CHAPTER

102

Hope and Hype of Adult Stem Cell Plasticity

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Introduction

Human embryonic stem cell (ESC) lines were first described in 1998.[1] Their ability to reproduce limitlessly and differentiate into almost all cell types has generated a lot of scientific and media interest. Scientific research into human ESC has been proliferating steadily in the last few years. This has led to premature and unjustified expectations that embryonic stem cells will cure a host of diseases affecting the heart, brain, endocrines, spinal cord. However, there are ethical issues involved in the use of ESC and research on embryonic cells has been banned in many countries.

Use of postnatal or adult stem cells, such as hematopoietic stem cells (HSCs), does not pose any ethical issue. Moreover, HSCs have been in use in hematopoietic stem cell transplantation for more than 30 years in a variety of malignant and non-malignant disorders. More recently, there have been reports based on laboratory and animal studies, suggesting that adult stem cells can also exhibit "plasticity" or the ability of "transdifferentiation", thereby allowing cells of one lineage to form cells belonging to another lineage. This has evoked excitement, as there would be no ethical obstacles in use of these cells.

Despite the enthusiasm based primarily on laboratory data or animal experiments, evidence for adult stem cell plasticity is itself controversial. There are no clinical indications for use of adult stem cells, except in hematopoietic stem cell transplantation. Umbilical cord blood cells, which are committed adult stem cells in a newborn, have also been studied in the laboratory. A number of private companies are also propagating cryopreservation of cord blood at birth for regenerative purposes. This does not have the sanction of any professional body. In this article plasticity of adult stem cells will be reviewed with emphasis on the current clinical trials involving HSCs. Many other aspects of stem cell research dealing with embryonic stem cells or tissue specific stem cells are not being addressed.

Stem cell development

A Stem Cell is defined as an undifferentiated cell capable of self-renewal and differentiation. Self-renewal is a process by which the stem cell produces daughter cells that are also stem cells. Differentiation implies commitment to a specific cell type via a differentiation pathway leading to production of mature progeny cells.[2] Stem cells have the ability to divide for indefinite periods in culture and to give rise to specialized cells, which gradually lose the ability to proliferate indefinitely. Stem cells may be classified according to their development

Table I : Definition of Stem Cells

Type	Characteristic
Totipotent	A totipotent cell is one that can give rise to a new individual if provided with appropriate maternal support. Totipotent cells persist only up to the 8-cell stage of mouse development. Examples: Zygote and immediate progeny around blastula stage (can give rise to all embryonic and extraembryonic tissues).
Pluripotent	Pluripotent cells can give rise to all tissues of the body plus many of the cells that support the pregnancy but are unable to produce a new individual on their own. After compaction and blastocyst formation occurs, cells of the inner cell mass (ICM) are destined to give rise to all the tissues of the body. These include cell types of the embryo proper, including somatic and germ layers. Example: Embryonal stem (ES) cells
Multipotent	Postnatal or Adult Stem cells are multipotent if they can differentiate into multiple cell types of a single tissue. In contrast to ES cells, adult stem cells have less self-renewal ability, in part because of lack of high levels of telomerase. Adult stem cells generate daughter cells that can differentiate into cells of the tissue of origin but not other cell types Example: Hematopoietic stem cells, Neural stem cells, other tissue specific stem cells isolated from epidermis, intestine, liver, lung, retina.
Unipotent	Cells capable of contributing to only one mature cell type. Example: myosattelite cell of muscle, endothelial progenitor cell, corneal epithelial cells

potential as totipotent, pluripotent, multipotent and unipotent as shown in Table 1.[2,3]

Cells from the very earliest embryo are totipotent stem cells. Those which are derived from the inner cell mass are pluripotent and are called embryonal stem cells. These pluripotent stem cells can give rise to the 3 germ layers- ectoderm, mesoderm and endoderm, which in turn give rise to the various tissues in the body. The human body continues to have some stem cells even after the complete anatomical development. These stem cells are called the "Adult" stem cells. Unlike embryonic stem cells, adult stem cells have a finite proliferative life in tissue culture possibly on account of lower telomerase levels.

