Advancing Stem Cell Science Without Destroying Human Life

Domestic Policy Council

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Domestic Policy Council
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Executive Summary

In 2001, President Bush established a policy on stem cell research that promotes scientific progress while respecting ethical boundaries. This policy is based on the President's firm belief that science and ethics need not be at odds, and that a balance can be struck between the natural desire for rapid scientific progress and the demands of conscience. Drawing careful distinctions between practices that avoid ongoing destruction of nascent human life and those that do not, the President's policy has allowed stem cell research to advance in rapid and promising ways—as the pages that follow will illustrate—without sacrificing the inherent dignity and matchless value of every human life.

Before President Bush took office, there was no federal funding for human embryonic stem cell research. Then on August 9, 2001, the President announced his decision to allow federal funds to be used for experiments on the embryonic stem cell lines that existed at the date of his announcement. To avoid encouraging further destruction of human embryos, no taxpayer funding could be expended on research using cell lines from embryos destroyed from that point forward.

The President's policy and new funding has produced considerable scientific investigation and progress. Over the past six years, more than $130 million of federal money has been devoted to human embryonic stem cell research consistent with the President's policy. Overall, more than $3 billion has gone to innovative research on all forms of stem cells, contributing to dozens of proven medical treatments.

In May of 2005, the President's Council on Bioethics issued a "White Paper"[1] reviewing possible non-destructive sources for additional stem cell lines in the future. *Alternative Sources of Human Pluripotent Stem Cells* examined the ethical, scientific, and practical prospects for a range of different approaches, and

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[1] President's Council of Bioethics*, White Paper: Alternative Sources of Human Pluripotent Stem Cells,* May 2005. (http://www.bioethics.gov/reports/white_paper/index.html)

recommended specific investigations that could advance these techniques. The goal of all of these alternative approaches is to produce the functional equivalent of embryonic stem cells, without harming embryos.

In the months since the Council's report, peer-reviewed studies on each of the approaches have been published in leading scientific journals, suggesting many opportunities for ethical creation of new stem cell lines. One of the most promising possibilities is adult-cell reprogramming. Recent work by two research teams—Kevin Eggan and Chad Cowan of the Harvard Stem Cell Institute, and Shinya Yamanaka and Kazutoshi Takahashi at Japan's Institute for Frontier Medical—suggests that it may be possible to use chemical and genetic factors to reprogram an adult cell to function like an embryonic stem cell. This approach could prove capable of creating cell lines for the study and treatment of disease without the many ethical dilemmas associated with the creation and destruction of embryos.

In January 2007, another very promising approach was outlined in the journal *Nature Biotechnology*. Dr. Anthony Atala of the Institute for Regenerative Medicine at Wake Forest University School of Medicine reported[2] that he and his colleagues had discovered a new and readily available source of stem cells in the amniotic fluid that cushions babies in the womb.[3] While the research is still developing, Dr. Atala and his team believe that these amniotic stem cells may be fully as flexible as embryonic stem cells, while having additional medical advantages: they are easier to grow than human embryonic stem cells, and they do not form tumors (a problem that has

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[2] Atala, A et al., Isolation of amniotic stem cell lines with potential for therapy, Nat Biotechnology. 2007 Jan 7; [Epub ahead of print].

[3] Mary Carmichael, N*ew Stem-Cell Source Could Alter Debate*, Newsweek.com, January, 7, 2007.

plagued embryonic stem cell use).[4] And since amniotic stem cells can be collected without destroying human life, they avoid the ethical dilemmas.[5]

In sum, it increasingly appears that the qualities researchers value in embryonic cells may also exist in other stem cells that are easier to procure, more stable to grow, safer to use in therapies, and free of the ethical violations of embryo destruction. There is a gathering consensus among experts, thanks to technical advances, that today's heated controversies over research that harms embryos could fade in the future.[6]

The dramatic advances in stem cell research since 2001 are evidence that the President's balanced policy is working. Scientists have shown they have the ingenuity and skill to pursue the potential benefits of embryonic stem cell research without endangering nascent human life in the process. In supporting these alternative approaches while maintaining longstanding bioethical guardrails which protect life and dignity, federal science-research funding can stay true to the ideals of a humane society.

[4] Mary Carmichael, *New Stem-Cell Source Could Alter Debate*, Newsweek, Web Exclusive, January 8, 2007; Maggie Fox, *Human stem cells found in amniotic fluid*, Reuters, January 8, 2007; *Breakthrough To Report In Stem Cell Research, The Discovery Of A New Source Of Stem Cells*, NBC's Nightly News, January 7, 2007; Dan Harris, *These Cells Can Be Gathered Without Hurting The Mother Or The Fetus, And Would Prove Much Less Controversial Than Research Done With Embryos,"* ABC's World News Sunday, January 7, 2007.
[5] Dan Harris, *These Cells Can Be Gathered Without Hurting The Mother Or The Fetus, And Would Prove Much Less Controversial Than Research Done With Embryos,* ABC's World News Sunday, January 7, 2007.
[6] Rick Weiss, *Scientists See Potential in Amniotic Stem Cells*, Washington Post, January 8, 2007; Rick Weiss, *Stem Cell Advances May Make Moral Issues Moot*, Washington Post , June 6, 2005.

1. Current Federal Law and Policy on Stem Cell Research

The History: From Clinton to Bush

Private-sector human embryonic stem cell research has been and continues to be permissible without restriction in the U.S. The national discussion over embryonic stem cells has largely taken the form of a debate over federal funding of this kind of research. Every year since 1995, the annual HHS appropriations bill has contained language (known as the Dickey-Wicker Amendment) that prohibits federal funding of research in which human embryos are destroyed or subjected to risk of injury or death.[7] In 1999 (a year after human embryonic stem cells were first derived in the lab), the Clinton Administration argued that as long as the actual destruction of embryos is performed with private dollars, research on the immediately resulting lines of stem cells could qualify for federal funding, since it technically would not be research in which embryos were directly destroyed with federal funding. That technical reading contradicted the intent of the law, and would have meant that taxpayer dollars would incentivize the destruction of embryos, since knowledge that federal funding would be available once the embryos were destroyed could provide a direct encouragement to engage in that destruction.

The Clinton Administration drafted rules along the lines described above, but their term ended before they could be put into effect. No federal dollars ever went to support human embryonic stem cell research. Upon entering office, President Bush examined the issue closely, seeking a way to advance potentially significant research without using taxpayer funds to support or encourage the destruction of nascent human lives.

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[7] The full text of the Dickey Amendment can be found in each year's Labor/HHS Appropriations Bill. The law states that "None of the funds made available in this Act may be used for—(1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204 and 46.207, and subsection 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)). (b) For purposes of this section, the term 'human embryo or embryos' includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of the governing appropriations act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells."

On August 9, 2001, President Bush announced his new policy: research on lines of human embryonic stem cells that already existed could qualify for funding, since those lines were created without any taxpayer-funded incentive, and the life-and-death decision on the embryos had already been made. Lines created subsequently would not be eligible, so that federal dollars would not further ongoing embryo destruction. The President's policy marked the first time the federal government ever made funding available for human embryonic stem cell research.

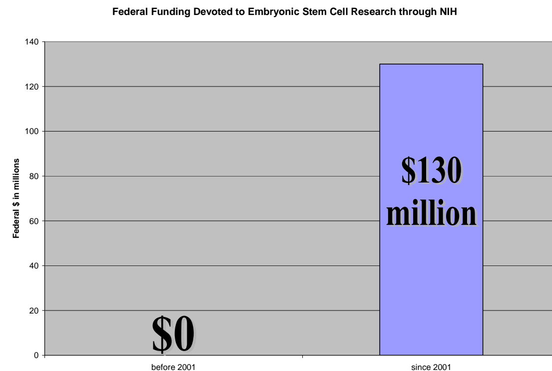
Embryonic Stem Cell Lines Available for Federal Funding

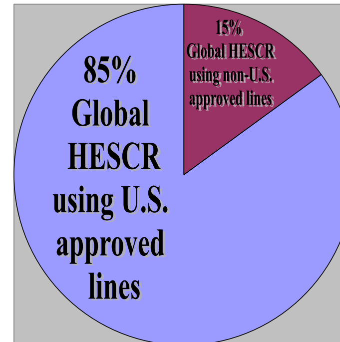
For more than half a century, the federal government has funded biomedical research through the National Institutes of Health. This support reflects the great value Americans place on the development of treatments and cures for those who suffer afflictions of health. But such support has never been offered indiscriminately or without ethical and moral restrictions. Researchers and institutions that accept federal funds must abide by many ethical strictures, including regulations governing the use of human subjects in research.

Controversies surrounding the morality of certain scientific undertakings have given rise to disputes over federal funding. When making decisions that involve moral and ethical issues plus taxpayer dollars, responsible public policy dictates caution. The debate about whether the federal government should support research that involves the creation or destruction of human embryos naturally falls into this category of policy.

This Administration is the first to provide federal funds for human embryonic stem cell research. And it has established this new funding within a careful framework that avoids encouraging the destruction of human embryos. Scientists can explore potential applications using existing cell lines—21 separate lines have been made available to researchers, in more than 1,000 shipments of cells, with over 3,000 more shipments available upon request from the NIH. To date, over 85 percent of

human embryonic stem cell research projects leading to publication worldwide have used these approved lines.[8]





Non-Federally Funded Embryonic Stem Cell Research

Contrary to common misperceptions, there is no Presidential ban on human embryonic stem cell research. No federal mandates constrain private or state-government funding of any element of this science. In fact, funding by individual states is expected to add up to several billion dollars in the next few years.

The President's policy has been built on the proposition that stem cell research is proceeding rapidly and will continue to make progress in the future without requiring ethical shortcuts that use taxpayer dollars in ways that tread on established protections for human life. There is no reason to sacrifice longstanding moral concerns in a shortsighted rush for therapeutic payoffs. The wisdom of a careful approach would seem to be underscored by the growing likelihood (based on the last year's research) that alternative sources of pluripotent stem cells may be available in the future without requiring the embryo destruction that has engendered so much controversy.

[8] Jason Woen-Smith and Jennifer McCormick, *An International gap in Human ES Cell Research*, Nature Biotechnology, 2006 Apr;24(4):391-2.

Rather than providing taxpayer dollars for methods that raise ethical concerns, the Administration has promoted intensified research into techniques to develop stem cells with the characteristics of those derived from embryos but without doing harm to embryos. And the ethical direction encouraged by the President in 2001 has begun to bear fruit. Scientists are now exploring a range of non-destructive techniques, with numerous articles in leading scientific journals reporting encouraging progress. Some examples are described in the second section of this report.

The Ethical Debate

Our nation has a long history of pioneering medical advances that improve life span and quality. We also have a deep tradition of protecting human life and dignity during scientific investigation.

Research on embryonic stem cells raises profound ethical quandaries. Most fundamentally, is an embryo a human life, and something therefore to be protected? This topic forces us to confront fundamental questions about the beginnings of life and the ends of science. These issues lie at a difficult moral intersection, where the desire to extend and improve life crosses the need to respect all human beings equally.

Embryos are humans in their earliest developmental stage. We do not have to think that human embryos are exactly the same in all ways as older humans to believe that they are entitled to respect and protection. Each of us originated as a single-celled embryo, and from that moment have developed along a continuous biological trajectory throughout our existence. To speak of “an embryo” is to designate a human being at a particular stage.

Our nation was founded on the principle that all of us are created equal, and endowed with a right to exist that is shared fully by all humans. There is no such thing as an excess life. And the fact that a human lacks some particular capacity, or

even is going to die, does not justify experimenting on that individual, or exploiting him or her as a natural resource. That has long been the standard in medical ethics—as encoded in the Hippocratic Oath, as well as more modern codes like the Physician's Oath in the 1948 Declaration of Geneva, which states: "I will maintain the utmost respect for human life from the time of conception."

To think of other human beings (and especially the weakest and most vulnerable of us at the beginning or end of life) as potential spare parts for use by others is a profound threat to human dignity and our society's broader respect for its citizens. It is something the U.S. government has scrupulously avoided to this point.

The destruction of embryos for experimental purposes could also open the way to more general and profound manipulations and reengineerings of human life. Without an understanding that life begins at conception, and that an embryo is a nascent human being, there will always be arguments that other uses, takeovers, and makeovers of embryos are justified by potential scientific and medical benefits. Crossing this line would needlessly encourage a conflict between science and ethics that can only do damage to both, and to our nation as a whole.

This potentially dangerous opening would establish exactly the wrong tone as the era of biotechnology dawns. This emerging age has golden promise. But we must pursue its great hope in ways consistent with our principles. That will sometimes require saying no. To find the right paths for medical research, we must be willing to reject paths that are morally wrong.

A policy that defends the inviolability of human life does not preclude the hopeful possibilities of new findings and new therapies. It simply means we must harness the creative powers of our advancing knowledge only to humane and morally balanced means and ends. Amidst today's dizzying pace of technological innovation, it is worth taking care to make sure that our moral and ethical policies keep up. The biotechnology revolution will bring sound and wholesome human results over the long run only if it is sensibly governed.

The stem cell debate is only the first in what will be an onrushing train of biotechnology challenges in our future. We must establish a constructive precedent here for taking the moral dimensions of these issues seriously. We must make certain we don't force ourselves into a false choice between science and ethics—because we need *both*.

And there is good reason, and growing scientific evidence, to believe that we can have both.

2. <u>Recent Developments in Non-Embryo-Destructive Stem Cell Research and Therapy</u>

In January 2004, the President's Council on Bioethics published a report entitled "Monitoring Stem Cell Research." In the introduction, the report made the following comment:

> Monitoring stem cell research can be a bit like watching Niagara Falls. Not only do scientific reports pour forth daily, as they do in many other areas of research, but a kind of mist rises up from the torrent of news flashes and editorials, making it difficult to separate knowledge from opinion and hope from hype. The underlying biology—whether viewed at the level of the gene, cell, tissue, organ, or organism—is dauntingly complex, as is all cell biology. At any of these levels, in this new and dynamic field, it is frequently difficult for even the most knowledgeable scientist to be truly certain of "what really causes what."[9]

The emotional appeal of stem cell research places further strains of exaggeration and misinterpretation on scientific claims. The cumulative effect is often a very foggy glass. In the two sections below—on developments in adult stem cell research, and on alternative sources of pluripotent stem cells—we offer a brief overview summarizing some significant recent research. Our aim is to build clearer public and legislative understanding that there are alternative scientific approaches which may be able to produce the functional equivalent of embryonic stem cells, but without harming embryos.

Advances in Adult Stem Cell Research

While research on adult stem cells began decades ago, important new discoveries have been made in just the past few years. Scientists have found adult stem cells in

[9] President's Council of Bioethics, *Monitoring Stem Cell Research,* January 2004, Pg. 15. (www.bioethics.gov/reports/stemcell/pcbe_final_version_monitoring_stem_cell_research.pdf)

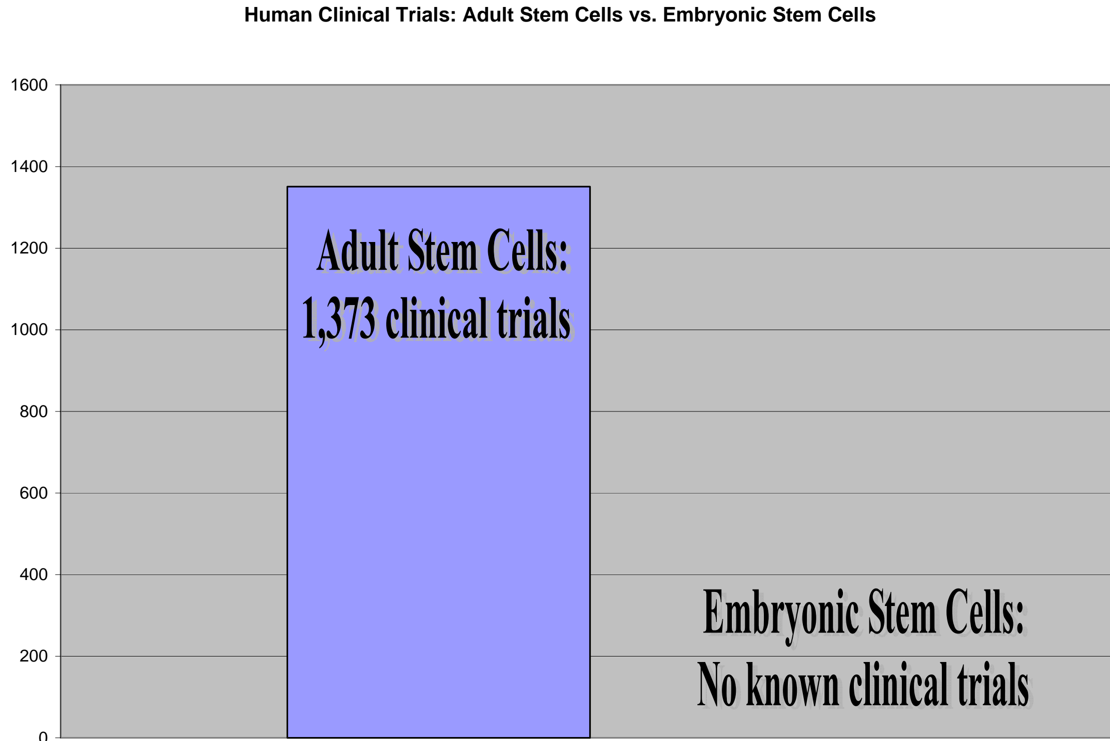
many more tissues than they once thought likely, including the brain, bone marrow, blood, blood vessels, skeletal muscle, skin, liver, and other body parts.[10] Given the right conditions, certain kinds of adult stem cells now seem to have the ability to differentiate into a number of different cell types. If this differentiation of adult stem cells can be controlled and sustained in the laboratory, these cells could become the basis of therapies for many serious common diseases and injuries. As examples of potential treatments, an NIH list includes replacing the dopamine-producing cells in the brains of Parkinson's patients, developing insulin-producing cells for type I diabetes, and repairing damaged heart muscle with new cardiac muscle cells following a heart attack.[11]

Scientists have also reported that adult stem cells may, in some cases, exhibit the ability to form specialized cell types of other tissues, a characteristic known as transdifferentiation, or plasticity. Some experiments have even suggested that certain adult stem cell types may be pluripotent.[12] Over the past few years, several examples of adult stem cell plasticity have been reported.[13] Most evidence indicates that while embryonic stem cells are capable of becoming all of the cell types of the body, adult stem cells may be more limited. Adult stem cells can also be difficult to isolate, and methods of growing them in culture are still a work in progress.

Adult stem cells, however, do have some important scientific advantages as potential therapies. For one, since adult stem cells can be taken from the patient's own tissue, they are genetically matched, and would not be rejected by that patient's own immune system. This is one reason they have already been successful in treating many patients, while there are no known human clinical trials or successful therapies derived from embryonic stem cells. As of April 2, 2007, there have been 1,373 publicly available clinical trials related to adult stem cells, including 671 that are currently recruiting patients.[14] Over the last couple of years, clinical trials using adult stem cells have produced encouraging improvements in patients suffering a

[10] http://stemcells.nih.gov/info/basics/basics4.asp
[11] http://stemcells.nih.gov/info/basics/basics4.asp
[12] http://stemcells.nih.gov/info/basics/basics4.asp
[13] http://stemcells.nih.gov/info/basics/basics4.asp
[14] http://www.clinicaltrials.gov/ct/search?term=stem+cell&submit=Search

range of diseases and disorders, including leukemia, lymphoma, diabetes, advanced kidney cancer, and several inherited blood disorders.[15]



It is important to note that in comparison to adult stem cell research, embryonic stem cell research is still in its infancy, and its potential should be understood in this context. Human embryonic stem cell studies are in early stages, and are currently being used to help understand the differentiation and functions of many human tissues, including neural, cardiac, vascular, pancreatic, hepatic, and bone. Embryonic stem cells have two properties that make them promising for research and cell therapy. First, they can be grown in tissue culture, and therefore provide an abundant, renewable source of cells. Second, they are pluripotent and can (theoretically) be directed to become virtually any cell type of the adult body.

But while much has been postulated about the ability of embryonic stem cells to generate transplantable cells for medical applications, many scientific hurdles

[15] http://stemcells.nih.gov/info/health.asp

remain before they could be used clinically in the United States. One of the difficulties specific to embryonic stem cells is their propensity to form tumors. This dangerous property is the corollary to their extensive growth potential. Other issues needing solutions include questions of how to generate functional differentiated cells, and how to solve immune rejection issues. Although there is high potential for treatment of degenerative disease with human embryonic stem cells, development of practical applications lags behind adult stem cells, and indeed has been limited to animal studies thus far.[16]

Advances in Alternative Sources of Pluripotent Cells

Recent biological advances have raised encouraging possibilities for producing powerful stem cells without harming human embryos. What scientists value most about embryonic stem cells is their pluripotency and expandability—that they have the potential to be teased into many, and perhaps all, of the different cell types in the body. But scientists have begun to find that this potential may also exist in certain cells derived without embryos. New ways of producing pluripotent cells that don't require the destruction (or even endangerment) of human embryos are now being investigated.

In May 2005, the President's Council on Bioethics published *Alternative Sources of Human Pluripotent Stem Cells*, a White Paper which suggested four possible approaches for alternative sources: (1) by extracting viable cells from embryos already dead; (2) by non-harmful biopsy of living embryos; (3) by extracting cells from artificially created non-embryonic cellular systems or entities (engineered to lack the essential elements of an embryo); (4) by reprogramming (or de-differentiation) of adult cells back to pluripotency. Each of these methods carries its own scientific and ethical uncertainties, but one or more may ultimately offer a path toward an ethically responsible source of pluripotent stem cells.

In January 2007, landmark research from Wake Forest University has suggested yet another alternative: Amniotic fluid, investigators discovered, contains highly flexible

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[16] http://stemcells.nih.gov/staticresources/info/scireport/PDFs/C.%20Chapter%201.pdf (Pg. 8)

stem cells shed by the fetus. These appear to have all the valuable qualities of embryonic stem cells, plus some advantages of their own—like greater ease and speed of culture, and no tendency to produce tumors. Meanwhile, they are comparatively easy to collect without harming human life.

We begin our review with the four methods identified by the Bioethics Council. Just in the short period since they were sketched out in May 2005, significant progress has been made on each of these techniques, as reported in a number of new, peer-reviewed research studies published in leading scientific journals. At this point, one of the most promising avenues appears to be somatic cell reprogramming, which uses chemical and genetic factors to reprogram an adult cell to function like an embryonic stem cell.

Each cell in an individual's body has the same DNA as every other cell. But in the course of developing into specialized adult tissues, different cells undergo different patterns of gene activation. Somatic cell reprogramming seeks to switch on or off the appropriate genes to transform an adult cell back into the equivalent of an embryonic stem cell. This might be accomplished by stimulating the adult cell with the right combination of chemicals and genes, or by exposing it to the cytoplasm of an existing line of stem cells. This method could, in theory, create stem cells in bulk while bypassing entirely the problem of creating and destroying embryos.

Two research teams that have demonstrated significant progress toward this sort of cell reprogramming are Kevin Eggan and Chad Cowan from the Harvard Stem Cell Institute, and Shinya Yamanaka and Kazutoshi Takahashi from the Department of Stem Cell Biology at Japan's Institute for Frontier Medical Science. In August of 2005 and August of 2006, respectively, each team published impressive results which seemed to produce pluripotent stem cells by reprogramming ordinary adult cells.

The project[17] conducted at the Harvard Stem Cell Institute fused a human adult cell with an embryonic stem cell. (These could come from one of the embryonic stem cell lines that President Bush has approved for use with federal funds.) That effectively turned back the clock on the adult cell such that it was reprogrammed to a pluripotent state. Eggan and Cowan believe that their research could lead to “an alternative route for creating genetically tailored human embryonic stem cells for use in the study and treatment of disease.”[18]

Drs. Yamanaka and Takahashi published research based on mouse cells.[19] Their complex study produced stunningly simple results: They reprogrammed adult cells into a pluripotent state simply by bathing them in four genetic factors. “The finding is an important step in controlling pluripotency, which may eventually allow the creation of pluripotent cells directly from somatic cells of patients,”[20] comments Dr. Yamanaka. If successful, this could offer all the benefits of embryonic stem cells and more—these cells could be genetically matched to any prospective patient—without the ethical dilemmas of embryonic destruction.

Other promising avenues are likely to open in the future. The latest alternative, published[21] on January 7, 2007, in *Nature Biotechnology*, involves amniotic-fluid stem cells. Dr. Anthony Atala and a team from the Institute for Regenerative Medicine at Wake Forest University School of Medicine and the Harvard Medical School reported on a new category of readily available stem cells extractable from the waters cushioning babies in utero, as well as the placenta. While this very new research will need to be replicated and confirmed, Dr. Atala and colleagues have

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[17] Cowan, C.A., Eggan, Kevin, et al., *Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells*, Science, 2005 Aug 26;309(5739):1369-73.
[18] Cowan, C.A., Eggan, Kevin, et al., *Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells*, Science, 2005 Aug 26;309(5739):1369-73.
[19] Takahashi, K. and S. Yamanaka, *Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors*, Cell, 2006 Aug 25;126(4):663-76. Epub 2006 Aug 10.
[20] http://www.eurekalert.org/pub_releases/2006-08cp-wff080906.php
[21] Atala, A et al., Isolation of amniotic stem cell lines with potential for therapy, Nat Biotechnology. 2007 Jan 7; [Epub ahead of print].

already managed to grow useful brain, bone, liver, muscle, and other replacement tissues using these stem cells.[22]

This discovery suggests that if all U.S. newborns had their amniotic stem cells frozen, they could be available for future tissue replacement without fear of immune rejection. Moreover, a bank of amniotic stem cells from the waters of 100,000 pregnancies could supply 99 percent of the U.S. population with genetically compatible stem cells for possible transplantation. More studies will be needed to confirm that amniotic stem cells can generate all other cell types, but so far every culture attempted has succeeded. It must also be determined that tests conducted in mice will translate to humans.

