satellite cells for myocardial regeneration have the potential to be a generally applied therapeutic procedure to treat the millions of Americans who have suffered and will suffer a heart attack or heart failure.</p>	
<p>Hypoxia Inducible Factor and Stem cells of the cardiovascular system</p>	<p>Decreased oxygen levels activate hypoxia-inducible factor (HIF-1) to induce genes involved in glycolysis, glucose transport, erythropoiesis, and angiogenesis. Mutations in various HIF-1 subunits have contributed to our understanding of the role hypoxia plays during early embryonic development in general and the cardiovascular system in particular. The authors propose that HIF-1 is important for the generation, proliferation, maintenance, and differentiation of the early cardiovascular system. Understanding aberrations in</p>	<p>Ramirez-Bergeron D, Simon M. "Hypoxia-Inducible Factor and the Development of Stem Cells of the Cardiovascular System." <i>Stem Cells</i> 2001; 19: 279-286.</p>

	<p>these hypoxic responses is important since they contribute to serious human disease such as ischemia and tumorigenesis. This article focuses on the critical role of oxygen in regulating cardiovascular events during early embryonic development.</p> <p>Summary: Although it has been suggested that a key feature of HIF-1 mutant mice is their lack of a mature vasculature, lethality may actually stem from improper production or differentiation of a common progenitor of the cardiovascular system, the hemangioblast. Various factors respond to hypoxia, ie. EPO, VEGF, bFGF, TGF-B3, and PDGF, and collectively regulate the generation and differentiation of these stem cells. The use of in vitro BL-CFU assays allows a further analysis of the role of HIF-1 in early development. Future studies include determining the physiological and molecular mechanism(s) by which hypoxia influences these early events. For example, does HIF-1: (a) influence the expression of early progenitor markers, (b) stimulate production of growth factors that influence mesoderm differentiation into early stem cells, or (c) mediate hemangioblast proliferation or differentiation during establishment of the cardiovascular system? A very important element to this puzzle is to identify hemangioblasts in the embryonic splanchnopleural region since mesoderm from this region gives rise to endothelial, hematopoietic, and cardiac progenitors. An understanding of the biological role of hypoxia in the early events of progenitor cell generation, lineage commitment, and development is critical to assess oxygen delivery during organogenesis and disease.</p>	
Tissue repair, regeneration and replacement	Powerful recent developments in the multidisciplinary field of tissue engineering have yielded a novel set of	Bottaro D, Heidaran M. "Engineered extracellular matrices: A biological solution for tissue

	<p>tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell and developmental biology, as well as materials science and bioinformatics.</p>	<p>repair, regeneration, and replacement." E-biomed, 2001; 2: 9-12.</p>
<p>Marrow reconstitution and nuclear accidents</p>	<p>Human umbilical cord blood has been shown to be an effective source of stem cells for marrow reconstitution in pediatric patients. Unfortunately, the quantity of stem cells obtained from an individual donor can be quite limited in both the total volume and the numbers of stem cells per ml of cord blood. HLA matching further limits the availability, but recent publications indicate close matching may be unnecessary. Therefore, if cord blood from different donors can be combined, larger numbers of stem cells can be available for clinical use provided pooling does not produce a negative effect. Storage of single cord blood specimens at 4 degrees C for 10-21 days in gas permeable bags produced an apparent increase in the percentage of immature cells (CD34, CD117, GPA) and mitotic activity (S+G2/M cells) over day 1. With similar storage of pooled specimens there was a further increase in the number of immature colonies cultured,</p>	<p>Ende N, Lu S, Alcid M, Chen R, Mack R. "Pooled umbilical cord blood as a possible universal donor for marrow reconstitution and use in nuclear accidents." Life Sciences, 2001; 69: 1531-1539.</p>

	<p>CD34, CD117, GPA, S+G2/M cells. In addition, nucleated red blood cells increased over the mean values obtained from single cord blood samples. The authors' previous studies have indicated that large numbers of human mononuclear cells are necessary to reconstitute an irradiated animal model. By combining multiple samples of human cord blood, adequate numbers of stem cells could be pooled for use in adults and would provide cells for megadose therapy, including those patients that had accidentally received lethal irradiation.</p>	
Nuclear Accidents	<p>For several years, the authors have been able experimentally to keep SJL/J mice alive with the use of human umbilical cord blood after the animals received lethal levels of irradiation. This finding suggests that under certain conditions human cord blood does not have to be HLA matched to facilitate rescue from irradiation. In addition, there are reports of unmatched HLA cord blood being used successfully for marrow transplantation. If human cord blood does not have to be matched for HLA, there may be emergency cataclysmic circumstances where the availability of umbilical cord blood may be of considerable value. To simulate a clinical situation such as a nuclear accident, in which human cord blood might serve as a source of stem cells for marrow transplantation, the authors attempted to rescue immunocompetent mice after 900 cGY of irradiation with the use of (nonfrozen) human cord blood stored in a blood bank. The blood was stored under routine conditions (3-6 degrees C) for 5-7 days in special bags that allow transmission of oxygen. Following lethal levels of irradiation, the cord blood was administered to the animals and a significant survival rate was obtained.</p>	<p>Ende N, Lu S, Ende M, Giuliani D, Ricafort R, Alcid M, Deladisma M, Bagtas-Ricafort L. "Potential Effectiveness of Stored Cord Blood (Non-frozen) for emergency use." J Emergency Medicine, 1996; 14(6): 673-677.</p>
CD4+ Epstein-Barr Virus-Specific	<p>Umbilical cord blood is increasingly used for allogeneic hematopoietic stem cell</p>	<p>Sun Q, Burton R, Pollok K, Emanuel D, Lucas K. "CD4+</p>