Concept of Adult Stem Cell Plasticity

The concept of adult stem cell plasticity or transdifferentiation arose from early transplantation studies. In sex mismatch bone marrow transplant patients, it was shown that donor cells could be detected in host non-hematopoietic tissues like the heart, neurons, liver, intestinal epithelium, and muscle. This suggested that bone marrow cells could form cells in non-hematopoietic tissues as well. Another recent development has been the identification of resident stem cells in a variety of organs such as:

a. mesenchymal stem cells (MSC) in bone marrow, human lipoaspirates, umbilical cord blood
b. neural stem cells (NSC) in the brain
c. skeletal muscle stem cells in muscle
d. adult cardiac stem cells in the heart.

Most of the reports of adult stem cell plasticity have been based on techniques, which are prone to error and observer bias. Thus the concept that adult stem cells have plasticity and that bone marrow derived progenitor cells have the capacity to regenerate cells, like the myocardial cells, is highly controversial with different observations and conclusions.

There is no consensus among basic scientists on the existence of adult stem cell plasticity.

The data in reports after bone marrow transplantation were obtained primarily from sex-mismatched transplantation studies in which immunohistochemistry (IHC) for tissue-specific antigens was combined with fluorescence in situ hybridization (FISH) for sex chromatin to identify donor-derived cells in nonhematopoietic tissues. However, the validity of these findings has remained controversial.[4] Ramakrishna et al obtained liver and intestinal tissue from a female patient at day 109 after allogeneic stem cell transplantation from a male donor. They prepared slides and on the same sections combined IHC for the CD45 antigen to distinguish cells from the hematopoietic lineage and FISH for X and Y chromatin to distinguish

between donor and host. Under high power, 200 nuclei were counted; the donor chromatin signal was, with few exceptions, always associated with CD45. Similar findings were obtained in the tissues of 2 other females who had also undergone sex-mismatched transplantation. The only exceptions were a few cells detected in liver sections that were CD45-negative and contained 2 X chromosomes and 1 Y chromosome, suggesting full fusion. These observations, together with previous studies in which the authors found no evidence of donor-derived stroma in patients at 0.15-27 years post-allogeneic transplantation, call into question the concept of plasticity in bone marrow stem cells.[4]

In sex mismatch heart transplants, it was possible to detect cells that engrafted after transplantation. In case a male patient received a heart from a female donor, presence in the heart of cells containing the Y chromosome would indicate the cellular engraftment from the male host.[5]

In one study, male recipients had myocardial infarction post transplantation and host derived non-inflammatory progenitor and endothelial cells were significantly increased, but only a small percentage (0.02-0.07%) was due to male derived cardiomyocytes.[6] Similar studies have been reported by other centers.[5]

This data suggests that a cell type present in the recipient patient can enter the host heart and can contribute to resident endothelial and myocardial cells, although at a very low level. These cells could be circulating progenitor cells, bone derived or organ-specific cells, which may trans-differentiate into endothelial or cardiomyocyte cells.

Orlic et al used bone marrow derived hematopoietic stem cells in mice experiments and showed that these cells have the capacity to regenerate lost myocardium.[7] Their study showed wide transdifferentiation of bone marrow derived HSCs into cardiomyocytes. However, in 2004 three studies directly contradicted these observations. They reported only rare events of so-called plasticity, which could be explained by cell fusion.[8-10] In 2003 it was reported that there is fusion of bone marrow derived cells with cardiomyocytes, Purkinje neurons and hepatocytes, explaining the phenomenon of transdifferentiation or plasticity.[11] Cell fusion was also reported as the mechanism of hepatocytes derived from bone marrow cells.[12]

Stem Cells and Cardiac Disease

Despite lack of evidence of stem cell plasticity, a large number of studies have been performed, with mixed results.[5] Bone marrow derived stem cells, isolated from whole bone marrow aspirate, remains the most commonly used cell type for human studies. Current methods of delivery include direct intramyocardial injection, via both endocardial catheter-based and epicardial surgical-based approaches. More recently, percutaneous, catheter based, intracoronary injections have been used. Alternatively, indirect mobilization has also been attempted with peripheral delivery of cytokines, notably G-CSF.[13] The benefit, when seen, is small and not sufficient for adequate functional recovery of the infarcted heart.