Meantime, that is but one of several promising glimmers in the latest research. What follows is a summary of the preliminary analysis from the Bioethics Council on the four alternative approaches they discussed in their May 2005 White Paper, supplemented by fresh additional findings from the most significant peer-reviewed studies published in each area since May 2005. It is important to note that while many of these alternative approaches show great promise, all of them are still being tested in animals and are thus in the early stages of development. The Bioethics Council has conducted only a preliminary analysis of the approaches, as used in animals. The Administration does not endorse any of these specific avenues of research in humans at this time. All new approaches will have to be carefully considered by scientists, regulators, and others as appropriate on a case-by-case basis, as further information and research becomes available. It may be that some of these research proposals could prove inconsistent with the Dickey-Wicker Amendment protections against creating, destroying, or harming human embryos, or they may fail some other test of ethical or scientific viability. But the cumulative weight of this emerging science is, as a *Washington Post* science journalist recently

[22] http://www.eurekalert.org/pub_releases/2007-01/wfub-sdn010207.php

summarized, "adding credence to an emerging consensus among experts that the popular distinction between embryonic and 'adult' stem cells…is artificial."[23]

a. Pluripotent Stem Cells Derived from Dead Embryos

Under this proposal, viable cells would be derived from early in vitro fertilization (IVF) embryos (of roughly 4-8 cells) that have spontaneously died (as large percentages of early embryos do). Crucial to this approach is defining and verifying what represents organismic death in an early embryo, which represents an ongoing challenge for ethics and science. This would be a determining factor in whether this approach could ever be used in humans. Drs. Donald Landry and Howard Zucker of the Columbia University College of Physicians and Surgeons presented this proposal to the Council.

The preliminary evaluation from the Council in 2005 was that while it raised "some serious ethical questions," the Council found the proposal "to be ethically acceptable for basic investigation in humans" as long as "stringent guidelines like those proposed by Drs. Landry and Zucker were strictly observed." At the time, the proposal had not been tested, even in animals. The Council suggested that certain non-invasive studies to explore and define organismic death could be pursued immediately.

In June 2006, Dr. Landry published a peer-reviewed study[24] that proposed applying "the ethical framework currently used for obtaining essential organs from deceased persons for transplantation" to the harvesting of stem cells from dead human embryos. The study presented a history of the concept of embryonic death and compared the function of viable embryos with non-viable embryos. Dr. Landry's research suggested that an irreversible loss of function could be documented and thus used as criteria that would permit a determination of embryonic death. Using

[23] Rick Weiss, *Scientists See Potential in Amniotic Stem Cells*, Washington Post, January 8, 2007.
[24] Don Landry et al., *Hypocellularity and Absence of Compaction as Criteria for Embryo Death*, Regenerative Medicine. Published in June 2006.

this criteria, Dr. Landry made the case that approximately a fifth of all embryos generated for in vitro fertilization could be re-classified as dead. This would enable thousands of new embryonic stem cell lines to be created without destroying a single living embryo.

In September 2006, scientists from the Centre for Stem Cell Biology and Developmental Genetics at the University of Newcastle in Britain published a peer-reviewed study[25] that described how embryos lacking essential function could be used as a source for the derivation of human embryonic stem cells.

Despite the promising work of Dr. Landry and the scientists at Newcastle, more work is required to address the concern that stem cells from dead embryos might not have the same pluripotent capacity as stem cells obtained from living embryos. In addition, more research is required on the concept and criteria that would permit determination of embryonic death in a way that is ethically unassailable for use in human embryos.

b. Pluripotent Stem Cells via Blastomere Extraction from Living Embryos

Under this proposal, pluripotent stem cells would be obtained through biopsy of an early human embryo. Crucial to this approach is finding a stage of early embryonic development at which one or a few cells can be removed without harming the embryo, with the removed cells being useable as a source of pluripotent stem cells.

The preliminary evaluation from the Council in 2005 was that this proposal was ethically unacceptable for humans. The Council stated that imposing "risks on living embryos destined to become children, for the sake of acquiring stem cells for research" could not be justified. While the approach could be attempted in animals, the Council did not anticipate that results from animal experimentation could alter its assessment that this method would be ethically unacceptable for humans.

[25] Zhang, X., P. Stojkovic, et al., *Derivation of human embryonic stem cells from developing and arrested embryos,* Stem Cells Express, 2006 Dec;24(12):2669-76. Epub 2006 Sep 21.

In August 2006, Dr. Robert Lanza and a team at the firm Advanced Cell Technology (ACT) made widely reported[26] claims that human embryonic stem cells could be derived from blastomeres without requiring the blastomeres' destruction. However, once the details of the study's methodology were reported, it became clear that the techniques used by the researchers did in fact destroy every one of the human embryos used, a fact ACT did not make clear in its initial press releases. This work thus neither proved nor disproved that human embryonic stem cells could be derived from individual cells extracted from an embryo without harming it.

c. Pluripotent Stem Cells Derived from Biological Artifacts

Under this proposal, pluripotent stem cells lines would be derived from an engineered "biological artifact" that lacks the organismal character of a human embryo. Crucial to this approach is demonstrating both that the developing entity is truly not a human embryo and that the cells derived from it are normal human pluripotent cells. In addition, one must show that creating such biological artifacts does not itself introduce other ethical problems. One such proposal, Altered Nuclear Transfer (ANT), was presented to the Council by member Dr. William Hurlbut of Stanford University.

The preliminary evaluation from the Council in 2005 was that this proposal would need to be carefully tested in animals before human trials could be considered, but that there were no insuperable ethical objections that would preclude pursuing it. The possibility of any future endorsement for trying this approach in humans would depend upon a more thorough ethical analysis made possible in part by the animal experiments.

[26] Klimanskya, I., Y. Chung, S. Becker, et al., *Human embryonic stem cell lines derived from single blastomeres,* Nature, Advance online publication, August 23, 2006.

In October 2005, Rudolf Jaenisch and Alex Meissner, scientists at the Whitehead Institute for Biomedical Research at MIT, conducted a peer-reviewed study[27] of ANT in mice. “To assess the validity” of ANT, the researchers conducted a study that used a process of gene silencing and nuclear transfer to generate a laboratory-constructed biological entity that could not implant in a uterus and was morphologically unlike a natural embryo. This non-embryonic entity nonetheless yielded fully functional pluripotent stem cells with the same characteristics as those obtained from embryos. The study in mice noted that this approach could produce pluripotent stem cells genetically matched to patients (because the nucleus transferred could be from one of their own cells).

More recently, in November 2006 testimony[28] to the Bioethics Council, Dr. Hans Scholer, Director of Cell and Developmental Biology at the Max Planck Institute in Muenster, Germany, discussed further advances that support the scientific feasibility and moral acceptability of ANT. Dr. Scholer used ANT techniques to preemptively silence a key factor essential for defining an embryonic organism. Using this technique he was able to obtain pluripotent stem cell lines at an earlier stage and at a rate 50 percent higher than from direct destruction of IVF-created embryos.

d. <u>Pluripotent Stem Cells via Somatic Cell De-differentiation</u>

Under this proposal, adult cells would be reprogrammed to “de-differentiate” back into pluripotent stem cells. Crucial to this approach is discovering a way to reverse cell differentiation all the way to pluripotency, but without creating an organism capable of developing as nascent life.

The preliminary evaluation from the Council in 2005 was that this approach was ethically acceptable for use in humans, if and when it becomes scientifically practical, provided the line between pluripotent cell and living organism can be

[27] Meissner, A. and R. Jaenisch, *Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocyst,* Nature, 239: 212-5 (2006).
[28] President’s Council on Bioethics, Transcript from November 16, 2006. (http://www.bioethics.gov/transcripts/nov06/session1.html)

maintained. The Council noted that while this approach was scientifically and technically uncertain, there were encouraging results appearing in the scientific literature.[29]

In 2005, scientists from the Harvard Stem Cell Institute published a peer-reviewed study[30] in which a fusion of human embryonic stem cells with adult cells suggested that adult cells could be reprogrammed. If the remaining technical barriers are overcome—and some of them are significant—the approaches discussed in this study could circumvent societal concerns over cloning or the destruction of human embryos. A year later, Drs. Takahashi and Yamanaka of Japan published their peer-reviewed mouse study[31] examining 24 genes as candidates for conveying pluripotency.[32] They discovered that altering just four genetic factors proved sufficient to change adult cells into pluripotent stem cells. Scientists will now try to determine if human adult cells can be similarly reprogrammed using this method.

In November 2006, scientists from the School of Biosciences and Institute of Genetics, University of Nottingham, Loughborough, reviewed[33] the recent scientific advancements in reprogramming and concluded there is sufficient evidence demonstrating that adult cells can be reprogrammed and that cell-based approaches for therapeutics may be a realistic expectation in the future. The study reviewed nine reprogramming approaches that are commonly used today and commented on their

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[29] Guan K *et al.*, *Pluripotency of spermatogonial stem cells from adult mouse testis,* Nature 440, 1199-1203, 27 April 2006; Kanatsu-Shinohara M & Shinohara T, *The germ of pluripotency*, Nature Biotechnology 24, 663-664, June 2006; Cyranoski D, *Stem cells from testes: could it work?* Nature 440, 586-587, March 30, 2006; Carlin R *et al.*, *Reproductive Biology and Endocrinology* 4:8, doi:10.1186/1477-7827-4-8, 6 February 2006.

[30] Cowan, C.A., Eggan, Kevin, et al., *Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells*, Science, 309: 1369-1373, 2005 Aug 26;309(5739):1369-73.

[31] Takahashi, K. and S. Yamanaka, *Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors*, Cell, 2006 Aug 25;126(4):663-76. Epub 2006 Aug 10.

[32] http://www.eurekalert.org/pub_releases/2006-08cp-wff080906.php

[33] Andrew Johnson et al., *Reprogramming Somatic Cells into Stem Cells*, Reproduction, November 2006.

respective advantages and limitations. While it is too early to tell which approaches may or may not work, the sheer number of methods makes clear that there may be several different ways to induce reprogramming of adult cells in the future.

3. Conclusion

As this report shows, there has been tremendous scientific progress of late in exploring methods of deriving pluripotent stem cells without destroying embryos. This groundbreaking alternative research has developed under President Bush's insistence on advancing stem cell research within clear ethical guidelines. With continued support for non-destructive alternatives, new developments will continue to unfold in this field in the years to come, holding the potential for innovative progress toward new medical cures, while at the same time upholding human dignity and the sanctity of innocent life.

Appendix 1

Embryo adoption as an alternative to embryo destruction

In the complex debate over human embryonic stem cell research, it is important to remember that real lives are involved—both the lives of those with diseases who might find therapies or cures from this research, and the lives of embryos who will be destroyed or harmed in the process.

President Bush strongly supports embryo adoption as a life-affirming alternative to embryo destruction. Embryo adoption provides an option for the survival and development of embryos frozen in fertility clinics, giving them a chance at life and giving infertile couples the opportunity to build a family.

The value in frozen embryos can be seen in each child who began his or her life as a product of *in vitro* fertilization, remained unused and frozen after the fertility treatments were complete, but now breathes and burgeons in an adopted family. These "snowflake" children remind us of what is lost when embryos are destroyed in the name of research. They remind us that all human life begins as but a tiny collection of cells. And, they remind us that in our zeal for new treatments and cures it is important for society not to abandon moral fundamentals.

Embryo adoption shows that frozen embryos do not have to be destroyed. The Administration has worked to inform doctors and infertile couples of embryo adoption programs, and the President signed a bill in 2002 granting $1 million per year for HHS grants to publicize embryo adoption opportunities. To date, the Nightlight Christian Adoptions agency has matched 289 placing families with 192 adopting families. The resulting new lives are not raw materials to be exploited, but rather gifts to be cherished.

Embryo adoptions programs have led to the birth of 118 babies, with 25 families currently expecting. These children are reminders that embryos are not raw materials to be exploited, but rather gifts to be cherished.

Appendix 2

What is the connection to human cloning?

Human cloning would involve creating a new human being who is genetically identical to an existing human. It would be done by replacing the nucleus of an egg with the nucleus of a cell taken from the body of a donor. The result would be a developing human embryo carrying the genetic identity of the nucleus donor. This is the same technique that was used to create Dolly the sheep.

A cloned embryo is produced in the lab, and so exists at first outside the body of a mother, like an IVF embryo. The question of what is done with that embryo is the same as what is done with IVF embryos. It could be implanted in a woman, to potentially produce a cloned child. Or it could be experimented on or destroyed for its stem cells. Neither of these options is morally acceptable. We do not want to produce cloned children, for a wide variety of ethical reasons with which nearly everyone agrees. Likewise, we should not create human life merely to use it for research. As the President has said, “We recoil at the idea of growing human beings for spare body parts, or creating life for our convenience.”

Since neither possible use of cloned embryos is morally acceptable, the President has consistently argued that the only appropriate, effective, and responsible policy toward human cloning is to prohibit it. To prevent either the creation of life for experimental destruction or the production of cloned children, he believes, cloned human embryos should never be created.

Advocates of cloning for research (often referred to by its technical name—somatic cell nuclear transfer, or SCNT) try to assuage concerns by assuring us that they do not plan to transfer the cloned embryos into the womb of a woman. But whether or not the embryo is implanted in a woman for gestation and birth is not the action that defines human cloning, and makes it repugnant to most observers. That is simply a transfer, something accomplished with IVF embryos routinely. The morally significant act in human cloning occurs before the transfer—at the point when the

embryo is manufactured. Intentionally creating human life to destroy it for laboratory research is itself a violation of an important moral principle. Moreover, the development of the cloning techniques involved will inevitably hasten the day when some practitioner oblivious to public recoil will arrange for embryos to be implanted, and cloned humans to be born.

Appendix 3

Significant Studies on Alternative Sources of Stem Cells Published Since 2005

This referenced collection of studies can be found on the website of the President's Bioethics Council as of April 2, 2007. The Council did not participate in the development of any other section or appendix of this report. This appendix includes the studies from the following three sections of the Council's site: Advances in Non-Embryonic Stem Cell Research, Recent Studies Suggesting that Some Adult Cells Have Pluripotent Characteristics, and Update of New Research on Alternative Sources of Pluripotent Stem Cells Since May 2005. The studies cited below are a sampling of the published and peer-reviewed material available on the subject. The list will be updated on a regular basis.

http://www.bioethics.gov/stemcells/index.html

Advances in Non-Embryonic Stem Cell Research

2007

Fusion of human hematopoietic progenitor cells and murine cardiomyocytes is mediated by alpha4beta1 integrin/vascular cell adhesion molecule-1 interaction.

Zhang S et al. Circ Res. 2007 Mar 16;100(5):693-702. Epub 2007 Feb 15.

Abstract: Fusion of transplanted stem cells and host cells has been proposed as a major mechanism for the generation of hepatocytes, Purkinje neurons, and cardiomyocytes. However, the mechanism of cell fusion has not been precisely defined. Furthermore, the consequence of cell fusion remains unclear. We have previously shown that adult peripheral blood CD34-positive cells injected into severe combined immune deficiency (SCID) mice can transform into cardiomyocytes, endothelial cells, and smooth muscle cells following experimentally induced myocardial infarction and that most of the newly formed cardiomyocytes result from cell fusion. We therefore undertook this study to define the mechanism and consequences of cell fusion. Here we show that hypoxia and cytokines increase fusion of human peripheral blood CD34-positive cells and murine cardiomyocytes in vitro by up to 7-fold, and this is blocked by anti-alpha4beta1 or anti-vascular cell adhesion molecule (VCAM)-1. In vivo, fusion of progenitor cells and cardiomyocytes can also be blocked by anti-alpha4beta1 or anti-VCAM-1, but not by anti-vascular endothelial growth factor. On the other hand, generation of human-derived endothelial cells is blocked by anti-vascular endothelial growth factor but not by anti-alpha4beta1 antibodies. Two months following transplant, a high percentage of fused cells expressed cyclin B1 and incorporated bromodeoxyuridine. Thus, hematopoietic progenitor cell and cardiomyocyte fusion is mediated by alpha4beta1/VCAM-1 interaction, leading to cell cycle reentry and cellular proliferation.

Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells.

Dellavalle A et al. Nat Cell Biol. 2007 Mar;9(3):255-67. Epub 2007 Feb 11.

Abstract: Cells derived from blood vessels of human skeletal muscle can regenerate skeletal muscle, similarly to embryonic mesoangioblasts. However, adult cells do not express endothelial markers, but instead express markers of pericytes, such as NG2 proteoglycan and alkaline phosphatase (ALP), and can be prospectively isolated from freshly dissociated ALP(+) cells. Unlike canonical myogenic precursors (satellite cells), pericyte-derived cells express myogenic markers only in differentiated myotubes, which they form spontaneously with high efficiency. When transplanted into severe combined immune deficient-X-linked, mouse muscular dystrophy (scid-mdx) mice, pericyte-derived cells colonize host muscle and generate numerous fibres expressing human dystrophin. Similar cells isolated from Duchenne patients, and engineered to express human mini-dystrophin, also give rise to many dystrophin-positive fibres in vivo. These data show that myogenic precursors, distinct from satellite cells, are associated with microvascular walls in the human skeletal muscle, may represent a correlate of embryonic 'mesoangioblasts' present after birth and may be a promising candidate for future cell-therapy protocols in patients.

Differentiation of human adult skin-derived neuronal precursors into mature neurons.

Gingras M et al. J Cell Physiol. 2007 Feb;210(2):498-506.

Abstract: The isolation of autologous neuronal precursors from skin-derived precursor cells extracted from adult human skin would be a very efficient source of neurons for the treatment of various neurodegenerative diseases. The purpose of this study was to demonstrate that these neuronal precursors were able to differentiate into mature neurons. We isolated neuronal precursors from breast skin and expanded them in vitro for over ten passages. We showed that 48% of these cells were proliferating after the first passage, while this growth rate decreased after the second passage. We demonstrated that 70% of these cells were nestin-positive after the third passage, while only 17% were neurofilament M-positive after 7 days of differentiation. These neuronal precursors expressed betaIII tubulin, the dendritic marker MAP2 and the presynaptic marker synaptophysin after 7 days of in vitro maturation. They also expressed the postsynaptic marker PSD95 and the late neuronal markers NeuN and neurofilament H after 21 days of differentiation, demonstrating they became terminally differentiated

neurons. These markers were still expressed after 50 days of culture. The generation of autologous neurons from an accessible adult human source opens many potential therapeutic applications and has a great potential for the development of experimental studies on normal human neurons.

The quantitative trait gene latexin influences the size of the hematopoietic stem cell population in mice.

Liang Y et al. Nat Genet. 2007 Feb;39(2):178-88. Epub 2007 Jan 14.

Abstract: We mapped quantitative trait loci that accounted for the variation in hematopoietic stem cell (HSC) numbers between young adult C57BL/6 (B6) and DBA/2 (D2) mice. In reciprocal chromosome 3 congenic mice, introgressed D2 alleles increased HSC numbers owing to enhanced proliferation and self-renewal and reduced apoptosis, whereas B6 alleles had the opposite effects. Using oligonucleotide arrays, real-time PCR and protein blots, we identified latexin (Lxn), a gene whose differential transcription and expression was associated with the allelic differences. Expression was inversely correlated with the number of HSCs; therefore, ectopic expression of Lxn using a retroviral vector decreased stem cell population size. We identified clusters of SNPs upstream of the Lxn transcriptional start site, at least two of which are associated with potential binding sites for transcription factors regulating stem cells. Thus, promoter polymorphisms between the B6 and D2 alleles may affect Lxn gene expression and consequently influence the population size of hematopoietic stem cells.

Tethered EGF Provides a Survival Advantage to Mesenchymal Stem Cells

Fan VH et al. Stem Cells 2007 Jan 18; [Epub ahead of print]

Abstract: Mesenchymal stem cells (MSC) can act as a pluripotent source of reparative cells during injury and therefore have great potential in regenerative medicine and tissue engineering. However, the response of MSC to many growth factors and cytokines is unknown. Many envisioned applications of MSC, such as treating large defects in bone, involve in vivo implantation of MSC attached to a scaffold, a process that creates an acute inflammatory environment that may be hostile to MSC survival. Here, we investigate cellular responses of MSC on a biomaterial surface covalently modified with epidermal growth factor (EGF). We find that surface-tethered EGF promotes both cell spreading and survival more strongly than saturating concentrations of soluble EGF. By sustaining MEK-ERK signaling, tethered EGF increases the contact of MSC with an otherwise moderately adhesive synthetic polymer and confers resistance to cell death induced by the proinflammatory cytokine, FasL. We conclude that tethered EGF may offer a protective advantage to MSC in vivo during acute inflammatory reactions to tissue engineering scaffolds. The tethered EGF-modified polymers described here could be used together with structural materials to construct MSC scaffolds for the treatment of hard-tissue lesions, such as large bony defects.

Hematopoietic reconstitution by multipotent adult progenitor cells: precursors to long-term hematopoietic stem cells

Marta Serafini and Catherine M. Verfaillie et. al., The Journal of Experimental Medicine; doi:10.1084/jem.20061115; Epub 2007 Jan 16.

Abstract: For decades, in vitro expansion of transplantable hematopoietic stem cells (HSCs) has been an elusive goal. Here, we demonstrate that multipotent adult progenitor cells (MAPCs), isolated from green fluorescent protein (GFP)-transgenic mice and expanded in vitro for >40–80 population doublings, are capable of multilineage hematopoietic engraftment of immunodeficient mice. Among MAPC-derived GFP+CD45.2+ cells in the bone marrow of engrafted mice, HSCs were present that could radioprotect and reconstitute multilineage hematopoiesis in secondary and tertiary recipients, as well as myeloid and lymphoid hematopoietic progenitor subsets and functional GFP+ MAPC-derived lymphocytes that were functional. Although hematopoietic contribution by MAPCs was comparable to control KTLS HSCs, approximately 103-fold more MAPCs were required for effi cient engraftment. Because GFP+ host-derived CD45.1+ cells were not observed, fusion is not likely to account for the generation of HSCs by MAPCs.

Isolation of amniotic stem cell lines with potential for therapy, Published online: 7 January 2007; | doi:10.1038/nbt1274

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Abstract: Stem cells capable of differentiating to multiple lineages may be valuable for therapy. We report the isolation of human and rodent amniotic fluid–derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral

marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. Examples of differentiated cells derived from human AFS cells and displaying specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue-engineered bone.

Isolation and functional characterization of murine prostate stem cells

Devon A. Lawson et al., Proc Natl Acad Sci U S A. 2007 Jan 2;104(1):181-6. Epub 2006 Dec 21.

Abstract: The ability to isolate prostate stem cells is essential to explore their role in prostate development and disease. In vitro prostate colony- and sphere-forming assays were used to quantitatively measure murine prostate stem/progenitor cell enrichment and self-renewal. Cell surface markers were screened for their ability to positively or negatively enrich for cells with enhanced growth potential in these assays. Immunohistochemical and FACS analyses demonstrate that specific cell surface markers can be used to discriminate prostate stromal (CD34(+)), luminal epithelial (CD24(+)CD49f(-)), basal epithelial (CD24(+)CD49f(+)), hematopoietic (CD45(+), Ter119(+)), and endothelial (CD31(+)) lineages. Sorting for cells with a CD45(-)CD31(-)Ter119(-)Sca-1(+)CD49f(+) antigenic profile results in a 60-fold enrichment for colony- and sphere-forming cells. These cells can self-renew and expand to form spheres for many generations and can differentiate to produce prostatic tubule structures containing both basal and luminal cells in vivo. These cells also localize to the basal cell layer within the region of the gland that is proximal to the urethra, which has been identified as the prostate stem cell niche. Prostate stem cells can be isolated to a purity of up to 1 in 35 by using this antigenic profile. The remarkable similarity in cell surface profile between prostate and mammary gland stem cells suggests these markers may be conserved among epithelial stem cell populations.

2006

Ex Vivo Large-Scale Generation of Human Platelets from Cord Blood CD34+ Cells

Takuya Matsunaga et al., Stem Cells. 2006 2006 Dec;24(12):2877-87. Epub 2006 Sep 7.

Abstract: In the present investigation, we generated platelets (PLTs) from cord blood (CB) CD34(+) cells using a three-phase culture system. We first cultured 500 CB CD34(+) cells on telomerase gene-transduced human stromal cells (hTERT stroma) in serum-free medium supplemented with stem cell factor (SCF), Flt-3/Flk-2 ligand (FL), and thrombopoietin (TPO) for 14 days. We then transferred the cells to hTERT stroma and cultured for another 14 days with fresh medium containing interleukin-11 (IL-11) in addition to the original cytokine cocktail. Subsequently, we cultured the cells in a liquid culture medium containing SCF, FL, TPO, and IL-11 for another 5 days to recover PLT fractions from the supernatant, which were then gel-filtered to purify the PLTs. The calculated yield of PLTs from 1.0 unit of CB (5 x 10(6) CD34(+) cells) was 1.26 x 10(11)-1.68 x 10(11) PLTs. These numbers of PLTs are equivalent to 2.5-3.4 units of random donor-derived PLTs or 2/5-6/10 of single-apheresis PLTs. The CB-derived PLTs exhibited features quite similar to those from peripheral blood in morphology, as revealed by electron micrographs, and in function, as revealed by fibrinogen/ADP aggregation, with the appearance of P-selectin and activated glycoprotein IIb-IIIa antigens. Thus, this culture system may be applicable for large-scale generation of PLTs for future clinical use.