Cytotoxic T-Lymphocytes	<p>transplantation. To determine whether viral antigen-specific cytotoxic T-lymphocytes (CTL) could be generated from the predominantly naïve T-cell populations in CB, CB-derived mononuclear cells were stimulated with autologous Epstein-Barr virus (EBV) transformed B-lymphoblastoid cell lines over several weeks in the presence of recombinant human interleukin-2. By 28 days of culture, T-lymphocytes from all six CB that had been treated with IL-2 displayed EBV-specific cytotoxicity. These cells were largely CD4+, with complete inhibition of cytotoxicity by anti-CD3 and variable inhibition by anti-HLA DR monoclonal antibodies. The EBV-specific effectors were cloned by limiting dilution, and most of the CTL clones were CD4+. The cytotoxicity of the CB-derived CD4+ CTL clones was inhibited by EGTA but not by anti-Fas ligand mAb, suggesting that this cytotoxicity was mediated by perforin/granzyme B. These data indicate that virus-specific CTL can be cultivated and cloned from CB, a human T-cell source that may not have prior in vivo antigenic exposure of reactivity. This finding may have applications in adoptive immunotherapy to recipients of CB transplants.</p>	<p>Epstein-Barr Virus-Specific Cytotoxic T-Lymphocytes from Human Umbilical Cord Blood." Cellular Immunology, 1999, 195: 81-88.</p>
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Gene Therapy - Animal

Gene Transfer to Hematopoietic stem cells	<p>Hematopoietic stem cells are desirable targets for gene therapy because of their self-renewal and multilineage differentiation abilities. Retroviral vectors are extensively used for HSC gene therapy. However, the initial human trials of HSC gene marking and therapy showed that the gene transfer efficiency into human HSCs with retroviral vectors was very low in contrast to the much higher efficiency observed in murine experiments. The more quiescent nature of human HSCs and the lower density of retroviral receptors on them hindered the efficient gene transfer with retroviral vectors. Since nonhuman primates have marked similarity to humans in all aspects including</p>	<p>Hanazono, Y., Terao K, Ozawa K. "Gene transfer into nonhuman primate Hematopoietic stem cells: Implications for Gene Therapy." Stem Cells, 2001; 19: 12-23.</p>
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	<p>the HSC biology, their models are considered to be important to evaluate and improve gene transfer into human HSCs. Using these models, clinically relevant levels (around 10% or even more) of gene-modified cells in peripheral blood have recently been achieved after gene transfer into HSCs and their autologous transplantation. This has been made possible by improving ex vivo transduction conditions such as introduction of Flt-3 ligand and specific fibronectin fragment (CH-296) into ex vivo culture during transduction, and the use of retroviral vectors pseudotyped with the gibbon ape leukemia virus or feline endogenous retrovirus envelope. Other strategies including the use of lentiviral vectors and in vivo selective expansion of gene-modified cells with the drug resistance gene or selective amplifier gene (also designated the molecular growth switch) are now being tested to further increase the fraction of gene-modified cells using nonhuman primate models. In addition to the high gene transfer efficiency, high-level and long-term expression of trans-genes in human HSCs and their progeny is also required for effective HSC gene therapy. For this purpose, other backbones of retroviral vectors such as the murine stem cell virus and ψ-DNA elements, such as the <i>B</i>-globin locus control region and the chromatin insulator, also need to be tested in nonhuman primate models. Nonhuman primate studies will continue to provide an important framework for human HSC gene therapy. Well-designed nonhuman primate studies will also offer unique insights into the HSCs, immune system, and transplantation biology characteristic of large animals.</p>	
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Gene Therapy - Human

<p>Project 90-C-195</p> <p>Gene therapy with T cell progenitors in Severe Combined Immunodeficiency Disease</p>	<p>Significant increases in lymphocyte adenosine deaminase activity, T cell numbers and immune function have been achieved in the two children with SCID thus far treated with autologous T cells genetically-corrected by retroviral-mediated insertion of a normal ADA gene. It is theoretically preferable to try to develop a treatment for these children that will result in stem cell gene correction. The genetic correction of T cell progenitors with long-term immune reconstituting ability would be more desirable because repeated infusions of genetically altered cells should not be necessary and the</p>	<p>Kohn, D. "Clinical Protocol. Amendment to Clinical Research Project, Project 90-C-195." Human Gene Therapy, 1999, 10: 477-488.</p>
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	generation of a more complete repertoire of T cell specificities might also be possible.	
Chemotherapy resistance HIV Tissue replacement and regeneration	<p>Goals for HSC gene therapy are transfer of the multi drug resistance gene 1 (MDR1) to HSC to render these and their progeny resistant to chemotherapeutic agents. In vivo data support this concept, but initial results from human clinical trials have been disappointing. In contrast to animal studies, transduction efficiency in enriched human precursor cell populations was extremely low and only transient. Overcoming this barrier by improving transduction efficiency to HSC with gene transfer vectors has therefore become a major goal of gene therapy research.</p> <p>Targeting HSC may be useful in the treatment of AIDS, where HSC are transduced with HIV decoys or RNase genes. Thus mature CD4+ T cells, the prime target cell for HIV could be made resistant towards virus uptake. Thorough understanding of the basic biology of HSC will directly guide the development of vectors to improve gene transfer to this critical cell population. During ontogeny, all somatic cells are derived from totipotent embryonal stem cells. The fact that cells derived from the bone marrow compartment are able to differentiate into non-hematopoietic tissues provides grounds for the intriguing hypothesis that totipotent stem cells persist in our body. Identification and understanding their biology may lead ultimately to means for tissue and organ replacement.</p>	Schwarzenberger P, Kolls J, La Russa V. "Hematopoietic Stem Cells." <i>Cancer Investigation</i> , 2002; 20(1): 124-138.

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