Five trials published in 2006 were reviewed in an editorial in the New England Journal of Medicine.[14] Overall, the results of three studies of a combined total of 376 patients do not promote the use of intracoronary infusions of autologous bone marrow to improve ventricular function. Clinical studies have suggested that only 1.3 to 2.6% of infused BMC are retained in the heart.[15]

Mobilisation of stem cells from the bone marrow represents another cell-based therapy. In one trial published in 2005, administration of G-CSF after reperfusion in myocardial infarction patients to mobilize bone marrow cells was safe and feasible. There was also a suggested potential for improvement in left ventricular ejection fraction and attenuation of left ventricular dilatation.[16] Two subsequent randomized trials failed to reproduce the benefits seen in early human studies.[17,18]

These studies suggest that bone marrow cell therapies are not sufficient for adequate functional recovery of the infarcted heart. Further work needs to be done to increase the ability of bone marrow cells to improve cardiac function. The mechanism through which the bone marrow cells act on the cardiac function needs to be further examined.[5] Recently, a number of studies have identified resident cardiac stem/progenitor cells. This has brought about a new wave of enthusiasm and scientific interest in the field.[13]

The functional benefit seen in some trials is not conclusive and the data should not be interpreted to suggest that infusion of autologous bone marrow derived cells in the setting of post myocardial infarction or cardiac failure is an approved therapy. There is a need for large randomized clinical trials to establish the role of stem cell infusion in this setting. At present autologous stem cell based therapy in cardiology cannot be considered as standard of care, and any such treatment should only be performed on the context of a clinical trial.

Mechanism of Benefit

The physiological benefit seen in some trials after infusion of bone marrow cells does not imply that this is due to stem cell plasticity. There is no clinical evidence of cardiomyogenesis after infusion of HSCs or MSCs in the various cardiac trials. This raises concerns for ongoing or pending clinical trials, many of which initially assumed that HSCs can differentiate into new cardiomyocytes. There are a number of other mechanisms, which could be responsible for the benefit.

The functional benefits may be mediated through paracrine secretion of growth factors or cytokines, which could indirectly promote survival of cardiomyocytes, mobilization of endogenous progenitor cells, or neovascularization.[14] Secreted angiogenic factors and/or activation of pathways that promote cell survival might protect and rescue hypoxic myocardium, thereby limiting damage to tissue and improving cardiac function. If paracrine factors are the key agents, isolating and delivering such factors at high concentrations or engineering Stem Cells to secrete larger amounts could result in more significant protection. Interestingly, thymosin β4, which is secreted in very large quantities by bone marrow stem cells, is cardioprotective after acute myocardial infarction and induces angiogenesis in mice. Future large-animal and clinical trials of thymosin β4 and other secreted factors hold promise and may obviate the need for cell-based therapy for the at-risk hypoxic myocardium.[19]

Stem Cells for the treatment of neurological disorders

The nervous system is a complex organ made up of neurons and glial cells, and the loss of any of these cell types may have catastrophic results on brain function. It is hoped that neural stem cells may be able to replenish those that are functionally lost in diseases such as Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis, as well as from brain and spinal cord injuries that result from stroke or trauma. The majority of stem cell studies of neurological disease have used rats and mice models, with some encouraging results. There are also side effects, which may have deleterious effects in humans.

Studies published in 2000 suggested that adult HSCs could trans-differentiate into neural cells,[20] but these were refuted by other subsequent publications.[21] There is likely greater role of using MSCs for this purpose, as these cells have a greater potential to differentiate into neural tissue and have a demonstrated immunomodulatory role.[22,23] More basic studies are required to understand the mechanisms involved before designing clinical trials.