Functional Neuronal Differentiation of Bone Marrow-Derived Mesenchymal Stem Cells

Philippe Tropel et al., Stem Cells. 2006 Dec;24(12):2868-76. Epub 2006 Aug 10.

Abstract: Recent results have shown the ability of bone marrow cells to migrate in the brain and to acquire neuronal or glial characteristics. In vitro, bone marrow-derived MSCs can be induced by chemical compounds to express markers of these lineages. In an effort to set up a mouse model of such differentiation, we addressed the neuronal potentiality of mouse MSCs (mMSCs) that we recently purified. These cells expressed nestin, a specific marker of neural progenitors. Under differentiating conditions, mMSCs display a distinct neuronal shape and express neuronal markers NF-L (neurofilament-light, or neurofilament 70 kDa) and class III beta-tubulin. Moreover, differentiated mMSCs acquire neuron-like functions characterized by a cytosolic calcium rise in response to various specific neuronal activators. Finally, we further demonstrated for the first time that clonal mMSCs and their progeny are competent to differentiate along the neuronal pathway, demonstrating that these bone marrow-derived stem cells share characteristics of widely multipotent stem cells unrestricted to mesenchymal differentiation pathways.

Bone Marrow Transplantation Attenuates the Myopathic Phenotype of a Muscular Mouse Model of Spinal Muscular Atrophy

Nouzha Salah-Mohellibi et al., Stem Cells. 2006 Dec;24(12):2723-32. Epub 2006 Aug 3.

Abstract: Bone marrow (BM) transplantation was performed on a muscular mouse model of spinal muscular atrophy that had been created by mutating the survival of motor neuron gene (Smn) in myofibers only. This model is characterized by a severe myopathy and progressive loss of muscle fibers leading to paralysis. Transplantation of wild-type BM cells following irradiation at a low dose (6 Gy) improved motor capacity (+85%). This correlated with a normalization of myofiber number associated with a

higher number of regenerating myofibers (1.6-fold increase) and an activation of CD34 and Pax7 satellite cells. However, BM cells had a very limited capacity to replace or fuse to mutant myofibers (2%). These data suggest that BM transplantation was able to attenuate the myopathic phenotype through an improvement of skeletal muscle regeneration of recipient mutant mice, a process likely mediated by a biological activity of BM-derived cells. This hypothesis was further supported by the capacity of muscle protein extracts from transplanted mutant mice to promote myoblast proliferation in vitro (1.6-fold increase). In addition, a tremendous upregulation of hepatocyte growth factor (HGF), which activates quiescent satellite cells, was found in skeletal muscle of transplanted mutants compared with nontransplanted mutants. Eventually, thanks to the Cre-loxP system, we show that BM-derived muscle cells were strong candidates harboring this biological activity. Taken together, our data suggest that a biological activity is likely involved in muscle regeneration improvement mediated by BM transplantation. HGF may represent an attractive paracrine mechanism to support this activity.

Increased generation of neuronal progenitors after ischemic injury in the aged adult human forebrain.

Macas J, et al., J Neurosci. 2006 Dec 13;26(50):13114-9.

Abstract: The adult human brain retains the capacity to generate new neurons in the hippocampal formation (Eriksson et al., 1998) and neuronal progenitor cells (NPCs) in the forebrain (Bernier et al., 2000), but to what extent it is capable of reacting to injuries, such as ischemia, is not known. We analyzed postmortem tissue from normal and pathological human brain tissue (n = 54) to study the cellular response to ischemic injury in the forebrain. We observed that cells expressing the NPC marker polysialylated neural adhesion cell molecule (PSA-NCAM) are continuously generated in the adult human subventricular zone (SVZ) and migrate along the olfactory tracts. These cells were not organized in migrating chains as in the adult rodent rostral migratory stream, and their number was lower in the olfactory tracts of brains from old (56-81 years of age) compared with young (29 + 36 years of age) individuals. Moreover, we show that in brains of patients of advanced age (60-87 years of age), ischemia led to an elevated number of Ki-67-positive cells in the ipsilateral SVZ without concomitant apoptotic cell death. Additionally, ischemia led to an increased number of PSA-NCAM-positive NPCs close to the lateral ventricular walls, compared with brains of comparable age without obvious neuropathologic changes. These results suggest that the adult human brain retains a capacity to respond to ischemic injuries and that this capacity is maintained even in old age.

A neurovascular niche for neurogenesis after stroke.

Ohab JJ, et al., J Neurosci. 2006 Dec 13;26(50):13007-16.

Abstract: Stroke causes cell death but also birth and migration of new neurons within sites of ischemic damage. The cellular environment that induces neuronal regeneration and migration after stroke has not been defined. We have used a model of long-distance migration of newly born neurons from the subventricular zone to cortex after stroke to define the cellular cues that induce neuronal regeneration after CNS injury. Mitotic, genetic, and viral labeling and chemokine/growth factor gain- and loss-of-function studies show that stroke induces neurogenesis from a GFAP-expressing progenitor cell in the subventricular zone and migration of newly born neurons into a unique neurovascular niche in peri-infarct cortex. Within this neurovascular niche, newly born, immature neurons closely associate with the remodeling vasculature. Neurogenesis and angiogenesis are causally linked through vascular production of stromal-derived factor 1 (SDF1) and angiopoietin 1 (Ang1). Furthermore, SDF1 and Ang1 promote post-stroke neuroblast migration and behavioral recovery. These experiments define a novel brain environment for neuronal regeneration after stroke and identify molecular mechanisms that are shared between angiogenesis and neurogenesis during functional recovery from brain injury.

Spontaneous Fusion and Non-clonal Growth of Adult Neural Stem Cells

Sebastian Jessberger et al., Stem Cells, published online December 21, 2006; doi:10.1634/stemcells.2006-0620.

Abstract: Multipotent neural stem cells (NSCs) can be isolated from various regions of the adult brain and propagated in vitro. Recent reports have suggested spontaneous fusion events among NSCs when grown as free-floating neurospheres that may affect the genetic composition of NSC cultures. We used adult NSCs expressing either red fluorescent protein (RFP) or green fluorescent protein (GFP) to analyze the fusion frequency of rat and mouse NSCs. Fluorescence activated cell sorting (FACS) revealed, that under proliferating conditions approximately 0.2% of rat and mouse NSCs coexpressed RFP and GFP, irrespective of whether the cells were grown as neurospheres (mouse NSCs) or as attached monolayers (rat and mouse NSCs). Fused cells did not proliferate and could not be propagated, suggesting that aberrantly fused cells are not viable. Furthermore, we found that neither neurospheres nor monolayers grew clonally, as even very low-density cultures had spheres containing both GFP- and RFP-expressing cells and monolayer patches with GFP- and RFP-expressing cells in close proximity. The non-clonal growth between distinct NSC populations strongly suggests the use of careful and precise culture conditions, such as single cell assays, to characterize potency and growth of NSCs in vitro.

Designer Self-Assembling Peptide Nanofiber Scaffolds for Adult Mouse Neural Stem Cell 3-Dimensional Cultures

Fabrizio Gelain et al., PLoS ONE 1. e119., i:10.1371/journal.pone.0000119, December 2006.

Abstract: Biomedical researchers have become increasingly aware of the limitations of conventional 2-dimensional tissue cell culture systems, including coated Petri dishes, multi-well plates and slides, to fully address many critical issues in cell biology, cancer biology and neurobiology, such as the 3-D microenvironment, 3-D gradient diffusion, 3-D cell migration and 3-D cell-cell contact interactions. In order to fully understand how cells behave in the 3-D body, it is important to develop a well-controlled 3-D cell culture system where every single ingredient is known. Here we report the development of a 3-D cell culture system using a designer peptide nanofiber scaffold with mouse adult neural stem cells. We attached several functional motifs, including cell adhesion, differentiation and bone marrow homing motifs, to a self-assembling peptide RADA16 (Ac-RADARADARADARADA-COHN2). These functionalized peptides undergo self-assembly into a nanofiber structure similar to Matrigel. During cell culture, the cells were fully embedded in the 3-D environment of the scaffold. Two of the peptide scaffolds containing bone marrow homing motifs significantly enhanced the neural cell survival without extra soluble growth and neurotrophic factors to the routine cell culture media. In these designer scaffolds, the cell populations with b-Tubulin+, GFAP+ and Nestin+ markers are similar to those found in cell populations cultured on Matrigel. The gene expression profiling array experiments showed selective gene expression, possibly involved in neural stem cell adhesion and differentiation. Because the synthetic peptides are intrinsically pure and a number of desired function cellular motifs are easy to incorporate, these designer peptide nanofiber scaffolds provide a promising controlled 3-D culture system for diverse tissue cells, and are useful as well for general molecular and cell biology.

Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine

Wataru Sonoyama et al., PLoS ONE 2006 Dec 20;1:e79.

Abstract: Mesenchymal stem cell-mediated tissue regeneration is a promising approach for regenerative medicine for a wide range of applications. Here we report a new population of stem cells isolated from the root apical papilla of human teeth (SCAP, stem cells from apical papilla). Using a minipig model, we transplanted both human SCAP and periodontal ligament stem cells (PDLSCs) to generate a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function. This work integrates a stem cell-mediated tissue regeneration strategy, engineered materials for structure, and current dental crown technologies. This hybridized tissue engineering approach led to recovery of tooth strength and appearance.

Transplantation of Mesenchymal Stem Cells is an Optimal Approach for Plastic Surgery

Dianji Fang et al., Stem Cells, published online December 14, 2006; doi:10.1634/stemcells.2006-0576.

Abstract: Mesenchymal stem cells (MSCs) are able to differentiate into a variety of cell types, offering promising approaches for stem-cell-mediated tissue regeneration. Here we explored the potential of utilizing MSCs to reconstruct orofacial tissue, thereby, altering the orofacial appearance. We demonstrated that bone marrow MSCs were capable of generating bone structures and bone-associated marrow elements on the surfaces of the orofacial bone. This resulted in significant re-contouring of the facial appearance in mouse and swine. Notably, the newly formed bone/marrow tissues integrated with the surfaces of the recipient bones and re-established a functional bone marrow organ-like system. These data suggested that MSC-mediated tissue regeneration led to a body structure extension, with the re-establishment of all functional components necessary for maintaining the bone/marrow organ. In addition, we found that the subcutaneous transplantation of another population of MSCs, the human periodontal ligament stem cells (PDLSCs) could form substantial amounts of collagen fibers and improve facial wrinkles in mouse. By contrast, bone marrow MSCs failed to survive at 8 weeks post-transplantation under the conditions used for the PDLSC transplantation. This study suggested that the mutual interactions between donor MSCs and recipient microenvironment determine long-term outcome of the functional tissue regeneration.

Granulocyte-Colony–Stimulating Factor Mobilizes Bone Marrow Stem Cells in Patients With Subacute Ischemic Stroke The Stem Cell Trial of Recovery EnhanceMent After Stroke (STEMS) Pilot Randomized, Controlled Trial (ISRCTN 16784092)

Nikola Sprigg et al., Stroke. 2006 Dec; 37(12):2979-83. Epub 2006 Nov 2.

Background and Purpose: Loss of motor function is common after stroke and leads to significant chronic disability. Stem cells are capable of self-renewal and of differentiating into multiple cell types, including neurones, glia, and vascular cells. We assessed the safety of granulocyte-colony-stimulating factor (G-CSF) after stroke and its effect on circulating CD34+ stem cells. **METHODS:** We performed a 2-center, dose-escalation, double-blind, randomized, placebo-controlled pilot trial (ISRCTN 16784092) of G-CSF (6 blocks of 1 to 10 microg/kg SC, 1 or 5 daily doses) in 36 patients with recent ischemic stroke. Circulating CD34+ stem cells were measured by flow cytometry; blood counts and measures of safety and functional outcome were also monitored. All measures were made blinded to treatment.
RESULTS: Thirty-six patients, whose mean+/-SD age was 76+/-8 years and of whom 50% were male, were recruited. G-CSF (5 days of 10 microg/kg) increased CD34+ count in a dose-dependent manner, from 2.5 to 37.7 at day 5 (area under curve, P=0.005). A dose-dependent rise in white cell count (P<0.001) was also seen. There was no difference between treatment groups in the number of patients with serious adverse events: G-CSF, 7/24 (29%) versus placebo 3/12 (25%), or in their dependence (modified Rankin Scale, median 4, interquartile range, 3 to 5) at 90 days.

CONCLUSIONS: G-CSF is effective at mobilizing bone marrow CD34+ stem cells in patients with recent ischemic stroke. Administration is feasible and appears to be safe and well tolerated. The fate of mobilized cells and their effect on functional outcome remain to be determined.

Successful treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation in patients over age 65

David C. Seldin et al., Blood. 2006 Dec 1;108(12):3945-7. Epub 2006 Aug 22.

Abstract: Recently, protocols using high-dose melphalan chemotherapy and autologous peripheral blood stem cell transplantation (HDM/SCT) have been developed for the treatment of patients with immunoglobulin light chain (AL) amyloidosis. Although peritransplantation mortality is greater than for other hematologic diseases, treatment leads to durable hematologic complete responses, improvements in organ function and quality of life, and extended survival in a substantial proportion of patients. To determine whether this treatment can be applied to older patients, we have analyzed HDM/SCT treatment outcomes for 65 patients (aged 65 years or older) with AL amyloidosis compared with outcomes for 280 younger patients. For patients over age 65 years who meet the same eligibility criteria as younger patients, toxicity, hematologic remission rate, and survival were not significantly different from those observed in younger patients, indicating that older patients should not be excluded a priori from consideration for HDM/SCT treatment.

Isolation of an adult blood-derived progenitor cell population capable of differentiation into angiogenic, myocardial and neural lineages

Yael Porat et al., British Journal of Haematology. 2006 Dec;135(5):703-14.

Abstract: Blood-derived adult stem cells were previously considered impractical for therapeutic use because of their small numbers. This report describes the isolation of a novel human cell population derived from the peripheral blood, termed synergetic cell population (SCP), and defined by the expression of CD31Bright, CD34+, CD45)/Dim and CD34Bright, but not lineage-specific features. The SCP was capable of differentiating into a variety of cell lineages upon exposure to defined culture conditions. The resulting cells exhibited morphological, immunocytochemical and functional characteristics of angiogenic, neural or myocardial lineages. Angiogenic cell precursors (ACPs) expressed CD34, CD133, KDR, Tie-2, CD144, von Willebrand factor, CD31Bright, concomitant binding of Ulex-Lectin and uptake of acetylated low density lipoprotein (Ac-LDL), secreted interleukin-8, vascular endothelial growth factor and angiogenin and formed tube-like structures in vitro. The majority of CD31Bright ACP cells demonstrated Ac-LDL uptake. Neural cell precursors (NCPs) expressed the neuronal markers Nestin, bIII-Tubulin, and Neu-N, the glial markers GFAP and O4, and responded to neurotransmitter stimulation. Myocardial cell precursors (MCPs) expressed Desmin, cardiac Troponin and Connexin 43. In conclusion, the simple and rapid method of SCP generation and the resulting considerable quantities of lineage-specific precursor cells makes it a potential source of autologous treatment for a variety of diseases.

Myeloid progenitors differentiate intomicroglia and promote vascular repair in a model of ischemic retinopathy

Matthew R. Ritter et al., J. Clin. Invest. 2006 Dec;116(12):3266-76. Epub 2006 Nov 16.

Abstract: Vision loss associated with ischemic diseases such as retinopathy of prematurity and diabetic retinopathy are often due to retinal neovascularization. While significant progress has been made in the development of compounds useful for the treatment of abnormal vascular permeability and proliferation, such therapies do not address the underlying hypoxia that stimulates the observed vascular growth. Using a model of oxygen-induced retinopathy, we demonstrate that a population of adult BM-derived myeloid progenitor cells migrated to avascular regions of the retina, differentiated into microglia, and facilitated normalization of the vasculature. Myeloid-specific hypoxia-inducible factor 1alpha (HIF-1alpha) expression was required for this function, and we also demonstrate that endogenous microglia participated in retinal vascularization. These findings suggest what we believe to be a novel therapeutic approach for the treatment of ischemic retinopathies that promotes vascular repair rather than destruction.

Cytokine-induced differentiation of multipotent adult progenitor cells into functional smooth muscle cells

J. Ross et al. J. Clin. Invest. 2006 Dec;116(12):3139-49. Epub 2006 Nov 9.

Abstract: Smooth muscle formation and function are critical in development and postnatal life. Hence, studies aimed at better understanding SMC differentiation are of great importance. Here, we report that multipotent adult progenitor cells (MAPCs) isolated from rat, murine, porcine, and human bone marrow demonstrate the potential to differentiate into cells with an SMC-like phenotype and function. TGF-beta1 alone or combined with PDGF-BB in serum-free medium induces a temporally correct expression of transcripts and proteins consistent with smooth muscle development. Furthermore, SMCs derived from MAPCs (MAPC-SMCs) demonstrated functional L-type calcium channels. MAPC-SMCs entrapped in fibrin vascular molds became circumferentially aligned and generated force in response to KCl, the L-type channel opener FPL64176, or the SMC agonists 5-HT and ET-1, and exhibited complete relaxation in response to the Rho-kinase inhibitor Y-27632. Cyclic distention (5% circumferential strain) for 3 weeks increased responses by 2- to 3-fold, consistent with what occurred in neonatal SMCs. These results provide evidence that MAPC-SMCs are phenotypically and functionally similar to neonatal SMCs and that the in vitro MAPC-SMC differentiation

system may be an ideal model for the study of SMC development. Moreover, MAPC-SMCs may lend themselves to tissue engineering applications.

Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice.

Munoz JR, et al. Proc Natl Acad Sci U S A. 2005 Dec 13;102(50):18171-6. Epub 2005 Dec 5.

Abstract: Stem/progenitor cells from bone marrow and other sources have been shown to repair injured tissues by differentiating into tissue-specific phenotypes, by secreting chemokines, and, in part, by cell fusion. Here we prepared the stem/progenitor cells from human bone marrow (MSCs) and implanted athem into the dentate gyrus of the hippocampus of immunodeficient mice. The implanted human MSCs markedly increased the proliferation of endogenous neural stem cells that expressed the stem cell marker Sox2. Labeling of the mice with BrdUrd demonstrated that, 7 days after implantation of the human MSCs, BrdUrd-labeled endogenous cells migrated throughout the dorsal hippocampus (positive for doublecortin) and expressed markers for astrocytes and for neural or oligodendrocyte progenitors. Subpopulations of BrdUrd-labeled cells exhibited short cytoplasmic processes immunoreactive for nerve growth factor and VEGF. By 30 days after implantation, the newly generated cells expressed markers for more mature neurons and astrocytes. Also, subpopulations of BrdUrd-labeled cells exhibited elaborate processes immunoreactive for ciliary neurotrophic factor, neurotrophin-4/5, nerve growth factor, or VEGF. Therefore, implantation of human MSCs stimulated proliferation, migration, and differentiation of the endogenous neural stem cells that survived as differentiated neural cells. The results provide a paradigm to explain recent observations in which MSCs or related stem/progenitor cells were found to produce improvements in disease models even though a limited number of the cells engrafted.

Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease.

Ang LP, et al. Arch Ophthalmol. 2006 Nov;124(11):1543-51.

OBJECTIVE: To evaluate the use of autologous serum (AS)-derived cultivated oral epithelial transplants for the treatment of severe ocular surface disease.
METHODS: We used AS from 10 patients with severe ocular surface disease and total limbal stem cell deficiency to develop autologous cultivated oral epithelial equivalents. These were compared with epithelial equivalents derived from conventional fetal bovine serum-supplemented medium. Surgery involved removal of the corneal pannus and surrounding diseased tissue and transplantation of the AS-derived epithelial equivalents. The oral equivalents were analyzed by review of histologic and immunohistochemical findings.
RESULTS: Oral epithelial sheets cultivated in AS- and fetal bovine serum-supplemented media were similar in morphology, and both formed basement membrane assembly proteins important for maintaining graft integrity. Complete corneal epithelialization was achieved within 2 to 5 days postoperatively. The ocular surface remained stable without major complications in all eyes during a mean +/- SD follow-up of 12.6 +/- 3.9 months. The visual acuity improved by more than 2 lines in 9 of 10 eyes, with transplanted oral epithelium surviving up to 19 months.
CONCLUSION: The successful use of an AS-derived oral epithelial equivalent to treat severe ocular surface disease represents an important advance in the pursuit of completely autologous xenobiotic-free bioengineered ocular equivalents for clinical transplantation.

Tracking Neural Stem Cells in Patients with Brain Trauma

Jianhong Zhu, et al. N Engl J Med. 2006 Nov 30;355(22):2376-8.

To the Editor: Regeneration of damaged brain tissue with neural stem cells is a promising strategy for reversing neurologic deficits.1 Superparamagnetic iron oxide nanoparticles have been used to label and track dendritic cells in the experimental treatment of melanoma2 and in experiments in animals. We report the feasibility of labeling neural stem cells from humans (two patients for whom written informed consent was provided by next of kin) with superparamagnetic iron oxide nanoparticles and tracking them with the use of magnetic resonance imaging (MRI).

Antibody Targeting of Stem Cells to Infarcted Myocardium

Randall J. Lee et al. Stem Cells, published online November 30, 2006; doi:10.1634/stemcells.2005-0602.

Abstract: Hematopoietic stem cell therapy for myocardial repair is limited by the number of stem cells that migrate to, engraft in and proliferate at sites of injured myocardium. To alleviate this limitation, we studied whether a strategy using a bispecific antibody could target human stem cells specifically to injuredmyocardium and preserve myocardial function. Using a xenogeneic rat model whereby ischemic injury was induced by transient ligation of the left anterior descending artery (LAD), we determined the ability of a bispecific antibody to target human CD34+ cells to specific antigens expressed in ischemic injured myocardium. A bispecific antibody comprised of an anti-CD45 antibody recognizing the common leukocyte antigen found on hematopoietic stem cells (HSC) and an antibody recognizing myosin light chain, an organ-specific injury antigen expressed by infarcted myocardium was prepared by chemical conjugation. CD34+ cells armed and unarmed with this BiAb were injected intravenously in rats 2 days post-myocardial injury. Immunohistochemistry studies showed that the armed CD34+ cells specifically localized to the infracted region of the heart, co-localized with troponin T stained cells and co-localized with vascular structures. Compared to unarmed CD34+ cells, the bispecific

antibody improved delivery of the stem cells to injured myocardium and such targeted delivery was correlated with improved myocardial function five weeksfollowing infarction (p<0.01). Bispecific antibody targeting offers a unique means to improve the delivery of stem cells to facilitate organ repair and a tool to study stem cell biology.

Stem Cells, Myocardial Regeneration and Methodological Artifacts

Piero Anversa et al. Stem Cells, published online November 30, 2006; doi:10.1634/stemcells.2006-0623.

Abstract: This review discusses the controversy that has permeated the field of myocardial regeneration in the last three decades. The notion of the heart as a terminally differentiated postmitotic organ has been so strong that observations promoting the opposite paradigm have been questioned technically and conceptually. The possibility of misinterpretation of results collected with cellular, molecular, and morphological methodologies has been the prevailing position in the scientific community. Myocardial regeneration mediated by activation of endogenous progenitor cells or by engraftment and differentiation of primitive cells from the bone marrow has been rejected strongly in an attempt to defend an unrealistic view of the heart. This article provides evidence in support of the notion that the heart is an organ regulated by a stem cell compartment responsible for cardiac homeostasis and repair.

Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial.

Schachinger V, et al. Eur Heart J. 2006 Dec;27(23):2775-83. Epub 2006 Nov 10.

AIMS: To investigate the clinical outcome after intracoronary administration of autologous progenitor cells in patients with acute myocardial infarction (AMI).
METHODS AND RESULTS: Using a double-blind, placebo-controlled multicentre trial design, we randomized 204 patients with successfully reperfused AMI to receive intracoronary infusion of bone-marrow-derived progenitor cells (BMCs) or placebo medium into the infarct artery 3-7 days after successful infarct reperfusion therapy. At 12 months, the pre-specified cumulative endpoint of death, myocardial infarction, or necessity for revascularization was significantly reduced in the BMC group compared with placebo (P=0.009). Likewise, the combined endpoint death, recurrence of myocardial infarction, and rehospitalization for heart failure was significantly (P=0.006) reduced in patients receiving intracoronary BMC administration. Intracoronary administration of BMC remained a significant predictor of a favourable clinical outcome by Cox regression analysis, adjusting for classical predictors of poor outcome after AMI.
CONCLUSION: Intracoronary administration of BMCs is associated with a significant reduction of the occurrence of major adverse cardiovascular events after AMI. Large-scale studies are warranted to confirm the effects of BMC administration on mortality and morbidity in patients with AMIs.

In Vitro and in Vivo Arterial Differentiation of Human Multipotent Adult Progenitor Cells

Xabier L. Aranguren et al. Blood First Edition Paper. prepublished online November 7, 2006; DOI 10.1182/blood-2006-06-030411.

Abstract: Many stem cell types have been shown to differentiate into endothelial cells (ECs), however, their specification to arterial or venous endothelium remains unexplored. We tested whether a specific arterial or venous EC fate could be induced in human Multipotent Adult Progenitor Cells (hMAPCs) and AC133+ cells (hAC133+). In vitro, in the presence of VEGF165, hAC133+ cells only adopted a venous and microvascular EC phenotype, while hMAPCs differentiated into both arterial and venous ECs, possibly because hMAPCs expressed significantly more sonic hedgehog (Shh) and its receptors, as well as Notch 1 and 3 receptors and some of their ligands. Accordingly, blocking either of those pathways attenuated in vitro arterial EC differentiation from hMAPCs. Complementarily, stimulating these pathways by addition of Delta-like 4 (Dll-4), a Notch ligand, and Shh to VEGF165 further boosted arterial differentiation in hMAPCs both in vitro and in an in vivo matrigel model. These results represent the first demonstration of adult stem cells with the potential to be differentiated into different types of ECs in vitro and in vivo and provide a useful human model to study arterio-venous specification.