Diabetes mellitus

Pancreatic islet transplantation has demonstrated that long-term insulin independence may be achieved in patients suffering from diabetes mellitus type 1. However, because of limited availability of islet tissue, new sources of insulin

producing cells are required. Development of pancreatic beta-cell lines from rodent or human origin has progressed slowly in recent years. Current experiments for ex vivo expansion of beta cells and in vitro differentiation of embryonic and adult stem cells into insulin producing beta-cell phenotypes led to promising results. Nevertheless, the cells generated to date lack important characteristics of mature beta cells and generally display reduced insulin secretion and loss of proliferative capacity. Therefore, much better understanding of the mechanisms that regulate expansion and differentiation of stem/progenitor cells is necessary.[24]

Animal studies suggested that bone marrow derived stem cells could transdifferentiate into beta-cells and correct the diabetic phenotype.[25] Subsequent studies failed to confirm these findings.[26,27] There is no peer reviewed published clinical data to suggest a clinical benefit from cell based therapy in diabetes mellitus. In mice, there are conflicting data as to whether hematopoietic stem cells contribute to pancreatic beta cells. To establish whether hematopoietic stem cells (derived from adult donors) transdifferentiate into pancreatic beta cells in adult humans, an autopsy study was carried out in hematopoietic stem cell transplant recipients. A study was done in 31 human pancreata obtained at autopsy from hematopoietic stem cell transplant recipients who had received their transplant from a donor of the opposite sex. Whereas some donor-derived cells were observed in the nonendocrine pancreata, no pancreatic beta-cells were identified that were derived from donor hematopoietic stem cells, including two cases with type 2 diabetes. The authors concluded that hematopoietic stem cells derived from adult donors contribute minimally to pancreatic beta-cells in nondiabetic adult humans. This data does not rule out the possibility that hematopoietic stem cells contribute to pancreatic beta-cells in childhood or in individuals with type 1 diabetes.[28]

Liver Disease

Initial publications on adult stem cell plasticity were greatly influenced by a publication by Lagasse et al, showing that HSCs could apparently transdifferentiate into hepatocytes in mice, with improvement of tyrosinemia.[29] Subsequently it was shown that these findings were due to cell fusion between the donor monocytes/macrophages rather than transdifferentiation.[12] A phase I study from Teheran involving infusion of autologous bone marrow HSCs through the hepatic artery in patients with decompensated cirrhosis was prematurely interrupted due to side effects.[30] There is no clinical data published in peer reviewed journals to suggest benefit of stem cell therapy in any hepatic disorder.

Autologous or Family Storage (Private or Commercial Cord Blood Banking)

Recently, a number of private companies have started facilities for commercial cord blood banking for autologous use. They advertise "cure for many life threatening diseases", speak of "unimaginable possibilities ……answers to curing diseases such as diabetes, breast cancer,……. rheumatoid arthritis, Parkinson's disease, regeneration of damaged heart tissue."[31] Such advertising induces parents to make a "one in a lifetime investment for their child". Recently a number of professional societies have issued guidelines or opinions on the subject. An opinion paper of the European Group on Ethics in Science and New Technologies and a Policy statement by the World Marrow Donors Association are freely available on the internet.[31,32] Most Professional bodies are against such banks and false advertising.[33-35]

Private cord blood storage companies have developed in many countries to sell cord blood storage to families for potential future autologous (patient's own cells) or family use. This is called "private storage" because the units are collected and stored solely to be available for the individual donor or the immediate family. These companies charge a collection fee, generally between $1000-