Therapeutic strategies for Parkinson.s disease based on the modulation of adult neurogenesis

Martine Geraerts et al. Stem Cells, published online November 2, 2006; doi:10.1634/stemcells.2006-0364.

Abstract: Parkinson.s disease (PD) is a progressive neurodegenerative disorder, affecting millions of people world-wide. To date, treatment strategies are mainly symptomatic and aimed at increasing dopamine levels in the degenerating nigrostriatal system. Hope rests upon the development of effective neurorestorative or neuroregenerative therapies based on gene and stem cell therapy or a combination of both. The results of experimental therapies based on transplanting exogenous dopamine-rich fetal cells or growth factor (GDNF) overexpression into the brain of Parkinson.s disease patients encourage future cell- and gene-based strategies. The endogenous neural stem cells of the adult brain provide an alternative and attractive cell source for neuroregeneration. Prior to designing endogenous stem cell therapies, one has to investigate the possible impact of PD on adult neuronal stem cell pools and their neurogenic potential. We review the experimental data obtained in animal models or based on analysis of patients. brain prior to describing different treatment strategies. Strategies aiming to enhance neuronal stem cell proliferation and/or differentiation in the

striatum or the substantia nigra will have to be compared in animal models and selected prior to clinical studies.

Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs.

Sampaolesi M et al. Nature. 2006 Nov 30;444(7119):574-9. Epub 2006 Nov 15.

Abstract: Duchenne muscular dystrophy remains an untreatable genetic disease that severely limits motility and life expectancy in affected children. The only animal model specifically reproducing the alterations in the dystrophin gene and the full spectrum of human pathology is the golden retriever dog model. Affected animals present a single mutation in intron 6, resulting in complete absence of the dystrophin protein, and early and severe muscle degeneration with nearly complete loss of motility and walking ability. Death usually occurs at about 1 year of age as a result of failure of respiratory muscles. Here we report that intra-arterial delivery of wild-type canine mesoangioblasts (vessel-associated stem cells) results in an extensive recovery of dystrophin expression, normal muscle morphology and function (confirmed by measurement of contraction force on single fibres). The outcome is a remarkable clinical amelioration and preservation of active motility. These data qualify mesoangioblasts as candidates for future stem cell therapy for Duchenne patients.

Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease.

Yasuhara T et al. J Neurosci. 2006 Nov 29;26(48):12497-511.

Abstract: Neural stem cells (NSCs) possess high potencies of self-renewal and neuronal differentiation. We explored here whether transplantation of human NSCs cloned by v-myc gene transfer, HB1.F3 cells, is a feasible therapeutic option for Parkinson's disease. In vivo, green fluorescent protein-labeled HB1.F3 cells (200,000 viable cells in 3 microl of PBS) when stereotaxically transplanted (same-day lesion-transplant paradigm) into the 6-hydroxydopamine-lesioned striatum of rats significantly ameliorated parkinsonian behavioral symptoms compared with controls (vehicle, single bolus, or continuous minipump infusion of trophic factor, or killed cell grafts). Such graft-derived functional effects were accompanied by preservation of tyrosine hydroxylase (TH) immunoreactivity along the nigrostriatal pathway. Grafted HB1.F3 cells survived in the lesioned brain with some labeled with neuronal marker mitogen-activated protein 2 and decorated with synaptophysin-positive terminals. Furthermore, endogenous neurogenesis was activated in the subventricular zone of transplanted rats. To further explore the neuroprotective mechanisms underlying HB1.F3 cell transplantation, we performed cell culture studies and found that a modest number of HB1.F3 cells were TH and dopamine and cAMP-regulated phosphoprotein 32 positive, although most cells were nestin positive, suggesting a mixed population of mature and immature cells. Administration of the HB1.F3 supernatant to human derived dopaminergic SH-SY5Y cells and fetal rat ventral mesencephalic dopaminergic neurons protected against 6-hydroxydopamine neurotoxicity by suppressing apoptosis through Bcl-2 upregulation, which was blocked by anti-stem cell factor antibody alone, the phosphatidylinositol 3-kinase/Akt inhibitor LY294002 [2-(4-morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one] alone, or a combination of both. These results suggest that HB1.F3 cell transplantation exerts neuroprotective effects against dopaminergic depletion in vitro and in vivo because of trophic factor secretion and neuronal differentiation.

Adipose Tissue-Derived Mesenchymal Stem Cells Have In Vivo Immunosuppressive Properties Applicable for the Control of the Graft-Versus-Host Disease

Rosa Yanez et al. Stem Cells. 2006 Nov;24(11):2582-91. Epub 2006 Jul 27.

Abstract: Previous studies have shown the relevance of bone marrow- derived MSCs (BM-MSCs) in controlling graft-versus- host disease (GVHD) after allogeneic transplantation. Since adipose tissue-derived MSCs (Ad-MSCs) may constitute a good alternative to BM-MSCs, we have expanded MSCs derived from human adipose tissue (hAd-MSCs) and mouse adipose tissue (mAd-MSCs), investigated the immunoregulatory properties of these cells, and evaluated their capacity to control GVHD in mice. The phenotype and immunoregulatory properties of expanded hAd-MSCs were similar to those of human BM-MSCs. Moreover, hAd-MSCs inhibited the proliferation and cytokine secretion of human primary T cells in response to mitogens and allogeneic T cells. Similarly, ex vivo expanded mAd-MSCs had an equivalent immunophenotype and exerted immunoregulatory properties similar to those of hAd-MSCs. Moreover, the infusion of mAd-MSCs in mice transplanted with haploidentical hematopoietic grafts controlled the lethal GVHD that occurred in control recipient mice. These findings constitute the first experimental proof that Ad-MSCs can efficiently control the GVHD associated with allogeneic hematopoietic transplantation, opening new perspectives for the clinical use of Ad-MSCs.

Side Population Cells Isolated from Porcine Dental Pulp Tissue with Self-Renewal and Multipotency for Dentinogenesis, Chondrogenesis, Adipogenesis, and Neurogenesis

Koichiro Iohara et al. Stem Cells. 2006 Nov;24(11):2493-503. Epub 2006 Jul 27.

Abstract: Dental pulp has the potential to form dentin as a regenerative response to caries. This regeneration is mediated by stem/progenitor cells. Thus, stem cell therapy might be of potential utility in induction of reparative dentin. We isolated side population (SP) cells from dental pulp based on the exclusion of the DNA binding dye Hoechst 33342 by flow cytometry and compared its self-renewal capacities and multipotency with non-SP cells and primary pulp cells. The cumulative cell number of the SP cells was greater than the non-SP cells and primary pulp cells. Bmi1 was continuously expressed

in SP cells, suggesting longer proliferative lifespan and self-renewal capacity of SP cells. Next, the maintenance of the multilineage differentiation potential of pulp SP cells was investigated. Expression of type II collagen and aggrecan confirmed chondrogenic conversion (30%) of SP cells. SP cells expressed peroxisome proliferator-activated receptor _ and adaptor protein 2, showing adipogenic conversion. Expression of mRNA and proteins of neurofilament and neuromodulin confirmed neurogenic conversion (90%). These results demonstrate that pulp SP cells maintain multilineage differentiation potential. We further examined whether bone morphogenetic protein 2 (BMP2) could induce differentiation of pulp SP cells into odontoblasts. BMP2 stimulated the expression of dentin sialophosphoprotein (Dspp) and enamelysin in three-dimensional pellet cultures. Autogenous transplantation of the Bmp2-supplemented SP cells on the amputated pulp stimulated the reparative dentin formation. Thus, adult pulp contains SP cells, which are enriched for stem cell properties and useful for cell therapy with BMP2 for dentin regeneration.

Bone Marrow-Derived Cells Contribute to Podocyte Regeneration and Amelioration of Renal Disease in a Mouse Model of Alport Syndrome

Evangelia I. Prodromidi et al. Stem Cells. 2006 Nov;24(11):2448-55. Epub 2006 Jul 27.

Abstract: In a model of autosomally recessive Alport syndrome, mice that lack the alpha3 chain of collagen IV (Col4alpha3(-/-)) develop progressive glomerular damage leading to renal failure. The proposed mechanism is that podocytes fail to synthesize normal glomerular basement membrane, so the collagen IV network is unstable and easily degraded. We used this model to study whether bone marrow (BM) transplantation can rectify this podocyte defect by correcting the deficiency in Col4alpha3. Female C57BL/6 Col4alpha3(-/-) (-/-) mice were transplanted with whole BM from male wild-type (+/+) mice. Control female -/- mice received BM from male -/- littermates. Serum urea and creatinine levels were significantly lower in recipients of +/+ BM compared with those of -/- BM 20 weeks post-transplant. Glomerular scarring and interstitial fibrosis were also significantly decreased. Donor-derived cells were detected by in situ hybridization (ISH) for the Y chromosome, and fluorescence and confocal microscopy indicated that some showed an apparent podocyte phenotype in mice transplanted with +/+ BM. Glomeruli of these mice showed small foci of staining for alpha3(IV) protein by immunofluorescence. alpha3(IV) mRNA was detectable by reverse transcription-polymerase chain reaction and ISH in some mice transplanted with +/+ BM but not -/- BM. However, a single injection of mesenchymal stem cells from +/+ mice to irradiated -/- recipients did not improve renal disease. Our data show that improved renal function in Col4alpha3(-/-) mice results from BM transplantation from wild-type donors, and the mechanism by which this occurs may in part involve generation of podocytes without the gene defect.

Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain.

Bauer S, Patterson PH. J Neurosci. 2006 Nov 15;26(46):12089-99.

Abstract: Although neural stem cells (NSCs) persist in various areas of the adult brain, their contribution to brain repair after injury is very limited. Treatment with exogenous growth factors can mitigate this limitation, suggesting that the brain environment is normally deficient in permissive cues and that it may be possible to stimulate the latent regenerative potential of endogenous progenitors with appropriate signals. We analyzed the effects of overexpressing the cytokine leukemia inhibitory factor (LIF) on adult neurogenesis in the normal brain. We found that LIF reduces neurogenesis in the olfactory bulb and subventricular zone by acting directly on NSCs. LIF appears to promote NSC self-renewal, preventing the emergence of more differentiated cell types. This ultimately leads to an expansion of the NSC pool. Our results have implications for the development of therapeutic strategies for brain repair and suggest that LIF may be useful, in combination with other factors, in promoting regeneration in the adult brain.

Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization.

Smart N, et al. Nature. 2006 Nov 15; [Epub ahead of print]

Abstract: Cardiac failure has a principal underlying aetiology of ischaemic damage arising from vascular insufficiency. Molecules that regulate collateral growth in the ischaemic heart also regulate coronary vasculature formation during embryogenesis. Here we identify thymosin beta4 (Tbeta4) as essential for all aspects of coronary vessel development in mice, and demonstrate that Tbeta4 stimulates significant outgrowth from quiescent adult epicardial explants, restoring pluripotency and triggering differentiation of fibroblasts, smooth muscle cells and endothelial cells. Tbeta4 knockdown in the heart is accompanied by significant reduction in the pro-angiogenic cleavage product N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). Although injection of AcSDKP was unable to rescue Tbeta4 mutant hearts, it significantly enhanced endothelial cell differentiation from adult epicardially derived precursor cells. This study identifies Tbeta4 and AcSDKP as potent stimulators of coronary vasculogenesis and angiogenesis, and reveals Tbeta4-induced adult epicardial cells as a viable source of vascular progenitors for continued renewal of regressed vessels at low basal level or sustained neovascularization following cardiac injury.

Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages.

Kattman SJ, et al. Dev Cell. 2006 Nov;11(5):723-32.

Abstract: Cell-tracing studies in the mouse indicate that the cardiac lineage arises from a population that expresses the vascular endothelial growth factor receptor 2 (VEGFR2,

Flk-1), suggesting that it may develop from a progenitor with vascular potential. Using the embryonic stem (ES) cell differentiation model, we have identified a cardiovascular progenitor based on the temporal expression of the primitive streak (PS) marker brachyury and Flk-1. Comparable progenitors could also be isolated from head-fold stage embryos. When cultured with cytokines known to function during cardiogenesis, individual cardiovascular progenitors generated colonies that displayed cardiomyocyte, endothelial, and vascular smooth muscle (VSM) potential. Isolation and characterization of this previously unidentified population suggests that the mammalian cardiovascular system develops from multipotential progenitors.

Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice.

Lee RH, et al. Proc Natl Acad Sci U S A. 2006 Nov 14;103(46):17438-43. Epub 2006 Nov 6.

Abstract: We tested the hypothesis that multipotent stromal cells from human bone marrow (hMSCs) can provide a potential therapy for human diabetes mellitus. Severe but nonlethal hyperglycemia was produced in NOD/scid mice with daily low doses of streptozotocin on days 1-4, and hMSCs were delivered via intracardiac infusion on days 10 and 17. The hMSCs lowered blood glucose levels in the diabetic mice on day 32 relative to untreated controls (18.34 mM +/- 1.12 SE vs. 27.78 mM +/- 2.45 SE, P = 0.0019). ELISAs demonstrated that blood levels of mouse insulin were higher in the hMSC-treated as compared with untreated diabetic mice, but human insulin was not detected. PCR assays detected human Alu sequences in DNA in pancreas and kidney on day 17 or 32 but not in other tissues, except heart, into which the cells were infused. In the hMSC-treated diabetic mice, there was an increase in pancreatic islets and beta cells producing mouse insulin. Rare islets contained human cells that colabeled for human insulin or PDX-1. Most of the beta cells in the islets were mouse cells that expressed mouse insulin. In kidneys of hMSC-treated diabetic mice, human cells were found in the glomeruli. There was a decrease in mesangial thickening and a decrease in macrophage infiltration. A few of the human cells appeared to differentiate into glomerular endothelial cells. Therefore, the results raised the possibility that hMSCs may be useful in enhancing insulin secretion and perhaps improving the renal lesions that develop in patients with diabetes mellitus.

Engraftment of Donor-Derived Epithelial Cells in Multiple Organs Following Bone Marrow Transplantation into Newborn Mice

Emanuela M. Bruscia et al. Stem Cells. 2006 Oct;24(10):2299-308. Epub 2006 Jun 22.

Abstract: Bone marrow-derived cells (BMDCs) can engraft as epithelial cells throughout the body, including in the lung, liver, and gastrointestinal (GI) tract following transplantation into lethally irradiated adult recipients. Except for rare disease models in which marrow-derived epithelial cells have a survival advantage over endogenous cells, the currently attained levels of epithelial engraftment of BMDCs are too low to be of therapeutic benefit. Here we tested whether the degree of bone marrow to epithelial engraftment would be higher if bone marrow transplantation (BMT) were performed on 1-day-old mice, when tissues are undergoing rapid growth and remodeling. BMT into newborn mice after multiple different regimens allowed for robust hematopoietic engraftment, as well as the development of rare donor-derived epithelial cells in the GI tract and lung but not in the liver. The highest epithelial engraftment (0.02%) was obtained in mice that received a preparative regimen of two doses of busulfan in utero. When BMDCs were transplanted into myelosuppressed newborn mice that lacked expression of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, the chloride channel that is not functional in patients with cystic fibrosis, the engrafted mice showed partial restoration of CFTR channel activity, suggesting that marrow-derived epithelial cells in the GI tract were functional. However, BMT into newborn mice, regardless of the myeloablative regimen used, did not increase the number of bone marrow-derived epithelial cells over that which occurs after BMT into lethally irradiated adult mice.

Improved Liver Function in Patients with Liver Cirrhosis After Autologous Bone Marrow Cell Infusion Therapy

Shuji Terai et al. Stem Cells. 2006 Oct;24(10):2292-8. Epub 2006 Jun 15.

Abstract: Supplementation of mesenchymal stem cells (MSCs) during hematopoietic stem cell (HSC) transplantation alleviates complications such as graft-versus-host disease, leading to a speedy recovery of hematopoiesis. To meet this clinical demand, a fast MSC expansion method is required. In the present study, we examined the feasibility of using a rotary bioreactor system to expand MSCs from isolated bone marrow mononuclear cells. The cells were cultured in a rotary bioreactor with Myelocult medium containing a combination of supplementary factors, including stem cell factor and interleukin-3 and -6. After 8 days of culture, total cell numbers, Stro-1(+)CD44(+)CD34(-) MSCs, and CD34(+)CD44(+)Stro-1(-) HSCs were increased 9-, 29-, and 8-fold, respectively. Colony-forming efficiency-fibroblast per day of the bioreactor-treated cells was 1.44-fold higher than that of the cells without bioreactor treatment. The bioreactor-expanded MSCs showed expression of primitive MSC markers endoglin (SH2) and vimentin, whereas markers associated with lineage differentiation, including osteocalcin (osteogenesis), type II collagen (chondrogenesis), and C/EBP-alpha (CCAAT/enhancer-binding protein-alpha) (adipogenesis), were not detected. Upon induction, the bioreactor-expanded MSCs were able to differentiate into osteoblasts, chondrocytes, and adipocytes. We conclude that the rotary bioreactor with the modified Myelocult medium reported in this study may be used to rapidly expand MSCs.

Transforming growth factor alpha promotes sequential conversion of mature astrocytes into neural progenitors and stem cells.

Sharif A, et al. Oncogene. 2006 Oct 23; [Epub ahead of print]

Abstract: An instability of the mature cell phenotype is thought to participate to the formation of gliomas, primary brain tumors deriving from astrocytes and/or neural stem cells. Transforming growth factor alpha (TGFalpha) is an erbB1 ligand overexpressed in the earliest stages of gliomas, and exerts trophic effects on gliomal cells and astrocytes. Here, we questioned whether prolonged TGFalpha exposure affects the stability of the normal mature astrocyte phenotype. We first developed astrocyte cultures devoid of residual neural stem cells or progenitors. We demonstrate that days of TGFalpha treatment result in the functional conversion of a population of mature astrocytes into radial glial cells, a population of neural progenitors. TGFalpha-generated radial glial cells support embryonic neurons migration, and give birth to cells of the neuronal lineage, expressing neuronal markers and the electrophysiological properties of neuroblasts. Lengthening TGFalpha treatment to months results in the delayed appearance of cells with neural stem cells properties: they form floating cellular spheres that are self-renewing, can be clonally derived from a single cell and differentiated into cells of the neuronal lineage. This study uncovers a novel population of mature astrocytes capable, in response to a single epigenetic factor, to regress progressively into a neural stem-like cell stage via an intermediate progenitor stage.Oncogene advance online publication, 23 October 2006; doi:10.1038/sj.onc.1210071.

Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial.

Gajjar A, et al. Lancet Oncol. 2006 Oct;7(10):813-20.

BACKGROUND: Current treatment for medulloblastoma, which includes postoperative radiotherapy and 1 year of chemotherapy, does not cure many children with high-risk disease. We aimed to investigate the effectiveness of risk-adapted radiotherapy followed by a shortened period of dose-intense chemotherapy in children with medulloblastoma.
METHODS: After resection, patients were classified as having average-risk medulloblastoma (< or = 1.5 cm2 residual tumour and no metastatic disease) or high-risk medulloblastoma (> 1.5 cm2 residual disease or metastatic disease localised to neuraxis) medulloblastoma. All patients received risk-adapted craniospinal radiotherapy (23.4 Gy for average-risk disease and 36.0-39.6 Gy for high-risk disease) followed by four cycles of cyclophosphamide-based, dose-intensive chemotherapy. Patients were assessed regularly for disease status and treatment side-effects. The primary endpoint was 5-year event-free survival; we also measured overall survival. This study is registered with ClinicalTrials.gov, number NCT00003211.
FINDINGS: Of 134 children with medulloblastoma who underwent treatment (86 average-risk, 48 high-risk), 119 (89%) completed the planned protocol. No treatment-related deaths occurred. 5-year overall survival was 85% (95% CI 75-94) in patients in the average-risk group and 70% (54-84) in those in the high-risk group (p=0.04); 5-year event-free survival was 83% (73-93) and 70% (55-85), respectively (p=0.046). For the 116 patients whose histology was reviewed centrally, histological subtype correlated with 5-year event-free survival (p=0.04): 84% (74-95) for classic histology, 77% (49-100) for desmoplastic tumours, and 57% (33-80) for large-cell anaplastic tumours.
INTERPRETATION: Risk-adapted radiotherapy followed by a shortened schedule of dose-intensive chemotherapy can be used to improve the outcome of patients with high-risk medulloblastoma.

Embryonic-like stem cells from umbilical cord blood and potential for neural modeling

McGuckin C et al. Acta Neurobiol. Exp (2006) 66: 321-329.

Abstract: Stem cells offer the distinct prospect of changing the face of human medicine. However, although they have potential to form different tissues, are still in the early stages of development as therapeutic interventions. The three most used stem cell sources are umbilical cord blood, bone marrow and human embryos. Whilst, cord blood is now used to treat over 70 disorders, at the time of writing this manuscript, not a single disease has been overcome or ameliorated using human embryonic stem cells. Advancing stem cell medicine requires ethically sound and scientifically robust models to develop tomorrow's medicines. Media attention, however, distracts from this reality; it is important to remember that stem cells are a new visitor to the medical world and require more research. Here we describe the utility of human cord blood to develop neural models that are necessary to take stem cells to the next level – into human therapies.

Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus

Bharathi Hattiangady, Ashok K. Shetty Neurobiol Aging (2006). doi:10.1016/j.neurobiolaging.2006.09.015.

Abstract: To investigate whether dramatically waned dentate neurogenesis during aging is linked to diminution in neural stem/progenitor cell (NSC) number, we counted cells immunopositive for Sox-2 (a putative marker of NSCs) in the subgranular zone (SGZ) of young, middle-aged and aged F344 rats. The young SGZ comprised ~50,000 Sox-2+ cells and this amount did not diminish with aging. Quantity of GFAP+ cells and vimentin+ radial glia also remained stable during aging in this region. Besides, in all age groups, analogous fractions of Sox-2+ cells expressed GFAP (astrocytes/NSCs), NG-2 (oligodendrocyte-progenitors/NSCs), vimentin (radial glia), S-100_ (astrocytes) and doublecortin (new neurons). Nevertheless, analyses of Sox-2+ cells with proliferative markers insinuated an increased quiescence of NSCs with aging. Moreover, the volume of rat-endothelial-cell-antigen-1+ capillaries (vascular-niches) within the SGZ exhibited an age-related decline, resulting in an increased expanse between NSCs and capillaries.

Thus, decreased dentate neurogenesis during aging is not attributable to altered number or phenotype of NSCs. Instead, it appears to be an outcome of increased quiescence of NSCs due to changes in NSC milieu.

MCP-3 is a myocardial mesenchymal stem cell homing factor

Soren Schenk, et al. Stem Cells, published online October 19, 2006; doi:10.1634/stemcells.2006-0293.

Abstract: Mesenchymal stem cells (MSC) have received attention for their therapeutic potential in a number of disease states including bone formation, diabetes, stem cell engraftment following marrow transplantation, graft verse host disease, and heart failure. Despite this diverse interest the molecular signals regulating MSC trafficking to sites of injury are unclear. MSC are known to transiently home to the freshly infracted myocardium. To identify MSC homing factors, we determined chemokine expression pattern as a function of time after MI. We merged these profiles with chemokine receptors expressed on MSC but not cardiac fibroblasts, which do not home following MI. This analysis identified MCP-3 as a potential MSC homing factor. Over-expression of MCP-3 1 month after MI restored MSC homing to the heart. Following serial infusions of MSC cardiac function improved in MCP-3 expressing hearts (88.7%, $p<0.001$), but not in control hearts (8.6%, $p=0.47$). MSC engraftment was not associated with differentiation into cardiac myocytes. Rather MSC engraftment appeared to result in recruitment of myofibroblasts and remodeling of the collagen matrix. These data indicate that MCP-3 is an MSC homing factor; local over-expression of MCP-3 recruits MSC to sites of injured tissue and improves cardiac remodeling independent of cardiac myocyte regeneration.

Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease

R. D. Lund et al. Stem Cells, published online October 19, 2006; doi:10.1634/stemcells.2006-0308.

Abstract: Progressive photoreceptor degeneration resulting from genetic and other factors is a leading and largely untreatable cause of blindness worldwide. The object of this study was to find a cell type that is effective in slowing the progress of such degeneration in an animal model of human retinal disease, is safe and could be generated in sufficient numbers for clinical application. We have compared efficacy of four human derived cell types in preserving photoreceptor integrity and visual functions after injection into the subretinal space of the Royal College of Surgeons rat early in the progress of degeneration. Umbilical tissue-derived cells, placenta-derived cells, and mesenchymal stem cells were studied; dermal fibroblasts served as cell controls. At various ages up to 100 days, electroretinogram responses, spatial acuity and luminance threshold were measured. Both umbilical-derived and mesenchymal cells significantly reduced the degree of functional deterioration in each test. The effect of placental cells was little better than controls. Umbilical tissue-derived cells gave large areas of photoreceptor rescue; mesenchymal stem cells gave only localized rescue. Fibroblasts gave sham levels of rescue. Donor cells were confined to the subretinal space. There was no evidence of cell differentiation into neurons, of tumor formation or other untoward pathology. Since the umbilical tissue-derived cells demonstrated the best photoreceptor rescue and unlike mesenchymal stem cells were capable of sustained population doublings without karyotypic changes, it is proposed that they may provide utility as a cell source for the treatment of retinal degenerative diseases such as retinitis pigmentosa.

Sarcoma Derived from Cultured Mesenchymal Stem Cells

Jakub Tolar et al. Stem Cells, published online October 12, 2006; doi:10.1634/stemcells.2005-0620.

Abstract: To study the biodistribution of Mesenchymal Stem Cells (MSCs), we labeled adult murine C57BL/6 MSCs with firefly luciferase and DsRed2 fluorescent protein using non-viral Sleeping Beauty transposons, and co-infused labeled MSCs with bone marrow into irradiated allogeneic recipients. Using in vivo whole body imaging, luciferase signals were shown to be increased between weeks 3 and 12. Unexpectedly, some mice with the highest luciferase signals died and all surviving mice developed foci of sarcoma in lungs. Two mice also developed sarcomas in their extremities. Common cytogenetic abnormalities were identified in tumor cells isolated from different animals. Original MSC cultures not labeled with transposons, as well as independently isolated cultured MSCs were found to be cytogenetically abnormal. Moreover, primary MSC.s derived from the bone marrow of both BALB/c and C57BL/6 mice showed cytogenetic aberrations after several passages in vitro, showing that transformation was not a strain specific nor rare event. Clonal evolution was observed in vivo suggesting that the critical transformation event(s) occurred before infusion. Mapping of the transposition insertion sites did not identify an obvious transposon related genetic abnormality and p53 was not overexpressed. Infusion of MSC-derived sarcoma cells resulted in malignant lesions in secondary recipients. This new sarcoma cell line, S1, is unique in having a cytogenetic profile similar to human sarcoma and contains bioluminescent and fluorescent genes making it useful for investigations of cellular biodistribution and tumor response to therapy in vivo. More importantly, our study indicates that sarcoma can evolve from MSC cultures.

Transcoronary transplantation of progenitor cells after myocardial infarction.

Assmus B, et al. N Engl J Med. 2006 Sep 21;355(12):1222-32.

BACKGROUND: Pilot studies suggest that intracoronary transplantation of progenitor cells derived from bone marrow (BMC) or circulating blood (CPC) may improve left ventricular function after acute myocardial infarction. The effects of cell transplantation in

patients with healed myocardial infarction are unknown.
METHODS: After an initial pilot trial involving 17 patients, we randomly assigned, in a controlled crossover study, 75 patients with stable ischemic heart disease who had had a myocardial infarction at least 3 months previously to receive either no cell infusion (23 patients) or infusion of CPC (24 patients) or BMC (28 patients) into the patent coronary artery supplying the most dyskinetic left ventricular area. The patients in the control group were subsequently randomly assigned to receive CPC or BMC, and the patients who initially received BMC or CPC crossed over to receive CPC or BMC, respectively, at 3 months' follow-up.
RESULTS: The absolute change in left ventricular ejection fraction was significantly greater among patients receiving BMC (+2.9 percentage points) than among those receiving CPC (-0.4 percentage point, P=0.003) or no infusion (-1.2 percentage points, P<0.001). The increase in global cardiac function was related to significantly enhanced regional contractility in the area targeted by intracoronary infusion of BMC. The crossover phase of the study revealed that intracoronary infusion of BMC was associated with a significant increase in global and regional left ventricular function, regardless of whether patients crossed over from control to BMC or from CPC to BMC.
CONCLUSIONS: Intracoronary infusion of progenitor cells is safe and feasible in patients with healed myocardial infarction. Transplantation of BMC is associated with moderate but significant improvement in the left ventricular ejection fraction after 3 months. 2006 Massachusetts Medical Society

Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction.

Schachinger V, et al. N Engl J Med. 2006 Sep 21;355(12):1210-21.

BACKGROUND: Pilot trials suggest that the intracoronary administration of autologous progenitor cells may improve left ventricular function after acute myocardial infarction.
METHODS: In a multicenter trial, we randomly assigned 204 patients with acute myocardial infarction to receive an intracoronary infusion of progenitor cells derived from bone marrow (BMC) or placebo medium into the infarct artery 3 to 7 days after successful reperfusion therapy.
RESULTS: At 4 months, the absolute improvement in the global left ventricular ejection fraction (LVEF) was significantly greater in the BMC group than in the placebo group (mean [+/-SD] increase, 5.5+/-7.3% vs. 3.0+/-6.5%; P=0.01). Patients with a baseline LVEF at or below the median value of 48.9% derived the most benefit (absolute improvement in LVEF, 5.0%; 95% confidence interval, 2.0 to 8.1). At 1 year, intracoronary infusion of BMC was associated with a reduction in the prespecified combined clinical end point of death, recurrence of myocardial infarction, and any revascularization procedure (P=0.01).
CONCLUSIONS: Intracoronary administration of BMC is associated with improved recovery of left ventricular contractile function in patients with acute myocardial infarction. Large-scale studies are warranted to examine the potential effects of progenitor-cell administration on morbidity and mortality. 2006 Massachusetts Medical Society

Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction.

Lunde K, et al. N Engl J Med. 2006 Sep 21;355(12):1199-209.

BACKGROUND: Previous studies have shown improvement in left ventricular function after intracoronary injection of autologous cells derived from bone marrow (BMC) in the acute phase of myocardial infarction. We designed a randomized, controlled trial to further investigate the effects of this treatment.
METHODS: Patients with acute ST-elevation myocardial infarction of the anterior wall treated with percutaneous coronary intervention were randomly assigned to the group that underwent intracoronary injection of autologous mononuclear BMC or to the control group, in which neither aspiration nor sham injection was performed. Left ventricular function was assessed with the use of electrocardiogram-gated single-photon-emission computed tomography (SPECT) and echocardiography at baseline and magnetic resonance imaging (MRI) 2 to 3 weeks after the infarction. These procedures were repeated 6 months after the infarction. End points were changes in the left ventricular ejection fraction (LVEF), end-diastolic volume, and infarct size.
RESULTS: Of the 50 patients assigned to treatment with mononuclear BMC, 47 underwent intracoronary injection of the cells at a median of 6 days after myocardial infarction. There were 50 patients in the control group. The mean (+/-SD) change in LVEF, measured with the use of SPECT, between baseline and 6 months after infarction for all patients was 7.6+/-10.4 percentage points. The effect of BMC treatment on the change in LVEF was an increase of 0.6 percentage point (95% confidence interval [CI], -3.4 to 4.6; P=0.77) on SPECT, an increase of 0.6 percentage point (95% CI, -2.6 to 3.8; P=0.70) on echocardiography, and a decrease of 3.0 percentage points (95% CI, 0.1 to -6.1; P=0.054) on MRI. The two groups did not differ significantly in changes in left ventricular end-diastolic volume or infarct size and had similar rates of adverse events.
CONCLUSIONS: With the methods used, we found no effects of intracoronary injection of autologous mononuclear BMC on global left ventricular function. 2006 Massachusetts Medical Society

Integrins Are Markers of Human Neural Stem Cells

Peter E. Hall et al. Stem Cells. 2006 Sep;24(9):2078-84. Epub 2006 May 11.

Abstract: The identification of markers for the isolation of human neural stem cells (hNSCs) is essential for studies of their biology and therapeutic applications. This study investigated expression of the integrin receptor family by hNSCs as potential markers. Selection of alpha6(hi) or beta1(hi) cells by fluorescence-activated cell sorting led to an enrichment of human neural precursors, as shown by both neurosphere forming assays

and increased expression of prominin-1, sox2, sox3, nestin, bmi1, and musashi1 in the beta1(hi) population. Cells expressing high levels of beta1 integrin also expressed prominin-1 (CD133), a marker previously used to isolate hNSCs, and selection using integrin beta1(hi) cells or prominin-1(hi) cells was found to be equally effective at enriching for hNSCs from neurospheres. Therefore, integrin subunits alpha6 and beta1 are highly expressed by human neural precursors and represent convenient markers for their prospective isolation.

High Yield of Cells Committed to the Photoreceptor Fate from Expanded Mouse Retinal Stem Cells

Faten Merhi-Soussi et al. Stem Cells. 2006 Sep;24(9):2060-70. Epub 2006 Apr 27.

Abstract: The purpose of the present work was to generate, from retinal stem cells (RSCs), a large number of cells committed toward the photoreceptor fate in order to provide an unlimited cell source for neurogenesis and transplantation studies. We expanded RSCs (at least 34 passages) sharing characteristics of radial glial cells and primed the cells in vitro with fibroblast growth factor (FGF)-2 for 5 days, after which cells were treated with the B27 supplement to induce cell differentiation and maturation. Upon differentiation, cells expressed cell type-specific markers corresponding to neurons and glia. We show by immunocytochemistry analysis that a subpopulation of differentiated cells was committed to the photoreceptor lineage given that these cells expressed the photoreceptor proteins recoverin, peripherin, and rhodopsin in a same ratio. Furthermore, cells infected during the differentiation procedure with a lentiviral vector expressing green fluorescent protein (GFP) under the control of either the rhodopsin promoter or the interphotoreceptor retinoidbinding protein (IRBP) promoter, expressed GFP. FGF-2 priming increased neuronal differentiation while decreasing glia generation. Reverse transcription-polymerase chain reaction analyses revealed that the differentiated cells expressed photoreceptor-specific genes such as Crx, rhodopsin, peripherin, IRBP, and phosphodiesterase-_. Quantification of the differentiated cells showed a robust differentiation into the photoreceptor lineage: Approximately 25%–35% of the total cells harbored photoreceptor markers. The generation of a significant number of nondifferentiated RSCs as well as differentiated photoreceptors will enable researchers to determine via transplantation studies which cells are the most adequate to integrate a degenerating retina.

Bioreactor Expansion of Human Adult Bone Marrow-Derived Mesenchymal Stem Cells

Xi Chen et al. Stem Cells. 2006 Sep;24(9):2052-9. Epub 2006 May 25.

Abstract: Supplementation of mesenchymal stem cells (MSCs) during hematopoietic stem cell (HSC) transplantation alleviates complications such as graft-versus-host disease, leading to a speedy recovery of hematopoiesis. To meet this clinical demand, a fast MSC expansion method is required. In the present study, we examined the feasibility of using a rotary bioreactor system to expand MSCs from isolated bone marrow mononuclear cells. The cells were cultured in a rotary bioreactor with Myelocult medium containing a combination of supplementary factors, including stem cell factor and interleukin-3 and -6. After 8 days of culture, total cell numbers, Stro-1(+)CD44(+)CD34(-) MSCs, and CD34(+)CD44(+)Stro-1(-) HSCs were increased 9-, 29-, and 8-fold, respectively. Colony-forming efficiency-fibroblast per day of the bioreactor-treated cells was 1.44-fold higher than that of the cells without bioreactor treatment. The bioreactor-expanded MSCs showed expression of primitive MSC markers endoglin (SH2) and vimentin, whereas markers associated with lineage differentiation, including osteocalcin (osteogenesis), type II collagen (chondrogenesis), and C/EBP-alpha (CCAAT/enhancer-binding protein-alpha) (adipogenesis), were not detected. Upon induction, the bioreactor-expanded MSCs were able to differentiate into osteoblasts, chondrocytes, and adipocytes. We conclude that the rotary bioreactor with the modified Myelocult medium reported in this study may be used to rapidly expand MSCs.

Multi-potent mesenchymal stromal cells in blood

Qiling He et al. Stem Cells. published online Sep 14, 2006; DOI: 10.1634/stemcells.2006-0335.

Abstract: Peripheral blood-derived multi-potent mesenchymal stromal cells circulate in low number. They share, though not all, but most of the surface markers with bone marrow-derived multi-potent mesenchymal stromal cells, possess diverse and complicated gene expression characteristics, and are capable of differentiating along and even beyond mesenchymal lineages. Although their origin and physio-pathological function are still unclear, their presence in the adult peripheral blood might relate to some interesting but controversial subjects in the filed of adult stem cell biology, such as systemic migration of bone marrowderived multi-potent mesenchymal stromal cells and the existence of common hematopoietic-mesenchymal precursors. In this review, current studies /knowledge about peripheral blood-derived multi-potent mesenchymal stromal cells is summarized and the above-mentioned topics are discussed.

Effective cell and gene therapy in a murine model of Gaucher disease.

Enquist IB, et al. Proc Natl Acad Sci U S A. 2006 Sep 12;103(37):13819-24. Epub 2006 Sep 5.

Abstract: Gaucher disease (GD) is a lysosomal storage disorder due to an inherited deficiency in the enzyme glucosylceramidase (GCase) that causes hepatosplenomegaly, cytopenias, and bone disease as key clinical symptoms. Previous mouse models with GCase deficiency have been lethal in the perinatal period or viable without displaying the clinical features of GD. We have generated viable mice with characteristic clinical symptoms of type 1 GD by conditionally deleting GCase exons 9-11 upon postnatal

induction. Both transplantation of WT bone marrow (BM) and gene therapy through retroviral transduction of BM from GD mice prevented development of disease and corrected an already established GD phenotype. The gene therapy approach generated considerably higher GCase activity than transplantation of WT BM. Strikingly, both therapeutic modalities normalized glucosylceramide levels and practically no infiltration of Gaucher cells could be observed in BM, spleen, and liver, demonstrating correction at 5-6 months after treatment. The findings demonstrate the feasibility of gene therapy for type 1 GD in vivo. Our type 1 GD mice will serve as an excellent tool in the continued efforts toward development of safe and efficient cell and gene therapy for type 1 GD.

Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys.

Sagrinati C, et al. J Am Soc Nephrol. 2006 Sep;17(9):2443-56. Epub 2006 Aug 2.

Abstract: Regenerative medicine represents a critical clinical goal for patients with ESRD, but the identification of renal adult multipotent progenitor cells has remained elusive. It is demonstrated that in human adult kidneys, a subset of parietal epithelial cells (PEC) in the Bowman's capsule exhibit coexpression of the stem cell markers CD24 and CD133 and of the stem cell-specific transcription factors Oct-4 and Bml-1, in the absence of lineage-specific markers. This CD24+CD133+ PEC population, which could be purified from cultured capsulated glomeruli, revealed self-renewal potential and a high cloning efficiency. Under appropriate culture conditions, individual clones of CD24+CD133+ PEC could be induced to generate mature, functional, tubular cells with phenotypic features of proximal and/or distal tubules, osteogenic cells, adipocytes, and cells that exhibited phenotypic and functional features of neuronal cells. The injection of CD24+CD133+ PEC but not of CD24-CD133- renal cells into SCID mice that had acute renal failure resulted in the regeneration of tubular structures of different portions of the nephron. More important, treatment of acute renal failure with CD24+CD133+ PEC significantly ameliorated the morphologic and functional kidney damage. This study demonstrates the existence and provides the characterization of a population of resident multipotent progenitor cells in adult human glomeruli, potentially opening new avenues for the development of regenerative medicine in patients who have renal diseases.

Human neural stem cells target experimental intracranial medulloblastoma and deliver a therapeutic gene leading to tumor regression.

Kim SK, et al. Clin Cancer Res. 2006 Sep 15;12(18):5550-6.

PURPOSE: Medulloblastoma, a malignant pediatric brain tumor, is incurable in about one third of patients despite multimodal treatments. In addition, current therapies can lead to long-term disabilities. Based on studies of the extensive tropism of neural stem cells (NSC) toward malignant gliomas and the secretion of growth factors common to glioma and medulloblastoma, we hypothesized that NSCs could target medulloblastoma and be used as a cellular therapeutic delivery system.
Experimental Design: The migratory ability of HB1.F3 cells (an immortalized, clonal human NSC line) to medulloblastoma was studied both in vitro and in vivo. As proof-of-concept, we used HB1.F3 cells engineered to secrete the prodrug activating enzyme cytosine deaminase. We investigated the potential of human NSCs to deliver a therapeutic gene and reduce tumor growth.
RESULTS: The migratory capacity of HB1.F3 cells was confirmed by an in vitro migration assay, and corroborated in vivo by injecting chloromethylbenzamido-Dil-labeled HB1.F3 cells into the hemisphere contralateral to established medulloblastoma in nude mice. In vitro studies showed the therapeutic efficacy of HB1.F3-CD on Daoy cells in coculture experiments. In vitro therapeutic studies were conducted in which animals bearing intracranial medulloblastoma were injected ipsilaterally with HB1.F3-CD cells followed by systemic 5-flourocytosine treatment. Histologic analyses showed that human NSCs migrate to the tumor bed and its boundary, resulting in a 76% reduction of tumor volume in the treatment group (P<0.01).
CONCLUSION: These studies show for the first time the potential of human NSCs as an effective delivery system to target and disseminate therapeutic agents to medulloblastoma.

A phase 2 study of high-activity(186)Re-HEDP with autologous peripheral blood stem cell transplant in progressive hormone-refractory prostate cancer metastatic to bone.

O'Sullivan JM, et al. Eur J Nucl Med Mol Imaging. 2006 Sep;33(9):1055-1061. Epub 2006 Mar 30.

PURPOSE: We investigated the potential for improvement in disease control by use of autologous peripheral blood stem cell transplant (PBSCT) to permit administration of high activities of (186)Re-hydroxyethylidene diphosphonate (HEDP) in patients with progressive hormone-refractory prostate cancer (HRPC).
METHODS: Eligible patients had progressive HRPC metastatic to bone, good performance status and minimal soft tissue disease. Patients received 5,000 MBq of (186)Re-HEDP i.v., followed 14 days later by PBSCT. Response was assessed using PSA, survival, pain scores and quality of life.
RESULTS: Thirty-eight patients with a median age of 67 years (range 50-77) and a median PSA of 57 ng/ml (range 4-3,628) received a median activity of 4,978 MBq (186)Re-HEDP (range 4,770-5,100 MBq). The most serious toxicity was short-lived grade 3 thrombocytopenia in 8 (21%) patients. The median survival of the group is 21 months (95%CI 18-24 months) with Kaplan-Meier estimated 1- and 2-year survival rates of 83% and 40% respectively. Thirty-one patients (81%, 95% CI 66-90%) had stable or reduced PSA levels 3 months post therapy while 11 (29%, 95% CI 15-49%) had PSA reductions of >50% lasting >4 weeks. Quality of life measures were stable or improved in 27 (66%) at 3 months.
CONCLUSION: We have shown that it is feasible and safe to deliver high-activity

radioisotope therapy with PBSCT to men with metastatic HRPC. Response rates and survival data are encouraging; however, further research is needed to define optimal role of this treatment approach.

Adipose-derived stem and progenitor cells as fillers in plastic and reconstructive surgery.

Moseley TA, et al. Plast Reconstr Surg. 2006 Sep;118(3 Suppl):121S-128S.

Abstract: Plastic surgeons are keenly aware of the principle "replace like with like." This principle underlies much of the rationale behind the clinical use of autologous fat transplantation, despite the procedure's drawbacks. Autologous fat transplantation is frequently used for a variety of cosmetic and reconstructive indications not limited to posttraumatic defects of the face and body, involutional disorders such as hemifacial atrophy, sequelae of radiation therapy, and many aesthetic uses such as lip and facial augmentation and wrinkle therapy. However, the limitations of fat transplantation are well known, particularly the long-term unpredictability of volume maintenance. Regenerative cell-based strategies such as those encompassing the use of stem cells hold tremendous promise for augmentation of the soft-tissue space. Preclinical studies and early clinical series show that adipose-derived stem cells offer the possibility of finally fulfilling the key principle of replacing like with like as an aesthetic filler, without the drawbacks of current technology.

Ex Vivo Expansion Does Not Alter the Capacity of Umbilical Cord Blood CD34_ Cells to Generate Functional T Lymphocytes and Dendritic Cells

LADAN KOBARI et al. Stem Cells. 2006;24:2150–2157.

Abstract: We examined whether ex vivo expansion of umbilical cord blood progenitor cells affected their capacity to generate immune cells such as T lymphocytes (TLs) and dendritic cells (DCs). The capacity to generate TLs from cord blood CD34_ cells expanded for 14 days (d14) was compared with that of nonexpanded CD34_ cells (d0) using fetal thymus organ cultures or transfer into nonobese diabetic/severe combined immunodeficient mice. The cell preparations yielded comparable percentages of immature (CD4_CD8_, CD4_CD8_) TLs and functional mature (CD3_CD4_, CD3_CD8_) TLs with an analogous TCR (T-cell receptor)-V_ repertoire pattern. As regards DCs, d0 and d14 CD34_ cells also yielded similar percentages of CD1a_ DCs with the same expression levels of HLA-DR, costimulatory and adhesion molecules, and chemokine receptors. DCs derived from either d14 or d0 CD34_ stimulated allogeneic TLs to the same extent, and the cytokine pattern production of these allogeneic TLs was similar with no shift toward a predominant Th1 or Th2 response. Even though the intrinsic capacity of d14 CD34_ cells to generate DCs was 13-fold lower than that of d0 CD34_ cells, this reduction was offset by the prior amplification of the CD34_ cells, resulting in the overall production of 15-fold more DCs. These data indicate that ex vivo expansion of CD34_ cells does not impair T lymphopoiesis nor DC differentiation capacity.

In Vivo Bone Formation by Human Bone Marrow Stromal Cells: Reconstruction of the Mouse Calvarium and Mandible

MAHESH H. MANKANI et al. Stem Cells. 2006 Sep;24(9):2140-9. Epub 2006 Jun 8.

Abstract: Bone marrow stromal cells (BMSCs) contain a subset of multipotent cells with the potential to repair hard-tissue defects. Mouse BMSCs, combined with a collagen carrier, can close critical-sized homologous mouse calvarial defects, but this new bone has a poor union with the adjacent calvarium. When human BMSCs are transplanted for the purpose of engineering new bone, best results can be achieved if the cells are combined with hydroxyapatite/tricalcium phosphate (HA/TCP) particles. Here, we demonstrate that transplantation of cultured human BMSCs in conjunction with HA/TCP particles can be used successfully to close mouse craniofacial bone defects and that removal of the periosteum from the calvarium significantly enhances union with the transplant. Transplants were followed for up to 96 weeks and were found to change in morphology but not bone content after 8 weeks; this constitutes the first description of human BMSCs placed long-term to heal bone defects. New bone formation continued to occur in the oldest transplants, confirmed by tetracycline labeling. Additionally, the elastic modulus of this engineered bone resembled that of the normal mouse calvarium, and our use of atomic force microscopy (AFM)-based nanoindentation offered us the first opportunity to compare these small transplants against equally minute mouse bones. Our results provide insights into the long-term behavior of newly engineered orthotopic bone from human cells and have powerful implications for therapeutic human BMSC transplantation.

Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats.

Kolb B, et al. J Cereb Blood Flow Metab. 2006 Sep 20; [Epub ahead of print]

Abstract: Recent studies suggest that proliferation in the adult forebrain subventricular zone increases in response to a forebrain stroke and intraventricular infusions of growth factors enhance this response. The potential for growth factor infusions to regenerate the damaged motor cortex and promote recovery of motor function after stroke has not been examined. Here, we report that intraventricular infusions of epidermal growth factor and erythropoietin together, but not individually, promote substantial regeneration of the damaged cerebral cortex and reverse impairments in spontaneous and skilled motor tasks, in a rat model of stroke. Cortical regeneration and functional recovery occurred even when growth factor administration was delayed for up to 7 days after the stroke-induced lesion. Cell tracking demonstrated the contribution of neural precursors originating in the forebrain subventricular zone to the regenerated cortex. Strikingly,

removal of the regenerated cortical tissue reversed the growth factor-induced functional recovery. These findings reveal that specific combinations of growth factors can mobilize endogenous adult neural stem cells to promote cortical tissue re-growth and functional recovery after stroke.Journal of Cerebral Blood Flow & Metabolism advance online publiaction, 20 September 2006; doi:10.1038/sj.jcbfm.9600402.

Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury.

Ziv Y, et al. Proc Natl Acad Sci U S A. 2006 Aug 29;103(35):13174-9. Epub 2006 Aug 22.

Abstract: The well regulated activities of microglia and T cells specific to central nervous system (CNS) antigens can contribute to the protection of CNS neural cells and their renewal from adult neural stem/progenitor cells (aNPCs). Here we report that T cell-based vaccination of mice with a myelin-derived peptide, when combined with transplantation of aNPCs into the cerebrospinal fluid (CSF), synergistically promoted functional recovery after spinal cord injury. The synergistic effect was correlated with modulation of the nature and intensity of the local T cell and microglial response, expression of brain-derived neurotrophic factor and noggin protein, and appearance of newly formed neurons from endogenous precursor-cell pools. These results substantiate the contention that the local immune response plays a crucial role in recruitment of aNPCs to the lesion site, and suggest that similar immunological manipulations might also serve as a therapeutic means for controlled migration of stem/progenitor cells to other acutely injured CNS sites.

Derivation and large-scale expansion of multipotent astroglial neural progenitors from adult human brain.

Walton NM, et al. Development. 2006 Sep;133(18):3671-81. Epub 2006 Aug 16.

Abstract: The isolation and expansion of human neural cell types has become increasingly relevant in restorative neurobiology. Although embryonic and fetal tissue are frequently envisaged as providing sufficiently primordial cells for such applications, the developmental plasticity of endogenous adult neural cells remains largely unclear. To examine the developmental potential of adult human brain cells, we applied conditions favoring the growth of neural stem cells to multiple cortical regions, resulting in the identification and selection of a population of adult human neural progenitors (AHNPs). These nestin(+) progenitors may be derived from multiple forebrain regions, are maintainable in adherent conditions, co-express multiple glial and immature markers, and are highly expandable, allowing a single progenitor to theoretically form sufficient cells for approximately 4x10(7) adult brains. AHNPs longitudinally maintain the ability to generate both glial and neuronal cell types in vivo and in vitro, and are amenable to genetic modification and transplantation. These findings suggest an unprecedented degree of inducible plasticity is retained by cells of the adult central nervous system.

Isolation of mesenchymal stem cells from human vermiform appendix.