Table 2 : Factors against Autologous Cord Blood Banking

Likelihood of Using an Autologous Cord Blood Unit Today
The probability of using autologous cord blood for transplantation is estimated as approximately 1 in 20 000 during the first 20 years of life.
Premalignant cells may be found in the cord blood of children who later develop childhood malignancies and result in recurrence of the disease.
Autologous cord blood cannot be used to treat genetic diseases like hemoglobinopathies, inherited immunodeficiencies, etc. as the cells carry the same genetic defect.
Potential of Future Use in Regenerative or Reparative Medicine
Cord blood cells are not embryonal stem cells and are not pluripotent.
Stem cells being used in clinical trials are derived from the patient's own bone marrow or blood and not from their cord blood
There is no clinical evidence that cord blood cells are capable of curing any of the diseases as claimed in advertisements
If a future use is discovered of adult hematopoietic stem cell use, stem cells can always be collected from a patient's bone marrow.

$1500 USD and an annual storage fee, often approximately $100. Some companies have used sales approaches that appear focused on making the family feel that they are not being good parents, if they don't store their baby's cord blood for future use.[33,34] Many such companies have started operating in India.

Commercial banking has been criticized by numerous medical bodies, including the UK's Royal College of Obstetricians and Gynecologists, American Academy of Pediatrics, American College of Obstetricians and Gynecologists, Society of Obstetricians and Gynecologists of Canada, French National Consultative Ethics Committee for Health and Life Sciences, and European Group on Ethics in Science and New Technologies. In Italy the practice has been banned.[34] A recent European Union report highlighted serious ethical concerns about commercial UCB banks and questioned their legitimacy in selling a service of no real use.[31] The World Medical Donor Association issued a policy statement on the utility of Autologous Cord Blood Unit Storage, strongly criticizing promotion of these commercial ventures.[32]

The reasons why this form of storage is not recommended is summarized in Table 2. It is unlikely that autologous cord blood will ever be used for transplant in the child. Another false advertisement given is that cord blood will, in future, treat a number of diseases like diabetes mellitus, Parkinson's disease, cardiac disease. The advertisements do not state that such work is at the experimental stage and involves embryonal stem cells. *Cord blood cells are not embryonal stem cells.* Most of the work in systemic diseases is derived from bone marrow cells, is still not of proven clinical benefit, and there is no definite role of cord blood for these illnesses. Even if future research shows a role of hematopoietic stem cells in regenerative research, these cells could be collected from the bone marrow of the individual.

Conclusions

At present, there is no definite evidence that adult stem cells exhibit plasticity. The phenomenon of extramedullary hematopoiesis, which occurs in severe hemolytic anemia and other conditions, has never been associated with anything but a histologic picture of bone marrow nestled in a foreign tissue. There is no visible differentiation into host tissue.[36]

In the past decade, the field of stem cell biology has undergone a remarkable change. There have been numerous publications describing methods for transforming adult stem cells into cells of another germinal layer, a process called as transdifferentiation. A number of studies have also studied the fate of adult stem cells administered in vivo and their effect on disease progression in animal models and human clinical trials. Most of the in vivo studies have shown that the phenomenon of engraftment and transdifferentiation, which occurs in injured or diseased tissue, is of very low

level. Thus these cells do not contribute physically to tissue regeneration to any significant extent. Therefore the prospects of using these cells to treat disease are doubtful

The cells are a rich source of chemokines and cytokines. These paracrine factors can stimulate regeneration of cells by a variety of mechanisms like increasing angiogenesis, inhibiting apoptosis and suppressing immune reactions. These cells can also enhance proliferation and differentiation of tissue-endogenous stem/progenitors cells as indicated by recent experiments in which human stem/progenitor cells were infused into the hippocampus of immunodeficient mice.[37] In addition, they may rescue cells with nonfunctioning mitochondria by transfer of either mitochondria or mitochondrial DNA, as was recently observed in coculture experiments. To some extent, they may also repair tissues by cell fusion.[38] Therefore, there may be benefit due to the effect of these cells on the tissue microenvironment rather than their capacity for transdifferentiation.