De Coppi P, et al. J Surg Res. 2006 Sep;135(1):85-91. Epub 2006 May 2.

Abstract: BACKGROUND: Recent findings have shown that pluripotent stem cells exist in areas outside the bone marrow (BM). Moreover, it has been demonstrated that the appendix is important for the development of mucosal gut immunity, and hematopoietic progenitors have been isolated from animal and human appendices.
MATERIALS AND METHODS: Non-inflamed appendices removed during laparotomy were processed and cultured until the appearance of adherent cells. Differentiations (performed under osteogenic, adipogenic, and myogenic conditions) were confirmed by immunohistochemistry and cytochemistry. Polymerase chain reaction and cytofluorimetric analyses were performed to evidence the presence of genes and protein specific lineages in appendix-derived mesenchymal stem cells (ADMCs). **RESULTS:** ADMCs were present in non-inflamed appendices. ADMCs under osteogenic conditions differentiated in osteoblasts and showed increased alkaline phosphatase expression; at the gene level, we observed the expression of Core binding factor alpha 1 (Cbfa1) and osteocalcin in osteogenic induced ADMCs. Under adipogenic conditions, lipidic drops in the cytoplasm, expression of lipoprotein lipase (LpL), and peroxisome proliferator-activated receptor gamma were observed; under myogenic conditions, myotubes expressing muscle specific proteins like desmin were formed. Myogenic regulatory factor 4 and MyoD were selectively induced in the ADMCs under myogenic conditions.
CONCLUSIONS: This study shows for the first time that mesenchymal stem cells can be isolated from normal appendices obtained from a pediatric and adult age group (0-18 years of age). This finding not only may further knowledge of the maturation of the intestinal immunesystem but also could indicate a new physiological role of the human vermiform appendix.

Differentiation of adult stem cells derived from bone marrow stroma into Leydig or adrenocortical cells.

Yazawa T, et al. Endocrinology. 2006 Sep;147(9):4104-11. Epub 2006 May 25.

Abstract: Adult stem cells from bone marrow, referred to as mesenchymal stem cells or marrow stromal cells (MSCs), are defined as pluripotent cells and have the ability to differentiate into multiple mesodermal cells. In this study, we investigated whether MSCs from rat, mouse, and human are able to differentiate into steroidogenic cells. When transplanted into immature rat testes, adherent marrow-derived cells (including MSCs) were found to be engrafted and differentiate into steroidogenic cells that were indistinguishable from Leydig cells. Isolated murine MSCs transfected with green fluorescence protein driven by the promoter of P450 side-chain cleaving enzyme gene

(CYP11A), a steroidogenic cell-specific gene, were used to detect steroidogenic cell production in vitro. During in vitro differentiation, green fluorescence protein-positive cells, which had characteristics similar to those of Leydig cells, were found. Stable transfection of murine MSCs with a transcription factor, steroidogenic factor-1, followed by treatment with cAMP almost recapitulated the properties of Leydig cells, including the production of testosterone. Transfection of human MSCs with steroidogenic factor-1 also led to their conversion to steroidogenic cells, but they appeared to be glucocorticoid- rather than testosterone-producing cells. These results indicate that MSCs represent a useful source of stem cells for producing steroidogenic cells that may provide basis for their use in cell and gene therapy.

Autologous adipose tissue-derived stromal cells for treatment of spinal cord injury.

Kang SK, et al. Stem Cells Dev. 2006 Aug;15(4):583-94.

Abstract: Isolated rat adipose tissue-derived stromal cells (rATSCs) contain pluripotent cells that can be differentiated into a variety of cell lineages, including neural cells. Recent work has shown that ATSCs can make neurosphere-like clumps and differentiate into neuron-like cells expressing neuronal markers, but their therapeutic effect is unclear. Here we report that intravenous infusion of oligodendrocyte precursor cells (OPCs) derived from rATSC autograft cells sources improve motor function in rat models of spinal cord injury (SCI). After 4-5 weeks, transplanted rATSC-OPC cells survived and migrated into the injured region of SCI very efficiently (30-35%) and migrated cells were partially differentiated into neurons and oligodendrocyte. Also, we found some of the engrafted OPCs migrated and integrated in the kidney, brain, lung, and liver through the intravenous system. Behavioral analysis revealed the locomotor functions of OPC-autografted SCI rats were significantly restored. Efficient migration of intravenously engrafted rATSC-OPCs cells into SCI lesion suggests that SCI-induced chemotaxic factors facilitate migration of rATSC-OPCs. Here, we verified that engrafted rATSCs and SCI-induced chemotaxic factors indeed play an important role in proliferation, migration, and differentiation of endogeneous spinal cord-derived neural progenitor cells in the injured region. In transplantation paradigms, the interaction between engrafted rATSC-OPCs and endogeneous spinal cord-derived neuronal progenitor cells will be important in promoting healing through fate decisions, resulting in coordinated induction of cell migration and differentiation.

Cord blood mesenchymal stem cells: Potential use in neurological disorders.

El-Badri NS, et al. Stem Cells Dev. 2006 Aug;15(4):497-506.

Abstract: Our previous studies demonstrate enhanced neural protective effects of cord blood (CB) cells in comparison to stem cells from adult marrow. To determine further whether mesenchymal stem cells (MSCs) derived from human umbilical cord blood (hUCB) possess optimal characteristics for neural therapy, we isolated populations of plastic-adherent CB MSCs. These cells generated CD34-, CD45-, CD11b-, CD3-, CD19- cells in culture and failed to produce CFU-M, CFU-GEMM, or CFU-GM hematopoietic colonies in methylcellulose. However, cultured CB MSCs possessed a remarkable ability to support proliferation as well as differentiation of hematopoietic cells in vitro. In addition, supernatants from cultured CB MSCs promoted survival of NT2 N neural cells and peripheral blood mononuclear cells (MNCs) cultured under conditions designed to induce cell stress and limit protein synthesis. After incubation in neural differentiation medium, CB MSCs expressed the neural cell-surface antigen A2B5, the neurofilament polypeptide NF200, the oligodendrocyte precursor marker 04, intermediate filament proteins characteristic of neural differentiation (nestin and vimentin), as well as the astrocyte marker glial fibrillary acidic protein (GFAP) and the neural progenitor marker TUJ-1. We examined the immunomodulatory effects of the CB MSCs after co-culture with murine splenocytes. Whereas spleen cells from normal C57Bl/6 mice exhibited a prominent immunoglobulin M (IgM) response after immunization with the T cell-dependent antigen sheep red blood cells, this response was significantly decreased after incubation with CB MSCs. These data indicate that CB MSCs possess multiple utilities that may contribute to their therapeutic potency in the treatment of neurological disorders.

Threshold of lung injury required for the appearance of marrow-derived lung epithelia.

Herzog EL, et al. Stem Cells. 2006 Aug;24(8):1986-92.

Abstract: Bone marrow-derived cells (BMDCs) can adopt an epithelial phenotype in the lung following bone marrow transplantation (BMT). This phenomenon has been assumed to result from the lung injury that occurs with myeloablative radiation. To date, no study has related the degree of epithelial chimerism following bone marrow transplantation to the lung damage induced by preconditioning for BMT. Such a goal is crucial to understanding the local host factors that promote the engraftment of BMDCs as lung epithelia. We undertook this aim by performing sex-mismatched bone marrow transplantation using a variety of preconditioning regimens and comparing measurements of lung injury (bronchoalveolar lavage [BAL] cell count, alveolar-capillary leak assayed by BAL protein levels, and terminal deoxynucleotidyl transferase dUTP nick-end labeling analysis on epithelial cells) with rigorous methods to quantify bone marrow-derived lung epithelia (costaining for epithelial and donor markers on tissue sections and isolated lung epithelia in recipient mice). We found that only at doses that induced lung injury could marrow derived lung epithelium be identified following BMT. With irradiation doses less than 1,000 centigray (cGy), there was little to no apparent injury to the lung, and there were no marrow-derived pneumocytes despite high levels of hematopoietic chimerism. In contrast, 4 days after either split or single-dose 1,000 cGy irradiation, nearly 15% of lung epithelia were apoptotic, and with this dose, marrow-derived type II pneumocytes (0.2%) were present at 28 days. These data indicate a

critical relationship between lung injury and the phenotypic change from BMDCs to lung epithelial cells.

The phenotypes of pluripotent human hepatic progenitors.

Schmelzer E, et al. Stem Cells. 2006 Aug;24(8):1852-8. Epub 2006 Apr 20.

Abstract: Human livers contain two pluripotent hepatic progenitors, hepatic stem cells and hepatoblasts, with size, morphology, and gene expression profiles distinct from that of mature hepatocytes. Hepatic stem cells, the precursors to hepatoblasts, persist in stable numbers throughout life, and those isolated from the livers of all age donors from fetal to adult are essentially identical in their gene and protein expression profiles. The gene expression profile of hepatic stem cells throughout life consists of high levels of expression of cytokeratin 19 (CK19), neuronal cell adhesion molecule (NCAM), epithelial cell adhesion molecule (EpCAM), and claudin-3 (CLDN-3); low levels of albumin; and a complete absence of expression of alpha-fetoprotein (AFP) and adult liver-specific proteins. By contrast, hepatoblasts, the dominant cell population in fetal and neonatal livers, decline in numbers with age and are found as <0.1% of normal adult livers. They express high levels of AFP, elevated levels of albumin, low levels of expression of adult liver-specific proteins, low levels of CK19, and a loss of NCAM and CLDN-3. Mature hepatocytes lack expression altogether of EpCAM, NCAM, AFP, CLDN-3, cytokeratin 19, and have acquired the well-known adult-specific profile that includes expression of high levels of albumin, cytochrome P4503A4, connexins, phosphoenolpyruvate carboxykinase, and transferrin. Thus, hepatic stem cells have a unique stem cell phenotype, whereas hepatoblasts have low levels of expression of both stem cell genes and genes expressed in high levels in mature hepatocytes.

Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells.

Rodriguez LV, et al. Proc Natl Acad Sci U S A. 2006 Aug 8;103(32):12167-72. Epub 2006 Jul 31.

Abstract: Smooth muscle is a major component of human tissues and is essential for the normal function of a multitude of organs including the intestine, urinary tract and the vascular system. The use of stem cells for cell-based tissue engineering and regeneration strategies represents a promising alternative for smooth muscle repair. For such strategies to succeed, a reliable source of smooth muscle precursor cells must be identified. Adipose tissue provides an abundant source of multipotent cells. In this study, the capacity of processed lipoaspirate (PLA) and adipose-derived stem cells to differentiate into phenotypic and functional smooth muscle cells was evaluated. To induce differentiation, PLA cells were cultured in smooth muscle differentiation medium. Smooth muscle differentiation of PLA cells induced genetic expression of all smooth muscle markers and further confirmed by increased protein expression of smooth muscle cell-specific alpha actin (ASMA), calponin, caldesmon, SM22, myosin heavy chain (MHC), and smoothelin. Clonal studies of adipose derived multipotent cells demonstrated differentiation of these cells into smooth muscle cells in addition to trilineage differentiation capacity. Importantly, smooth muscle-differentiated cells, but not their precursors, exhibit the functional ability to contract and relax in direct response to pharmacologic agents. In conclusion, adipose-derived cells have the potential to differentiate into functional smooth muscle cells and, thus, adipose tissue can be a useful source of cells for treatment of injured tissues where smooth muscle plays an important role.

Differentiation of umbilical cord blood-derived multilineage progenitor cells into respiratory epithelial cells.

Berger MJ, et al. Cytotherapy. 2006;8(5):480-7.

BACKGROUND: Umbilical cord blood (UCB) has been examined for the presence of stem cells capable of differentiating into cell types of all three embryonic layers (i.e. endo-, ecto- and mesoderm). The few groups reporting success have typically confirmed endodermal potential using hepatic differentiation. We report differentiation of human UCB-derived multipotent stem cells, termed multilineage progenitor cells (MLPC), into respiratory epithelial cells (i.e. type II alveolar cells).
METHODS: Using a cell separation medium (PrepaCyte-MLPC; BioE Inc.) and plastic adherence, MLPC were isolated from four of 16 UCB units (American Red Cross) and expanded. Cultures were grown to 80% confluence in mesenchymal stromal cell growth medium (MSCGM; Cambrex BioScience) prior to addition of small airway growth medium (SAGM; Cambrex BioScience), an airway maintenance medium. Following a 3-8-day culture, cells were characterized by light microscopy, transmission electron microscopy, immunofluorescence and reverse transcriptase (RT)-PCR.
RESULTS: MLPC were successfully differentiated into type II alveolar cells (four of four mixed lines; two of two clonal lines). Differentiated cells were characterized by epithelioid morphology with lamellar bodies. Both immunofluorescence and RT-PCR confirmed the presence of surfactant protein C, a protein highly specific for type II cells.
DISCUSSION: MLPC were isolated, expanded and then differentiated into respiratory epithelial cells using an off-the-shelf medium designed for maintenance of fully differentiated respiratory epithelial cells. To the best of our knowledge, this is the first time human non-embryonic multipotent stem cells have been differentiated into type II alveolar cells. Further studies to evaluate the possibilities for both research and therapeutic applications are necessary.

Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability.

Galvez BG, et al. J Cell Biol. 2006 Jul 17;174(2):231-43. Epub 2006 Jul 10.

Abstract: Efficient delivery of cells to target tissues is a major problem in cell therapy. We report that enhancing delivery of mesoangioblasts leads to a complete reconstitution of downstream skeletal muscles in a mouse model of severe muscular dystrophy (alpha-sarcoglycan ko). Mesoangioblasts, vessel-associated stem cells, were exposed to several cytokines, among which stromal- derived factor (SDF) 1 or tumor necrosis factor (TNF) alpha were the most potent in enhancing transmigration in vitro and migration into dystrophic muscle in vivo. Transient expression of alpha4 integrins or L-selectin also increased several fold migration both in vitro and in vivo. Therefore, combined pretreatment with SDF-1 or TNF-alpha and expression of alpha4 integrin leads to massive colonization (>50%) followed by reconstitution of >80% of alpha-sarcoglycan-expressing fibers, with a fivefold increase in efficiency in comparison with control cells. This study defines the requirements for efficient engraftment of mesoangioblasts and offers a new potent tool to optimize future cell therapy protocols for muscular dystrophies.

Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation.

Zurita M, Vaquero J. Neurosci Lett. 2006 Jul 10;402(1-2):51-6. Epub 2006 May 19.

Abstract: Chronic paraplegia resulting from severe spinal cord injury (SCI) is considered to be an irreversible condition. Nevertheless, recent studies utilizing adult stem cells appear to offer promise in the treatment of this and other neurological diseases. Here, we show that progressive functional motor recovery is achieved over the course of the year following the administration of bone marrow stromal cells (BMSC) in traumatic central spinal cord cavities of adult rats with chronic paraplegia. At this time, functional recovery is almost complete and associated with evident nervous tissue regeneration in the previously injured spinal cord.

Maturation of tissue engineered cartilage implanted in injured and osteoarthritic human knees.

Hollander AP, et al. Tissue Eng. 2006 Jul;12(7):1787-98.

Abstract: The regeneration of damaged organs requires that engineered tissues mature when implanted at sites of injury or disease. We have used new analytic techniques to determine the extent of tissue regeneration after treatment of knee injury patients with a novel cartilage tissue engineering therapy and the effect of pre-existing osteoarthritis on the regeneration process. We treated 23 patients, with a mean age of 35.6 years, presenting with knee articular cartilage defects 1.5 cm2 to 11.25 cm2 (mean, 5.0 cm2) in area. Nine of the patients had X-ray evidence of osteoarthritis. Chondrocytes were isolated from healthy cartilage removed at arthroscopy. The cells were cultured for 14 days, seeded onto esterified hyaluronic acid scaffolds (Hyalograft C), and grown for a further 14 days before implantation. A second-look biopsy was taken from each patient after 6 to 30 months (mean, 16 months). After standard histological analysis, uncut tissue was further analyzed using a newly developed biochemical protocol involving digestion with trypsin and specific, quantitative assays for type II collagen, type I collagen, and proteoglycan, as well as mature and immature collagen crosslinks. Cartilage regeneration was observed as early as 11 months after implantation and in 10 out of 23 patients. Tissue regeneration was found even when implants were placed in joints that had already progressed to osteoarthrosis. Cartilage injuries can be effectively repaired using tissue engineering, and osteoarthritis does not inhibit the regeneration process.

Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor.

Gordon MY, et al. Stem Cells. 2006 Jul;24(7):1822-30. Epub 2006 Mar 23.

Abstract: A phase I study was performed to determine the safety and tolerability of injecting autologous CD34(+) cells into five patients with liver insufficiency. The study was based on the hypothesis that the CD34(+) cell population in granulocyte colony-stimulating factor (G-CSF)-mobilized blood contains a subpopulation of cells with the potential for regenerating damaged tissue. We separated a candidate CD34(+) stem cell population from the majority of the CD34(+) cells (99%) by adherence to tissue culture plastic. The adherent and nonadherent CD34(+) cells were distinct in morphology, immunophenotype, and gene expression profile. Reverse transcription-polymerase chain reaction-based gene expression analysis indicated that the adherent CD34(+) cells had the potential to express determinants consistent with liver, pancreas, heart, muscle, and nerve cell differentiation as well as hematopoiesis. Overall, the characteristics of the adherent CD34(+) cells identify them as a separate putative stem/progenitor cell population. In culture, they produced a population of cells exhibiting diverse morphologies and expressing genes corresponding to multiple tissue types. Encouraged by this evidence that the CD34(+) cell population contains cells with the potential to form hepatocyte-like cells, we gave G-CSF to five patients with liver insufficiency to mobilize their stem cells for collection by leukapheresis. Between 1 x 10(6) and 2 x 10(8) CD34(+) cells were injected into the portal vein (three patients) or hepatic artery (two patients). No complications or specific side effects related to the procedure were observed. Three of the five patients showed improvement in serum bilirubin and four of five in serum albumin. These observations warrant further clinical trials.

Regeneration and transdifferentiation potential of muscle-derived stem cells propagated as myospheres.

Sarig R, et al. Stem Cells. 2006 Jul;24(7):1769-78. Epub 2006 Mar 30.

Abstract: We have isolated from mouse skeletal muscle a subpopulation of slow adherent myogenic cells that can proliferate for at least several months as suspended

clusters of cells (myospheres). In the appropriate conditions, the myospheres adhere to the plate, spread out, and form a monolayer of MyoD(+) cells. Unlike previously described myogenic cell lines, most of the myosphere cells differentiate, without cell fusion, into thin mononucleated contractile fibers, which express myogenin and skeletal muscle myosin heavy chain. The presence of Pax-7 in a significant proportion of these cells suggests that they originate from satellite cells. The addition of leukemia inhibitory factor to the growth medium of the myospheres enhances proliferation and dramatically increases the proportion of cells expressing Sca-1, which is expressed by several types of stem cells. The capacity of myosphere cells to transdifferentiate to other mesodermal cell lineages was examined. Exposure of cloned myosphere cells to bone morphogenetic protein resulted in suppression of myogenic differentiation and induction of osteogenic markers such as alkaline phosphatase and osteocalcin. These cells also sporadically differentiated to adipocytes. Myosphere cells could not, so far, be induced to transdifferentiate to hematopoietic cells. When inoculated into injured muscle, myosphere-derived cells participated in regeneration, forming multinucleated cross-striated mature fibers. This suggests a potential medical application.

In vitro differentiation of human placenta-derived multipotent cells into hepatocyte-like cells.

Chien CC, et al. Stem Cells. 2006 Jul;24(7):1759-68.

Abstract: Multipotent cells isolated from human term placenta (placenta-derived multipotent cells [PDMCs]) have been known to be able to differentiate into mesodermal lineage cells, including adipocytes and osteoclasts. The low infection rate and young age of placenta compared with other tissue origins of adult stem cells make theses cells attractive target for cell-based therapy. However, the differentiation potential of PDMCs toward hepatic cells has not been evaluated yet. In this study, we cultivated PDMCs with hepatic differentiation medium to evaluate the ability of these cells in differentiating toward hepatic cells. After treatment, the morphologies of differentiated PDMCs changed to polygonal epithelial cell-like. The differentiated cells not only show the hepatocyte-like morphologies but also express hepatocyte-specific markers, including albumin and cytokeratin 18. The bioactivity assays revealed that these hepatocyte-like cells could uptake lipoprotein and store glycogen. Furthermore, the addition of rifampicin increased the gene expression of CYP3A4, which is similar with the activities of human liver cells. According to our previous results, PDMCs were capable of differentiating into mesodermal and ectodermal lineage cells. Our results indicate that PDMCs can differentiate into three germ layer cells, which is similar to embryonic stem cells. In conclusion, placenta might be an easily accessible source for progenitor cells that are capable of differentiating toward hepatocyte-like cells in vitro.

The defined combination of growth factors controls generation of long-term-replicating islet progenitor-like cells from cultures of adult mouse pancreas.

Ta M, et al. Stem Cells. 2006 Jul;24(7):1738-49. Epub 2006 Mar 23.

Abstract: Application of pancreatic islet transplantation to treatment of diabetes is severely hampered by the inadequate islet supply. This problem could in principle be overcome by generating islet cells from adult pancreas in vitro. Although it is possible to obtain replicating cells from cultures of adult pancreas, these cells, when significantly expanded in vitro, progressively lose pancreatic-specific gene expression, including that of a "master" homeobox transcription factor Pdx1. Here we show for the first time that long-term proliferating islet progenitor-like cells (IPLCs) stably expressing high levels of Pdx1 and other genes that control early pancreatic development can be derived from cultures of adult mouse pancreas under serum-free defined culture conditions. Moreover, we show that cells derived thus can be maintained in continuous culture for at least 6 months without any substantial loss of early pancreatic phenotype. Upon growth factor withdrawal, the IPLCs organize into cell clusters and undergo endocrine differentiation of various degrees in a line-dependent manner. We propose that our experimental strategy will provide a framework for developing efficient approaches for ex vivo expansion of islet cell mass.

Allogeneic haematopoietic stem cell transplantation for metastatic renal carcinoma in Europe.

Barkholt L, et al. Ann Oncol. 2006 Jul;17(7):1134-40. Epub 2006 Apr 28.

BACKGROUND: An allogeneic antitumour effect has been reported for various cancers. We evaluated the experience of allogeneic haematopoietic stem cell transplantation (HSCT) for renal cell carcinoma (RCC) in 124 patients from 21 European centres.
PATIENTS AND METHODS: Reduced intensity conditioning and peripheral blood stem cells from an HLA-identical sibling (n = 106), a mismatched related (n = 5), or an unrelated (n = 13) donor were used. Immunosuppression was cyclosporine alone, or combined with methotrexate or mycophenolate mofetil. Donor lymphocyte infusions (DLI) were given to 42 patients. The median follow-up was 15 (range 3-41) months.
RESULTS: All but three patients engrafted. The cumulative incidence of moderate to severe, grades II-IV acute GVHD was 40% and for chronic GVHD it was 33%. Transplant-related mortality was 16% at one year. Complete (n = 4) or partial (n = 24) responses, median 150 (range 42-600) days post-transplant, were associated with time from diagnosis to HSCT, mismatched donor and acute GVHD II-IV. Factors associated with survival included chronic GVHD (hazards ratio, HR 4.12, P < 0.001), DLI (HR 3.39, P < 0.001), <3 metastatic sites (HR 2.61, P = 0.002) and a Karnofsky score >70 (HR 2.33, P = 0.03). Patients (n = 17) with chronic GVHD and given DLI had a 2-year survival of 70%.
CONCLUSION: Patients with metastatic RCC, less than three metastatic locations and a Karnofsky score >70% can be considered for HSCT. Posttransplant DLI and limited chronic GVHD improved the patient survival.

Derivation of male germ cells from bone marrow stem cells.

Nayernia K, et al. Lab Invest. 2006 Jul;86(7):654-63. Epub 2006 May 1.

Abstract: Recent studies have demonstrated that somatic stem cells have a more flexible potential than expected, whether put into tissue or cultured under different conditions. Bone marrow (BM)-derived stem cells can transdifferentiate into multilineage cells, such as muscle of mesoderm, lung and liver of endoderm, and brain and skin of ectoderm origin. Here we show that BM stem cells are able to transdifferentiate into male germ cells. For derivation of male germ cells from adult BM stem (BMS) cells, we used the Stra8-enhanced green fluoresence protein (EGFP) transgenic mouse line expressing EGFP specifically in male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells, such as fragilis, stella, Rnf17, Mvh and Oct4; as well as molecular markers of spermatogonial stem cells and spermatogonia including Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90alpha, beta1- and alpha6-integrins. Our ability to derive male germ cells from BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine.

Living autologous heart valves engineered from human prenatally harvested progenitors.

Schmidt D, et al. Circulation. 2006 Jul 4;114(1 Suppl):I125-31.

BACKGROUND: Heart valve tissue engineering is a promising strategy to overcome the lack of autologous growing replacements, particularly for the repair of congenital malformations. Here, we present a novel concept using human prenatal progenitor cells as new and exclusive cell source to generate autologous implants ready for use at birth.
METHODS AND RESULTS: Human fetal mesenchymal progenitors were isolated from routinely sampled prenatal chorionic villus specimens and expanded in vitro. A portion was cryopreserved. After phenotyping and genotyping, cells were seeded onto synthetic biodegradable leaflet scaffolds (n=12) and conditioned in a bioreactor. After 21 days, leaflets were endothelialized with umbilical cord blood-derived endothelial progenitor cells and conditioned for additional 7 days. Resulting tissues were analyzed by histology, immunohistochemistry, biochemistry (amounts of extracellular matrix, DNA), mechanical testing, and scanning electron microscopy (SEM) and were compared with native neonatal heart valve leaflets. Fresh and cryopreserved cells showed comparable myofibroblast-like phenotypes. Genotyping confirmed their fetal origin. Neo-tissues exhibited organization, cell phenotypes, extracellular matrix production, and DNA content comparable to their native counterparts. Leaflet surfaces were covered with functional endothelia. SEM showed cellular distribution throughout the polymer and smooth surfaces. Mechanical profiles approximated those of native heart valves.
CONCLUSIONS: Prenatal fetal progenitors obtained from routine chorionic villus sampling were successfully used as an exclusive, new cell source for the engineering of living heart valve leaflets. This concept may enable autologous replacements with growth potential ready for use at birth. Combined with the use of cell banking technology, this approach may be applied also for postnatal applications.

Autologous human tissue-engineered heart valves: prospects for systemic application.

Mol A, et al. Circulation. 2006 Jul 4;114(1 Suppl):I152-8.

BACKGROUND: Tissue engineering represents a promising approach for the development of living heart valve replacements. In vivo animal studies of tissue-engineered autologous heart valves have focused on pulmonary valve replacements, leaving the challenge to tissue engineer heart valves suitable for systemic application using human cells.
METHODS AND RESULTS: Tissue-engineered human heart valves were analyzed up to 4 weeks and conditioning using bioreactors was compared with static culturing. Tissue formation and mechanical properties increased with time and when using conditioning. Organization of the tissue, in terms of anisotropic properties, increased when conditioning was dynamic in nature. Exposure of the valves to physiological aortic valve flow demonstrated proper opening motion. Closure dynamics were suboptimal, most likely caused by the lower degree of anisotropy when compared with native aortic valve leaflets.
CONCLUSIONS: This study presents autologous tissue-engineered heart valves based on human saphenous vein cells and a rapid degrading synthetic scaffold. Tissue properties and mechanical behavior might allow for use as living aortic valve replacements.

Autologous hematopoietic stem cell transplantation for older patients with relapsed non-Hodgkin's lymphoma.

Buadi FK, et al. Bone Marrow Transplant. 2006 Jun;37(11):1017-22.

Abstract: To evaluate autologous stem cell transplant (ASCT) in older patients with intermediate grade non-Hodgkin's lymphoma (NHL), the Mayo Clinic Rochester BMT database was reviewed for all patients 60 years of age and older who received ASCT for NHL between September 1995 and February 2003. Factors evaluated included treatment-related mortality (TRM), event-free survival (EFS) and overall survival (OS). Ninety-three patients were identified, including twenty-four (26%) over the age of 70 years. Treatment-related mortality (5.4%) was not significantly different when compared to a younger cohort (2.2%). At a median follow-up of 14 months (0.6-87.6 months), the estimated median survival is 25 months (95% confidence interval (CI) 12-38) in the older group compared to 56 months (95% CI 37-75) (P=0.037) in the younger group. The estimated 4-year EFS was 38% for the older group compared to 42% in the younger cohort (P=0.1). By multivariate analysis, the only factor found to influence survival in the older group was age-adjusted International Prognostic Index at relapse, 0-1 better than

2-3 (P=0.03). Autologous stem-cell transplant can be safely performed in patients 60 years or older with chemotherapy sensitive relapsed or first partial remission NHL. The outcome may not be different from that of younger patients in terms of TRM and EFS.

Isolation of a novel population of multipotent adult stem cells from human hair follicles.

Yu H, et al. Am J Pathol. 2006 Jun;168(6):1879-88.

Abstract: Hair follicles are known to contain a well-characterized niche for adult stem cells: the bulge, which contains epithelial and melanocytic stem cells. Using human embryonic stem cell culture conditions, we isolated a population of adult stem cells from human hair follicles that are distinctively different from known epithelial or melanocytic stem cells. These cells do not express squamous or melanocytic markers but express neural crest and neuron stem cell markers as well as the embryonic stem cell transcription factors Nanog and Oct4. These precursor cells proliferate as spheres, are capable of self-renewal, and can differentiate into multiple lineages. Differentiated cells not only acquire lineage-specific markers but also demonstrate appropriate functions in ex vivo conditions. Most of the Oct4-positive cells in human skin were located in the area highlighted by cytokeratin 15 staining in vivo. Our data suggest that human embryonic stem cell medium can be used to isolate and expand human adult stem cells. Using this method, we isolated a novel population of multipotent adult stem cells from human hair follicles, and these cells appear to be located in the bulge area. Human hair follicles may provide an accessible, autologous source of adult stem cells for therapeutic application.

Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model.

Kim SW, et al. Stem Cells. 2006 Jun;24(6):1620-6. Epub 2006 Feb 23.

Abstract: Buerger's disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vasoocclusive disease. It is characterized pathologically as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially the distal extremities (the feet and the hands). There is no curative medication or surgery for this disease. In the present study, we transplanted human leukocyte antigen-matched human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) into four men with Buerger's disease who had already received medical treatment and surgical therapies. After the stem cell transplantation, ischemic rest pain suddenly disappeared from their affected extremities. The necrotic skin lesions were healed within 4 weeks. In the follow-up angiography, digital capillaries were increased in number and size. In addition, vascular resistance in the affected extremities, compared with the preoperative examination, was markedly decreased due to improvement of the peripheral circulation. Because an animal model of Buerger's disease is absent and also to understand human results, we transplanted human UCB-derived MSCs to athymic nude mice with hind limb ischemia by femoral artery ligation. Up to 60% of the hind limbs were salvaged in the femoral artery-ligated animals. By in situ hybridization, the human UCB-derived MSCs were detected in the arterial walls of the ischemic hind limb in the treated group. Therefore, it is suggested that human UCB-derived MSC transplantation may be a new and useful therapeutic armament for Buerger's disease and similar ischemic diseases.

Formation of a functional thymus initiated by a postnatal epithelial progenitor cell.

Bleul CC, et al. Nature. 2006 Jun 22;441(7096):992-6.

Abstract: The thymus is essential for the generation of self-tolerant effector and regulatory T cells. Intrathymic T-cell development requires an intact stromal microenvironment, of which thymic epithelial cells (TECs) constitute a major part. For instance, cell-autonomous genetic defects of forkhead box N1 (Foxn1) and autoimmune regulator (Aire) in thymic epithelial cells cause primary immunodeficiency and autoimmunity, respectively. During development, the thymic epithelial rudiment gives rise to two major compartments, the cortex and medulla. Cortical TECs positively select T cells, whereas medullary TECs are involved in negative selection of potentially autoreactive T cells. It has long been unclear whether these two morphologically and functionally distinct types of epithelial cells arise from a common bi-potent progenitor cell and whether such progenitors are still present in the postnatal period. Here, using in vivo cell lineage analysis in mice, we demonstrate the presence of a common progenitor of cortical and medullary TECs after birth. To probe the function of postnatal progenitors, a conditional mutant allele of Foxn1 was reverted to wild-type function in single epithelial cells in vivo. This led to the formation of small thymic lobules containing both cortical and medullary areas that supported normal thymopoiesis. Thus, single epithelial progenitor cells can give rise to a complete and functional thymic microenvironment, suggesting that cell-based therapies could be developed for thymus disorders.

Stem cell niches in the adult mouse heart.

Urbanek K, et al. Proc Natl Acad Sci U S A. 2006 Jun 13;103(24):9226-31. Epub 2006 Jun 5.

Abstract: Cardiac stem cells (CSCs) have been identified in the adult heart, but the microenvironment that protects the slow-cycling, undifferentiated, and self-renewing CSCs remains to be determined. We report that the myocardium possesses interstitial structures with the architectural organization of stem cell niches that harbor long-term BrdU-retaining cells. The recognition of long-term label-retaining cells provides functional evidence of resident CSCs in the myocardium, indicating that the heart is an organ regulated by a stem cell compartment. Cardiac niches contain CSCs and lineage-committed cells, which are connected to supporting cells represented by myocytes and fibroblasts. Connexins and cadherins form gap and adherens junctions at the interface of

CSCs-lineage-committed cells and supporting cells. The undifferentiated state of CSCs is coupled with the expression of alpha(4)-integrin, which colocalizes with the alpha(2)-chain of laminin and fibronectin. CSCs divide symmetrically and asymmetrically, but asymmetric division predominates, and the replicating CSC gives rise to one daughter CSC and one daughter committed cell. By this mechanism of growth kinetics, the pool of primitive CSCs is preserved, and a myocyte progeny is generated together with endothelial and smooth muscle cells. Thus, CSCs regulate myocyte turnover that is heterogeneous across the heart, faster at the apex and atria, and slower at the base-midregion of the ventricle.

Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system.

McKenzie IA, et al. J Neurosci. 2006 Jun 14;26(24):6651-60.

Abstract: Although neural stem cells hold considerable promise for treatment of the injured or degenerating nervous system, their current human sources are embryonic stem cells and fetally derived neural tissue. Here, we asked whether rodent and human skin-derived precursors (SKPs), neural crest-related precursors found in neonatal dermis, represent a source of functional, myelinating Schwann cells. Specifically, cultured SKPs responded to neural crest cues such as neuregulins to generate Schwann cells, and these Schwann cells proliferated and induced myelin proteins when in contact with sensory neuron axons in culture. Similar results were obtained in vivo; 6 weeks after transplantation of naive SKPs or SKP-derived Schwann cells into the injured peripheral nerve of wild-type or shiverer mutant mice (which are genetically deficient in myelin basic protein), the majority of SKP-derived cells had associated with and myelinated axons. Naive rodent or human SKPs also generated Schwann cells that myelinated CNS axons when transplanted into the dysmyelinated brain of neonatal shiverer mice. Thus, neonatal SKPs generate functional neural progeny in response to appropriate neural crest cues and, in so doing, provide a highly accessible source of myelinating cells for treatment of nervous system injury, congenital leukodystrophies, and dysmyelinating disorders.

Angiogenesis facilitated by autologous whole bone marrow stem cell transplantation for Buerger's disease.

Kim DI, et al. Stem Cells. 2006 May;24(5):1194-200. Epub 2006 Jan 26.

Abstract: We hypothesized that angiogenesis can be triggered by autologous whole bone marrow stem cell transplantation. Twenty-seven patients (34 lower limbs) with Buerger's disease, who were not candidates for surgical revascularization or radiologic intervention, were enrolled in this study. Six sites of the tibia bone were fenestrated using a 2.5-mm-diameter screw under fluoroscopic guidance. Clinical status and outcome were determined using the "Recommended Standards for Reports." To mobilize endothelial progenitor cells (EPCs) from bone marrow, recombinant human granulocyte colony-stimulating factor (r-HuG-CSF) was injected subcutaneously as a dose of 75 microg, preoperatively. During the follow-up period (19.1 +/- 3.5 months), one limb showed a markedly improved outcome (+3), and 26 limbs showed a moderately improved outcome (+2). Thirteen limbs among 17 limbs with nonhealing ulcers healed. Postoperative angiograms were obtained for 22 limbs. Two limbs showed marked (+3), five limbs moderate (+2), and nine limbs slight (+1) collateral development. However, six limbs showed no collateral development (0). Peripheral blood and bone marrow samples were analyzed for CD34 and CD133 molecules to enumerate potential EPCs, and EPC numbers were found to be increased in peripheral blood and in bone marrow after r-HuG-CSF injection. In conclusion, the transplantation of autologous whole BMCs by fenestration of the tibia bone represents a simple, safe, and effective means of inducing therapeutic angiogenesis in patients with Buerger's disease.

Transplantation of human hematopoietic stem cells into ischemic and growing kidneys suggests a role in vasculogenesis but not tubulogenesis.

Dekel B, et al. Stem Cells. 2006 May;24(5):1185-93. Epub 2006 Jan 12.

Abstract: Transplantation of murine bone marrow-derived stem cells has been reported recently to promote regeneration of the injured kidney. We investigated the potential of human adult CD34(+) progenitor cells to undergo renal differentiation once xenotransplanted into ischemic and developing kidneys. Immunostaining with human-specific antibodies for tubular cells (broad-spectrum cytokeratin), endothelial cells (CD31, PECAM), stromal cells (vimentin), and hematopoietic cells (pan-leukocyte CD45) demonstrated that although kidney ischemia enhanced engraftment of human cells, they were mostly hematopoietic cells (CD45(+)) residing in the interstitial spaces. Few other engrafted cells demonstrated an endothelial phenotype (human CD31(+)in morphologically appearing peritubular capillaries), but no evidence of tubular or stromal cells of human origin was found. Upregulation of SDF1 and HIF1 transcript levels in the ischemic kidneys might explain the diffuse engraftment of CD45(+)cells following injury. Similarly, when embryonic kidneys rudiments were co-transplanted with human CD34(+)cells in mice, we found both human CD45(+)and CD31(+)cells in the periphery of the developing renal grafts, whereas parenchymal elements failed to stain. In addition, human CD34(+)cells had no effect on kidney growth and differentiation. This first demonstration of human CD34(+)stem cell transplantation into injured and developing kidneys indicates that these cells do not readily acquire a tubular phenotype and are restricted mainly to hematopoietic and, to a lesser extent, to endothelial lineages. Efforts should be made to identify additional stem cell sources applicable for kidney growth and regeneration.

Bone marrow-derived cells home to and regenerate retinal pigment epithelium after injury.

Harris JR, et al. Invest Ophthalmol Vis Sci. 2006 May;47(5):2108-13.

PURPOSE: To determine whether hematopoietic stem and progenitor cells (HSCs/HPCs) can home to and regenerate the retinal pigment epithelium (RPE) after induced injury.
METHODS: Enriched HSCs/HPCs from green fluorescent protein (gfp) transgenic mice were transplanted into irradiated recipient mice to track bone marrow-derived cells. Physical damage was induced by breaching Bruch's membrane and inducing vascular endothelial growth factor A (VEGFa) expression to promote neovascularization. RPE damage was also induced by sodium iodate injection (40 mg/kg) into wild-type or albino C57Bl/6 mice. Cell morphology, gfp expression, the presence of the Y chromosome, and the presence of melanosomes were used to determine whether the injured RPE was being repaired by the donor bone marrow.
RESULTS: Injury to the RPE recruits HSC/HPC-derived cells to incorporate into the RPE layer and differentiate into an RPE phenotype. A portion of the HSCs/HPCs adopt RPE morphology, express melanosomes, and integrate into the RPE without cell fusion.
CONCLUSIONS: HSCs/HPCs can migrate to the RPE layer after physical or chemical injury and regenerate a portion of the damaged cell layer.

Unilateral intraputaminal glial cell line-derived neurotrophic factor in patients with Parkinson disease: response to 1 year each of treatment and withdrawal.

Slevin JT, et al. Neurosurg Focus. 2006 May 15;20(5):E1.

OBJECT: Glial cell line-derived neurotrophic factor (GDNF) infused unilaterally into the putamen for 6 months was previously shown to improve motor functions and quality of life measures significantly in 10 patients with Parkinson disease (PD) in a Phase I trial. In this study the authors report the safety and efficacy of continuous treatment for 1 year or more. After the trial was halted by the sponsor, the patients were monitored for an additional year to evaluate the effects of drug withdrawal.
METHODS: During the extended study, patients received unilateral intraputaminal infusion of 30 mg/day GDNF at a basal infusion rate supplemented with pulsed boluses every 6 hours at a convection-enhanced delivery rate to increase tissue penetration of the protein. When the study was stopped, the delivery system was reprogrammed to deliver sterile saline at the basal infusion rate of 2 ml/hour. The Unified PD Rating Scale (UPDRS) total scores after 1 year of therapy were improved by 42 and 38%, respectively, in the "off" and "on" states. Motor UPDRS scores were also improved: 45 and 39% in the off and on conditions, respectively. Benefits from treatment were lost by 9 to 12 months after GDNF infusion was halted. At that time, the patients had returned to their baseline UPDRS scores and required higher levels of conventional antiparkinsonian drugs to treat symptoms. After 11 months of treatment, the delivery system had to be removed in one patient because of the risk of infection. In seven patients antibodies to GDNF developed, with no evidence of clinical sequelae. There was also no evidence of GDNF-induced cerebellar toxicity, as evaluated using magnetic resonance imaging analysis and clinical testing.
CONCLUSIONS: Unilateral administration of GDNF results in significant, sustained bilateral benefits. These improvements are lost within 9 months after drug withdrawal. Safety concerns with GDNF therapy can be closely monitored and managed.

Targeting of melanoma brain metastases using engineered neural stem/progenitor cells.

Aboody KS, et al. Neuro-oncol. 2006 Apr;8(2):119-26. Epub 2006 Mar 8.

Abstract: Brain metastases are an increasingly frequent and serious clinical problem for cancer patients, especially those with advanced melanoma. Given the extensive tropism of neural stem/progenitor cells (NSPCs) for pathological areas in the central nervous system, we expanded investigations to determine whether NSPCs could also target multiple sites of brain metastases in a syngeneic experimental melanoma model. Using cytosine deaminase-expressing NSPCs (CD-NSPCs) and systemic 5-fluorocytosine (5-FC) pro-drug administration, we explored their potential as a cell-based targeted drug delivery system to disseminated brain metastases. Our results indicate a strong tropism of NSPCs for intracerebral melanoma metastases. Furthermore, in our therapeutic paradigm, animals with established melanoma brain metastasis received intracranial implantation of CD-NSPCs followed by systemic 5-FC treatment, resulting in a significant (71%) reduction in tumor burden. These data provide proof of principle for the use of NSPCs for targeted delivery of therapeutic gene products to melanoma brain metastases.

Tissue-engineered autologous bladders for patients needing cystoplasty.

Atala A, et al. Lancet. 2006 Apr 15;367(9518):1241-6.

BACKGROUND: Patients with end-stage bladder disease can be treated with cystoplasty using gastrointestinal segments. The presence of such segments in the urinary tract has been associated with many complications. We explored an alternative approach using autologous engineered bladder tissues for reconstruction.
METHODS: Seven patients with myelomeningocele, aged 4-19 years, with high-pressure or poorly compliant bladders, were identified as candidates for cystoplasty. A bladder biopsy was obtained from each patient. Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted either with or without an omental wrap. Serial urodynamics, cystograms, ultrasounds, bladder biopsies, and serum analyses were done.
RESULTS: Follow-up range was 22-61 months (mean 46 months). Post-operatively, the mean bladder leak point pressure decrease at capacity, and the volume and compliance increase was greatest in the composite engineered bladders with an omental wrap (56%,

1.58-fold, and 2.79-fold, respectively). Bowel function returned promptly after surgery. No metabolic consequences were noted, urinary calculi did not form, mucus production was normal, and renal function was preserved. The engineered bladder biopsies showed an adequate structural architecture and phenotype.
CONCLUSIONS: Engineered bladder tissues, created with autologous cells seeded on collagen-polyglycolic acid scaffolds, and wrapped in omentum after implantation, can be used in patients who need cystoplasty.

Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial.

Shyu WC, et al. CMAJ. 2006 Mar 28;174(7):927-33. Epub 2006 Mar 3.

BACKGROUND: Because granulocyte colony-stimulating factor (G-CSF) has anti-inflammatory and neuroprotective properties and is known to mobilize stem cells, it may be useful in the treatment of acute ischemic stroke. We sought to examine the feasibility, safety and efficacy of using G-CSF to treat acute stroke.
METHODS: We conducted a randomized, blinded controlled trial involving 10 patients with acute cerebral infarction (middle cerebral artery territory as documented by the admission MRI) who presented within 7 days of onset and whose scores on the National Institutes of Health Stroke Scale (NIHSS) were between 9 and 20. Patients were assigned to either G-CSF therapy or usual care. The G-CSF group (n = 7) received subcutaneous G-CSF injections (15 microg/kg per day) for 5 days. The primary outcome was percentage changes between baseline and 12-month follow-up in mean group scores on 4 clinical scales: the NIHSS, European Stroke Scale (ESS), ESS Motor Subscale (EMS) and Barthel Index (BI). We also assessed neurologic functioning using PET to measure cerebral uptake of fluorodeoxyglucose in the cortical areas surrounding the ischemic core.
RESULTS: All of the patients completed the 5-day course of treatment, and none were lost to follow-up. No severe adverse effects were seen in patients receiving G-CSF. There was greater improvement in neurologic functioning between baseline and 12-month follow-up in the G-CSF group than in the control group (NIHSS: 59% change in the mean G-CSF group score v. 36% in the mean control group score, ESS: 33% v. 20%, EMS: 106% v. 58%, BI: 120% v. 60%). Although at 12 months there was no difference between the 2 groups in cerebral uptake of fluorodeoxyglucose in the ischemic core, uptake in the area surrounding the core was significantly improved in the G-CSF group compared with the control group. There was positive correlation between metabolic activity and EMS score following simple linear correlation analysis.
INTERPRETATION: Our preliminary evidence suggests that using G-CSF as therapy for acute stroke is safe and feasible and leads to improved neurologic outcomes.

Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury.

Karimi-Abdolrezaee S, et al. J Neurosci. 2006 Mar 29;26(13):3377-89.

Abstract: Spinal cord injury (SCI) results in loss of oligodendrocytes demyelination of surviving axons and severe functional impairment. Spontaneous remyelination is limited. Thus, cell replacement therapy is an attractive approach for myelin repair. In this study, we transplanted adult brain-derived neural precursor cells (NPCs) isolated from yellow fluorescent protein-expressing transgenic mice into the injured spinal cord of adult rats at 2 and 8 weeks after injury, which represents the subacute and chronic phases of SCI. A combination of growth factors, the anti-inflammatory drug minocycline, and cyclosporine A immunosuppression was used to enhance the survival of transplanted adult NPCs. Our results show the presence of a substantial number of surviving NPCs in the injured spinal cord up to 10 weeks after transplantation at the subacute stage of SCI. In contrast, cell survival was poor after transplantation into chronic lesions. After subacute transplantation, grafted cells migrated >5 mm rostrally and caudally. The surviving NPCs integrated principally along white-matter tracts and displayed close contact with the host axons and glial cells. Approximately 50% of grafted cells formed either oligodendroglial precursor cells or mature oligodendrocytes. NPC-derived oligodendrocytes expressed myelin basic protein and ensheathed the axons. We also observed that injured rats receiving NPC transplants had improved functional recovery as assessed by the Basso, Beattie, and Bresnahan Locomotor Rating Scale and grid-walk and footprint analyses. Our data provide strong evidence in support of the feasibility of adult NPCs for cell-based remyelination after SCI.

Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease.

Weiss ML, et al. Stem Cells. 2006 Mar;24(3):781-92. Epub 2005 Oct 13.

Abstract: The umbilical cord contains an inexhaustible, noncontroversial source of stem cells for therapy. In the U.S., stem cells found in the umbilical cord are routinely placed into bio-hazardous waste after birth. Here, stem cells derived from human umbilical cord Wharton's Jelly, called umbilical cord matrix stem (UCMS) cells, are characterized. UCMS cells have several properties that make them of interest as a source of cells for therapeutic use. For example, they 1) can be isolated in large numbers, 2) are negative for CD34 and CD45, 3) grow robustly and can be frozen/thawed, 4) can be clonally expanded, and 5) can easily be engineered to express exogenous proteins. UCMS cells have genetic and surface markers of mesenchymal stem cells (positive for CD10, CD13, CD29, CD44, and CD90 and negative for CD14, CD33, CD56, CD31, CD34, CD45, and HLA-DR) and appear to be stable in terms of their surface marker expression in early passage (passages 4-8). Unlike traditional mesenchymal stem cells derived from adult bone marrow stromal cells, small populations of UCMS cells express endoglin (SH2, CD105) and CD49e at passage 8. UCMS cells express growth factors and angiogenic factors, suggesting that they may be used to treat neurodegenerative disease. To test the therapeutic value of UCMS cells, undifferentiated human UCMS cells were

transplanted into the brains of hemiparkinsonian rats that were not immune-suppressed. UCMS cells ameliorated apomorphine-induced rotations in the pilot test. UCMS cells transplanted into normal rats did not produce brain tumors, rotational behavior, or a frank host immune rejection response. In summary, the umbilical cord matrix appears to be a rich, noncontroversial, and inexhaustible source of primitive mesenchymal stem cells.

Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study.

Lima C, et al. J Spinal Cord Med. 2006;29(3):191-203; discussion 204-6.

BACKGROUND/OBJECTIVE: Olfactory mucosa is a readily accessible source of olfactory ensheathing and stem-like progenitor cells for neural repair. To determine the safety and feasibility of transplanting olfactory mucosa autografts into patients with traumatically injured spinal cords, a human pilot clinical study was conducted.
METHODS: Seven patients ranging from 18 to 32 years of age (American Spinal Injury Association [ASIA] class A) were treated at 6 months to 6.5 years after injury. Olfactory mucosa autografts were transplanted into lesions ranging from 1 to 6 cm that were present at C4-T6 neurological levels. Operations were performed from July 2001 through March 2003. Magnetic resonance imaging (MRI), electromyography (EMG), and ASIA neurological and otolaryngological evaluations were performed before and after surgery.
RESULTS: MRI studies revealed moderate to complete filling of the lesion sites. Two patients reported return of sensation in their bladders, and one of these patients regained voluntary contraction of anal sphincter. Two of the 7 ASIA A patients became ASIA C. Every patient had improvement in ASIA motor scores. The mean increase for the 3 subjects with tetraplegia in the upper extremities was 6.3 +/- 1.2 (SEM), and the mean increase for the 4 subjects with paraplegia in the lower extremities was 3.9 +/- 1.0. Among the patients who improved in their ASIA sensory neurological scores (all except one patient), the mean increase was 20.3 +/- 5.0 for light touch and 19.7 +/- 4.6 for pinprick. Most of the recovered sensation below the initial level of injury was impaired. Adverse events included sensory decrease in one patient that was most likely caused by difficulty in locating the lesion, and there were a few instances of transient pain that was relieved by medication. EMG revealed motor unit potential when the patient was asked to perform movement.
CONCLUSION: This study shows that olfactory mucosa autograft transplantation into the human injured spinal cord is feasible, relatively safe, and potentially beneficial. The procedure involves risks generally associated with any surgical procedure. Long-term patient monitoring is necessary to rule out any delayed side effects and assess any further improvements.