As these trials proceed, it seems imperative that some of the potential dangers should also be highlighted. One potential danger is that the clinical trials will be performed without appropriate controls or without well-defined end points. The danger seems particularly apparent in trials such as those in acute myocardial infarction in which there is great variability in the size and location of the lesions, the outcomes are difficult to predict, and different parameters have been used to assess heart function.[39]

A second potential risk arises from the striking ability of stem/progenitors cells to enhance repair of tissues and to suppress immune reactions. Several reports demonstrated that MSCs stimulate the growth of cancers in mice. The cells apparently enhance growth of the cancer by decreasing immune reactions. Therefore, there is a risk that administering MSCs or similar cells will enhance the growth of a previously undetected cancer in a patient.[39] By alerting everyone to the possibility, researchers may be able to avoid repeating the sad episode in the history of viral gene therapy in which a shadow was cast over the whole field by a trial in which 9 of 10 patients with severe combined immunodeficiency disease were cured but 2 patients subsequently developed leukemia because of an unanticipated insertional mutation from a retrovirus.[40]

Another risk is that stem/progenitor cells that are extensively expanded in culture may themselves generate tumors in patients.[39] Another potential danger is when cells that are injected in high concentrations into tissues. Concentrated cells can form aggregates, particularly if sheared by passage through small needles under pressure. Therefore, if they are not handled with extreme care, they can produce pulmonary emboli or infarctions after infusion into patients. In the wave of enthusiasm for using adult stem cells for a variety of disorders, several essential precautions are not being fully addressed. Therefore, there is a great danger that potentially important new therapies will be discarded prematurely because of poorly designed clinical trials.[39]

It is important to play down promises to the public that the work will produce anything of clinical value in the foreseeable future. It is not known whether human marrow contains a pluripotent stem cell that can transdifferentiate. We should guard against both premature declarations of victory and premature abandonment of a promising therapeutic strategy. The ultimate success of this strategy is likely to depend on continued and effective coordination of rigorous basic and clinical investigations.[14]

Summary

Since the discovery of embryonic stem cells, which are pluripotent, research workers have been excited by the possibility of utilizing stem cells for regenerative medicine. Use of human stem cells has raised ethical objections and the practice is banned in many countries. Observations based on hematopoietic

and cardiac transplantation suggested that adult stem cells also possess the property of 'plasticity' or 'trans-differentiation', thereby forming cells beyond the limitations of their cell line commitment. A number of laboratory experiments seemed to confirm this, raising hopes of using cells like the hematopoietic stem cells to generate tissues of any organ. Many of these observations and experiments utilized techniques, which were prone to error. Subsequent studies in the laboratory, animal experiments, and review of human tissue data failed to confirm the original observations. There is a major debate among basic scientists about the very existence of adult stem cell plasticity. Despite lack of definite data, a large number of clinical trials were started utilizing bone marrow cells for a number of diseases with special emphasis on cardiology disorders. The results have been mixed. Some studies have shown benefit, which is usually minor, whereas others have shown no difference. Any benefit does not imply that this is due to the property of 'plasticity'. A number of paracrine effects of infusing or transplanting adult stem cells can contribute to tissue repair. It is important to continue basic research in stem cell biology and conduct well planned human trials to increase our understanding of this exciting field. At present use of stem cells is not standard of care except for hematopoietic stem cell transplantation. The hype of stem cell therapy appears premature but the hope remains that continued efforts in this field will result in clinical benefit.

References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282: 1145-7.
2. Serafini M, Verfaillie CM. Pluripotency in adult stem cells: state of the art. Semin Reprod Med 2006;24: 379-88.
3. Verfaillie CM, Pera MF, Lansdorp PM. Stem cells: hype and reality. Hematology Am Soc Hematol Educ Program 2002: 369-91.
4. Ramakrishnan A, Shi D, Torok-Storb B. Characterizing donor-derived cells in nonhematopoietic tissue. Biol Blood Marrow Transplant 2006;12: 990-2.
5. Christoforou N, Gearhart JD. Stem cells and their potential in cell-based cardiac therapies. Prog Cardiovasc Dis 2007;49: 396-413.
6. Hocht-Zeisberg E, Kahnert H, Guan K, et al. Cellular repopulation of myocardial infarction in patients with sex-mismatched heart transplantation. Eur Heart J 2004;25: 749-58.
7. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410: 701-5.
8. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428: 664-8.
9. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004;428: 668-73.
10. Nygren JM, Jovinge S, Breitbach M, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med 2004;10: 494-501.
11. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425: 968-73.
12. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422: 897-901.
13. Liao R, Pfister O, Jain M, Mouquet F. The bone marrow--cardiac axis of myocardial regeneration. Prog Cardiovasc Dis 2007;50: 18-30.
14. Rosenzweig A. Cardiac cell therapy--mixed results from mixed cells. N Engl J Med 2006;355(12):1274-7.
15. Hofmann M, Wollert KC, Meyer GP, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation 2005;111: 2198-202.
16. Ince H, Petzsch M, Kleine HD, et al. Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. Circulation 2005;112(9 Suppl): I73-80.
17. Ripa RS, Jorgensen E, Wang Y, et al. Stem cell mobilization induced by subcutaneous granulocyte-colony stimulating factor to improve cardiac regeneration after acute ST-elevation myocardial infarction: result of the double-blind, randomized, placebo-controlled stem cells in myocardial infarction (STEMMI) trial. Circulation 2006;113: 1983-92.
18. Zohlnhofer D, Ott I, Mehilli J, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. Jama 2006;295: 1003-10.
19. Srivastava D, Ivey KN. Potential of stem-cell-based therapies for heart disease. Nature 2006;441: 1097-9.
20. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science 2000;290: 1775-9.

21. Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells in vivo. Science 2002;297: 1299.

22. Wislet-Gendebien S, Hans G, Leprince P, Rigo JM, Moonen G, Rogister B. Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 2005;23: 392-402.

23. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106: 1755-61.

24. Limbert C, Path G, Jakob F, Seufert J. Beta-cell replacement and regeneration: Strategies of cell-based therapy for type 1 diabetes mellitus. Diabetes Res Clin Pract 2007. Sep 11; [Epub ahead of print]

25. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003;111: 843-50.

26. Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA. Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. Diabetes 2004;53: 91-8.

27. Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF. No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells in vivo. Diabetes 2004;53: 616-23.

28. Butler AE, Huang A, Rao PN, et al. Hematopoietic stem cells derived from adult donors are not a source of pancreatic beta-cells in adult nondiabetic humans. Diabetes 2007;56: 1810-6.

29. Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6: 1229-34.

30. Mohamadnejad M, Namiri M, Bagheri M, et al. Phase 1 human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. World J Gastroenterol 2007;13: 3359-63.

31. http://ec.europa.eu/european group ethics/docs/avis 19 en.pdf (accessed 26 Nov 2003)

32. www.worldmarrow.org/…/DRWG/Cord Blood Registries/ WMDA Policy Statement Final 02062006.pdf (accessed 26 Nov 2006)

33. Edozien LC. NHS maternity units should not encourage commercial banking of umbilical cord blood. BMJ 2006;333: 801-4.

34. Fisk NM, Roberts IA, Markwald R, Mironov V. Can routine commercial cord blood banking be scientifically and ethically justified? PLoS Med 2005;2: e44.

35. Lubin BH, Shearer WT. Cord blood banking for potential future transplantation. Pediatrics 2007;119: 165-70.

36. Schwartz RS. The politics and promise of stem-cell research. N Engl J Med 2006;355: 1189-91.

37. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci U S A 2005;102: 18171-6.

38. Ogle BM, Cascalho M, Platt JL. Biological implications of cell fusion. Nat Rev Mol Cell Biol 2005;6: 567-75.

39. Prockop DJ, Olson SD. Clinical trials with adult stem/ progenitor cells for tissue repair: let's not overlook some essential precautions. Blood 2007;109: 3147-51.

40. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302: 415-9.