Induction of neuronal differentiation of adult human olfactory neuroepithelial-derived progenitors.

Zhang X, et al. Brain Res. 2006 Feb 16;1073-1074:109-19. Epub 2006 Feb 7.

Abstract: Neurosphere forming cells (NSFCs) have been established from cultures of adult olfactory neuroepithelium obtained from patients and cadavers as described previously. They remained undifferentiated in serum or defined media with or without neurotrophic factors. Many factors affect the differentiation of stem cells along a neuronal pathway. Retinoic acid (RA), forskolin (FN), and sonic hedgehog (Shh) have been reported to act as growth promoters during neurogenesis of embryonic CNS in vivo. The effect of RA, FN, and Shh on NSFCs' neuronal lineage restriction has not been described. The application of RA, FN, and Shh to NSFCs induced the expression of motoneuronal transcription factors, tyrosine hydroxylase, an indicator of dopamine production, and neurite formation. These studies further heighten the potential for using olfactory neuroepithelial progenitors for future autologous cell replacement strategies in neurodegenerative conditions and trauma as well as for use in diagnostic evaluation.

Role of transcription factors in motoneuron differentiation of adult human olfactory neuroepithelial-derived progenitors.

Zhang X, et al. Stem Cells. 2006 Feb;24(2):434-42. Epub 2005 Sep 1.

Abstract: Neurosphereforming cell (NSFC) lines have been established from cultures of human adult olfactory neuroepithelium. Few of these cells ever express mature neuronal or glial markers in minimal essential medium supplemented with 10% fetal bovine serum or defined medium. However, these neural progenitors have the potential to differentiate along glial or neuronal lineages. To evaluate the potential of NSFCs to form motoneurons, transcription factors Olig2, Ngn2, and HB9 were introduced into NSFCs to determine if their expression is sufficient for motoneuron specification and differentiation, as has been shown in the early development of the avian and murine central nervous systems in vivo. NSFCs transfected with Olig2, Ngn2, and HB9 alone exhibited no phenotypic lineage restriction. In contrast, simultaneous transfection of Ngn2 and HB9 cDNA increased the expression of Isl1/2, a motoneuron marker, when the cells were maintained in medium supplemented with retinoic acid, forskolin, and sonic hedgehog. Furthermore, a population of Olig2-expressing NSFCs also expressed Ngn2. Cotransfection of NSFCs with Olig2 and HB9, but not Olig2 and Ngn2, increased Isl1/2 expression. Coculture of NSFCs trans-fected with Ngn2-HB92 or Olig2 and HB9 with purified chicken skeletal muscle demonstrated frequent contacts that resembled neuromuscular junctions. These studies demonstrate that transcription factors governing the early development of chick and mouse motoneuron formation are able to drive human adult olfactory neuroepithelial progenitors to differentiate into motoneurons in vitro. Our long-term goal is to develop cell populations for future studies of the therapeutic utility of these olfactory-derived NSFCs for autologous cell replacement strategies for central nervous system trauma and neurodegenerative diseases.

Bone marrow production of lung cells: the impact of G-CSF, cardiotoxin, graded doses of irradiation, and subpopulation phenotype.

Aliotta JM, et al. Exp Hematol. 2006 Feb;34(2):230-41.

OBJECTIVE: Previous studies have demonstrated the production of various types of lung cells from marrow cells under diverse experimental conditions. Our aim was to identify some of the variables that influence conversion in the lung.
METHODS: In separate experiments, mice received various doses of total-body irradiation followed by transplantation with whole bone marrow or various subpopulations of marrow cells (Lin(-/+), c-kit(-/+), Sca-1(-/+)) from GFP(+) (C57BL/6-TgN[ACTbEGFP]1Osb) mice. Some were given intramuscular cardiotoxin and/or mobilized with granulocyte colony-stimulating factor (G-CSF).
RESULTS: The production of pulmonary epithelial cells from engrafted bone marrow was established utilizing green fluorescent protein (GFP) antibody labeling to rule out autofluorescence and deconvolution microscopy to establish the colocaliztion of GFP and cytokeratin and the absence of CD45 in lung samples after transplantation. More donor-derived lung cells (GFP(+)/CD45(-)) were seen with increasing doses of radiation (5.43% of all lung cells, 1200 cGy). In the 900-cGy group, 61.43% of GFP(+)/CD45(-) cells were also cytokeratin(+). Mobilization further increased GFP(+)/CD45(-) cells to 7.88% in radiation-injured mice. Up to 1.67% of lung cells were GFP(+)/CD45(-) in radiation-injured mice transplanted with Lin(-), c-kit(+), or Sca-1(+) marrow cells. Lin(+), c-kit(-), and Sca-1(-) subpopulations did not significantly engraft the lung.
CONCLUSIONS: We have established that marrow cells are capable of producing pulmonary epithelial cells and identified radiation dose and G-CSF mobilization as variables influencing the production of lung cells from marrow cells. Furthermore, the putative lung cell-producing marrow cell has the phenotype of a hematopoietic stem cell.

Results of the cord blood transplantation study (COBLT): outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with lysosomal and peroxisomal storage diseases.

Martin PL, et al. Biol Blood Marrow Transplant. 2006 Feb;12(2):184-94.

Abstract: The Cord Blood Transplantation Study (COBLT), sponsored by the National Heart, Lung, and Blood Institute, is a phase II multicenter study designed to evaluate the use of cord blood in allogeneic transplantation. In this report, we evaluated the outcomes of cord blood transplantation in 69 patients with lysosomal and peroxisomal storage diseases. Patients with mucopolysaccharidoses I to III, mucolipidoses (ML) II (n = 36), adrenoleukodystrophy (n = 8), metachromatic leukodystrophy (n = 6), Krabbe disease (n = 16), and Tay-Sachs disease (n = 3) were enrolled between August 1999 and June 2004. All patients received the same preparative regimen, graft-versus-host disease (GVHD) prophylaxis, and supportive care. End points included survival, engraftment, GVHD, and toxicity. Sixty-nine patients (64% men; 81% white) with a median age of 1.8 years underwent transplantation with a median cell dose of 8.7 x 10(7)/kg. One-year survival was 72% (95% confidence interval, 61%-83%). The cumulative incidence of neutrophil engraftment by day 42 was 78% (95% confidence interval, 67%-87%) at a median of 25 days. Grade II to IV acute GVHD occurred in 36% of patients. Cord blood donors are readily available for rapid transplantation. Cord blood transplantation should be considered as frontline therapy for young patients with lysosomal and peroxisomal storage diseases.

Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus.

Burt RK, et al. JAMA. 2006 Feb 1;295(5):527-35.

CONTEXT: Manifestations of systemic lupus erythematosus (SLE) may in most patients be ameliorated with medications that suppress the immune system. Nevertheless, there remains a subset of SLE patients for whom current strategies are insufficient to control disease.
OBJECTIVE: To assess the safety of intense immunosuppression and autologous hematopoietic stem cell support in patients with severe and treatment-refractory SLE.
DESIGN, SETTING, AND PARTICIPANTS: A single-arm trial of 50 patients with SLE refractory to standard immunosuppressive therapies and either organ- or life-threatening visceral involvement. Patients were enrolled from April 1997 through January 2005 in an autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT) study at a single US medical center.
INTERVENTIONS: Peripheral blood stem cells were mobilized with cyclophosphamide (2.0 g/m2) and granulocyte colony-stimulating factor (5 microg/kg per day), enriched ex vivo by CD34+ immunoselection, cryopreserved, and reinfused after treatment with cyclophosphamide (200 mg/kg) and equine antithymocyte globulin (90 mg/kg).
MAIN OUTCOME MEASURES: The primary end point was survival, both overall and disease-free. Secondary end points included SLE Disease Activity Index (SLEDAI), serology (antinuclear antibody [ANA] and anti-double-stranded (ds) DNA), complement C3 and C4, and changes in renal and pulmonary organ function assessed before treatment and at 6 months, 12 months, and then yearly for 5 years.
RESULTS: Fifty patients were enrolled and underwent stem cell mobilization. Two patients died after mobilization, one from disseminated mucormycosis and another from active lupus after postponing the transplantation for 4 months. Forty-eight patients underwent nonmyeloablative HSCT. Treatment-related mortality was 2% (1/50). By intention to treat, treatment-related mortality was 4% (2/50). With a mean follow-up of 29 months (range, 6 months to 7.5 years) for patients undergoing HSCT, overall 5-year survival was 84%, and probability of disease-free survival at 5 years following HSCT was 50%. Secondary analysis demonstrated stabilization of renal function and significant improvement in SLEDAI score, ANA, anti-ds DNA, complement, and carbon monoxide diffusion lung capacity adjusted for hemoglobin.
CONCLUSIONS: In treatment-refractory SLE, autologous nonmyeloablative HSCT results in amelioration of disease activity, improvement in serologic markers, and either stabilization or reversal of organ dysfunction. These data are nonrandomized and thus

preliminary, providing the foundation and justification for a definitive randomized trial. Clinical Trial Registration ClinicalTrials.gov Identifier: NCT00271934.

Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial.

Janssens S, et al. Lancet. 2006 Jan 14;367(9505):113-21.

BACKGROUND: The benefit of reperfusion therapies for ST-elevation acute myocardial infarction (STEMI) is limited by post-infarction left-ventricular (LV) dysfunction. Our aim was to investigate the effect of autologous bone marrow-derived stem cell (BMSC) transfer in the infarct-related artery on LV function and structure.
METHODS: We did a randomised, double-blind, placebo-controlled study in 67 patients from whom we harvested bone marrow 1 day after successful percutaneous coronary intervention for STEMI. We assigned patients optimum medical treatment and infusion of placebo (n=34) or BMSC (n=33). Our primary endpoint was the increase in LV ejection fraction and our secondary endpoints were change in infarct size and regional LV function at 4 months' follow-up, all assessed by MRI. We assessed changes in myocardial perfusion and oxidative metabolism with serial 1-[11C]acetate PET. Analyses were per protocol. This study is registered with , number NCT00264316.
FINDINGS: Mean global LV ejection fraction 4 days after percutaneous coronary intervention was 46.9% (SD 8.2) in controls and 48.5% (7.2) in BMSC patients, and increased after 4 months to 49.1% (10.7) and 51.8% (8.8; OR for treatment effect 1.036, 95% CI 0.961-1.118, p=0.36). Compared with placebo infusion, BMSC transfer was associated with a significant reduction in myocardial infarct size (BMSC treatment effect 28%, p=0.036) and a better recovery of regional systolic function. Myocardial perfusion and metabolism increased similarly in both groups. We noted no complications associated with BMSC transfer and all but one patient in the BMSC group completed the 4 months' follow-up.
INTERPRETATION: Intracoronary transfer of autologous bone marrow cells within 24 h of optimum reperfusion therapy does not augment recovery of global LV function after myocardial infarction, but could favourably affect infarct remodelling.

2005

Clonal analysis of adult human olfactory neurosphere forming cells.

Othman M, et al. Biotech Histochem. 2005 Sep-Dec;80(5-6):189-200.

Abstract: Olfactory neuroepithelium (ONe) is unique because it contains progenitor cells capable of mitotic division that replace damaged or lost neurons throughout life. We isolated populations of ONe progenitors from adult cadavers and patients undergoing nasal sinus surgery that were heterogeneous and consisted of neuronal and glial progenitors. Progenitor lines have been obtained from these cultures that continue to divide and form nestin positive neurospheres. In the present study, we used clonal and population analyses to probe the self-renewal and multipotency of the neurosphere forming cells (NSFCs). NSFCs plated at the single cell level produced additional neurospheres; dissociation of these spheres resulted in mitotically active cells that continued to divide and produce spheres as long as they were subcultured. The mitotic activity of clonal NSFCs was assessed using bromodeoxyuridine (BrdU) incorporation. Lineage restriction of the clonal cultures was determined using a variety of antibodies that were characteristic of different levels of neuronal commitment: ss-tubulin isotype III, neural cell adhesion molecule (NCAM) and microtubule associated protein (MAP2), or glial restriction: astrocytes, glial fibrillary acidic protein (GFAP); and oligodendrocytes, galactocerebroside (GalC). Furthermore, nestin expression, a marker indicative of progenitor nature, decreased in defined medium compared to serum-containing medium. Therefore, adult human ONe-derived neural progenitors retain their capacity for self-renewal, can be clonally expanded, and offer multipotent lineage restriction. Therefore, they are a unique source of progenitors for future cell replacement strategies in the treatment of neurotrauma and neurodegenerative diseases.

Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms

Florian Togel, et al. Am J Physiol Renal Physiol 2005 Jul;289(1):F31-42. Epub 2005 Feb 15.

Abstract: Severe acute renal failure (ARF) remains a common, largely treatment-resistant clinical problem with disturbingly high mortality rates. Therefore, we tested whether administration of multipotent mesenchymal stem cells (MSC) to anesthetized rats with ischemia-reperfusion-induced ARF (40-min bilateral renal pedicle clamping) could improve the outcome through amelioration of inflammatory, vascular, and apoptotic/necrotic manifestations of ischemic kidney injury. Accordingly, intracarotid administration of MSC (approximately 10(6)/animal) either immediately or 24 h after renal ischemia resulted in significantly improved renal function, higher proliferative and lower apoptotic indexes, as well as lower renal injury and unchanged leukocyte infiltration scores. Such renoprotection was not obtained with syngeneic fibroblasts. Using in vivo two-photon laser confocal microscopy, fluorescence-labeled MSC were detected early after injection in glomeruli, and low numbers attached at microvasculature sites. However, within 3 days of administration, none of the administered MSC had differentiated into a tubular or endothelial cell phenotype. At 24 h after injury, expression of proinflammatory cytokines IL-1beta, TNF-alpha, IFN-gamma, and inducible nitric oxide synthase was significantly reduced and that of anti-inflammatory IL-10 and bFGF, TGF-alpha, and Bcl-2 was highly upregulated in treated kidneys. We conclude that the early, highly significant renoprotection obtained with MSC is of considerable therapeutic promise for the cell-based management of clinical ARF. The beneficial effects of MSC are primarily mediated via complex paracrine actions and not by their differentiation into

target cells, which, as such, appears to be a more protracted response that may become important in late-stage organ repair.

Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery.

Xiao M, et al. Exp Neurol. 2005 Jul;194(1):12-30.

Abstract: Previously, our lab reported the isolation of patient-specific neurosphere-forming progenitor lines from human adult olfactory epithelium from cadavers as well as patients undergoing nasal sinus surgery. RT-PCR and ELISA demonstrated that the neurosphere-forming cells (NSFCs) produced BDNF. Since rubrospinal tract (RST) neurons have been shown to respond to exogenous BNDF, it was hypothesized that if the NSFCs remained viable following engraftment into traumatized spinal cord, they would rescue axotomized RS neurons from retrograde cell atrophy and promote functional recovery. One week after a partial cervical hemisection, GFP-labeled NSFCs suspended in Matrigel matrix or Matrigel matrix alone was injected into the lesion site. GFP-labeled cells survived up to 12 weeks in the lesion cavity or migrated within the ipsilateral white matter; the apparent number and mean somal area of fluorogold (FG)-labeled axotomized RST neurons were greater in the NSFC-engrafted rats than in lesion controls. Twelve weeks after engraftment, retrograde tracing with FG revealed that some RST neurons regenerated axons 4-5 segments caudal to the engraftment site; anterograde tracing with biotinylated dextran amine confirmed regeneration of RST axons through the transplants within the white matter for 3-6 segments caudal to the grafts. A few RST axons terminated in gray matter close to motoneurons. Matrix alone did not elicit regeneration. Behavioral analysis revealed that NSFC-engrafted rats displayed better performance during spontaneous vertical exploration and horizontal rope walking than lesion Matrigel only controls 11 weeks post transplantation. These results emphasize the unique potential of human olfactory neuroepithelial-derived progenitors as an autologous source of stem cells for spinal cord repair.

Generation of islet-like hormone-producing cells in vitro from adult human pancreas.

Atouf F, et al. Cell Transplant. 2005;14(10):735-48.

Abstract: Transplantation of pancreatic islets can provide long-lasting insulin independence for diabetic patients, but the current islet supply is limited. Here we describe a new in vitro system that utilizes adult human pancreatic islet-enriched fractions to generate hormone-producing cells over 3-4 weeks of culture. By labeling proliferating cells with a retrovirus-expressing green fluorescent protein, we show that in this system hormone-producing cells are generated de novo. These hormone-producing cells aggregate to form islet-like cell clusters. The cell clusters, when tested in vitro, release insulin in response to glucose and other secretagogues. After transplantation into immunodeficient, nondiabetic mice, the islet-like cell clusters survive and release human insulin. We propose that this system will be useful as an experimental tool for investigating mechanisms for generating new islet cells from the postnatal pancreas, and for designing strategies to generate physiologically competent pancreatic islet cells ex vivo.

Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas.

Nakamizo A, et al. Cancer Res. 2005 Apr 15;65(8):3307-18.

Abstract: The poor survival of patients with human malignant gliomas relates partly to the inability to deliver therapeutic agents to the tumor. Because it has been suggested that circulating bone marrow-derived stem cells can be recruited into solid organs in response to tissue stresses, we hypothesized that human bone marrow-derived mesenchymal stem cells (hMSC) may have a tropism for brain tumors and thus could be used as delivery vehicles for glioma therapy. To test this, we isolated hMSCs from bone marrow of normal volunteers, fluorescently labeled the cells, and injected them into the carotid artery of mice bearing human glioma intracranial xenografts (U87, U251, and LN229). hMSCs were seen exclusively within the brain tumors regardless of whether the cells were injected into the ipsilateral or contralateral carotid artery. In contrast, intracarotid injections of fibroblasts or U87 glioma cells resulted in widespread distribution of delivered cells without tumor specificity. To assess the potential of hMSCs to track human gliomas, we injected hMSCs directly into the cerebral hemisphere opposite an established human glioma and showed that the hMSCs were capable of migrating into the xenograft in vivo. Likewise, in vitro Matrigel invasion assays showed that conditioned medium from gliomas, but not from fibroblasts or astrocytes, supported the migration of hMSCs and that platelet-derived growth factor, epidermal growth factor, or stromal cell-derived factor-1alpha, but not basic fibroblast growth factor or vascular endothelial growth factor, enhanced hMSC migration. To test the potential of hMSCs to deliver a therapeutic agent, hMSCs were engineered to release IFN-beta (hMSC-IFN-beta). In vitro coculture and Transwell experiments showed the efficacy of hMSC-IFN-beta against human gliomas. In vivo experiments showed that treatment of human U87 intracranial glioma xenografts with hMSC-IFN-beta significantly increase animal survival compared with controls ($P < 0.05$). We conclude that hMSCs can integrate into human gliomas after intravascular or local delivery, that this engraftment may be mediated by growth factors, and that this tropism of hMSCs for human gliomas can be exploited to therapeutic advantage.

Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI).

Ince H, et al. Circulation. 2005 Nov 15;112(20):3097-106. Epub 2005 Nov 7.

BACKGROUND: Considering experimental evidence that stem cells enhance myocardial regeneration and granulocyte colony-stimulating factor (G-CSF) mediates mobilization of CD34+ mononuclear blood stem cells (MNCCD34+), we tested the impact of G-CSF integrated into primary percutaneous coronary intervention (PCI) management of acute myocardial infarction in man.
METHODS AND RESULTS: Fifty consecutive patients with ST-segment elevation myocardial infarction were subjected to primary PCI stenting with abciximab and followed up for 6 months; 89+/-35 minutes after successful PCI, 25 patients were randomly assigned in this pilot study (PROBE design) to receive subcutaneous G-CSF at 10 microg/kg body weight for 6 days in addition to standard care, including aspirin, clopidogrel, an ACE inhibitor, beta-blocking agents, and statins. By use of CellQuest software on peripheral blood samples incubated with CD45 and CD34, mobilized MNCCD34+ were quantified on a daily basis. With homogeneous demographics and clinical and infarct-related characteristics, G-CSF stimulation led to mobilization of MNCCD34+ to between 3.17+/-2.93 MNCCD34+/microL at baseline and 64.55+/-37.11 MNCCD34+/microL on day 6 (P<0.001 versus control); there was no indication of leukocytoclastic effects, significant pain, impaired rheology, inflammatory reactions, or accelerated restenosis at 6 months. Within 35 days, G-CSF and MNCCD34+ liberation led to enhanced resting wall thickening in the infarct zone of between 0.29+/-0.22 and 0.99+/-0.32 mm versus 0.49+/-0.29 mm in control subjects (P<0.001); under inotropic challenge with dobutamine (10 microg.kg(-1).min(-1)), wall motion score index showed improvement from 1.66+/-0.23 to 1.41+/-0.21 (P<0.004 versus control) and to 1.35+/-0.24 after 4 months (P<0.001 versus control), respectively, coupled with sustained recovery of wall thickening to 1.24+/-0.31 mm (P<0.001 versus control) at 4 months. Accordingly, resting wall motion score index improved with G-CSF to 1.41+/-0.25 (P<0.001 versus control), left ventricular end-diastolic diameter to 55+/-5 mm (P<0.002 versus control), and ejection fraction to 54+/-8% (P<0.001 versus control) after 4 months. Morphological and functional improvement with G-CSF was corroborated by enhanced metabolic activity and 18F-deoxyglucose uptake in the infarct zone (P<0.001 versus control).
CONCLUSIONS: G-CSF and mobilization of MNC(CD34+) after reperfusion of infarcted myocardium may offer a pragmatic strategy for preservation of myocardium and prevention of remodeling without evidence of aggravated restenosis.

Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor.

Slevin JT, et al. J Neurosurg. 2005 Feb;102(2):216-22.

OBJECT: Glial cell line-derived neurotrophic factor (GDNF) has demonstrated significant antiparkinsonian actions in several animal models and in a recent pilot study in England in which four of five patients received bilateral putaminal delivery. In the present study the authors report on a 6-month unilateral intraputaminal GDNF infusion in 10 patients with advanced Parkinson disease (PD).
METHODS: Patients with PD in a functionally defined on and off state were evaluated 1 week before and 1 and 4 weeks after intraputaminal catheter implantation in the side contralateral to the most affected side. Each patient was placed on a dose-escalation regimen of GDNF: 3, 10, and 30 microg/day at successive 8-week intervals, followed by a 1-month wash-out period. The Unified Parkinson's Disease Rating Scale (UPDRS) total scores in the on and off states significantly improved 34 and 33%, respectively, at 24 weeks compared with baseline scores (95% confidence interval [CI] 18-47% for off scores and 16-51% for on scores). In addition, UPDRS motor scores in both the on and off states significantly improved by 30% at 24 weeks compared with baseline scores (95% CI 15-48% for off scores and 5-61% for on scores). Improvements occurred bilaterally, as measured by balance and gait and increased speed of hand movements. All significant improvements of motor function continued through the wash-out period. The only observed side effects were transient Lhermitte symptoms in two patients.
CONCLUSIONS: Analysis of the data in this open-label study demonstrates the safety and potential efficacy of unilateral intraputaminal GDNF infusion. Unilateral administration of the protein resulted in significant, sustained bilateral effects.

Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study.

Strauer BE, et al. J Am Coll Cardiol. 2005 Nov 1;46(9):1651-8.

OBJECTIVES: Stem cell therapy may be useful in chronic myocardial infarction (MI); this is conceivable, but not yet demonstrated in humans.
BACKGROUND: After acute MI, bone marrow-derived cells improve cardiac function.
METHODS: We treated 18 consecutive patients with chronic MI (5 months to 8.5 years old) by the intracoronary transplantation of autologous bone marrow mononuclear cells and compared them with a representative control group without cell therapy.
RESULTS: After three months, in the transplantation group, infarct size was reduced by 30% and global left ventricular ejection fraction (+15%) and infarction wall movement velocity (+57%) increased significantly, whereas in the control group no significant changes were observed in infarct size, left ventricular ejection fraction, or wall movement velocity of infarcted area. Percutaneous transluminal coronary angioplasty alone had no effect on left ventricular function. After bone marrow cell transplantation, there was an improvement of maximum oxygen uptake (VO2max, +11%) and of regional 18F-fluor-desoxy-glucose uptake into infarct tissue (+15%).
CONCLUSIONS: These results demonstrate that functional and metabolic regeneration of infarcted and chronically avital tissue can be realized in humans by bone marrow mononuclear cell transplantation.

Surgical ventricular remodeling for patients with clinically advanced congestive heart failure and severe left ventricular dysfunction.

Patel ND, et al. J Heart Lung Transplant. 2005 Dec;24(12):2202-10. Epub 2005 Sep 15.

BACKGROUND: Surgical ventricular remodeling (SVR) is an accepted therapy for post-infarction ventricular remodeling. Current literature on SVR outcomes has focused on heterogeneous populations with regard to left ventricular function and New York Heart Association (NYHA) class. We assessed outcomes after SVR in patients with advanced congestive heart failure (CHF) (NYHA Class III/IV) and a pre-operative ejection fraction (EF) < or =20%.
METHODS: Data were analyzed for 51 consecutive SVR patients from January 2002 to June 2004. Cardiac catheterization, echocardiography and magnetic resonance imaging (MRI) identified 62.7% (32 of 51) of patients with an EF < or =20%, with the majority having an EF < or =15% (65.6%; 21 of 32). Cox regression analysis was performed to determine predictors of mortality in patients with an EF < or =20%. Follow-up was 100% (32 of 32) complete.
RESULTS: Mean age was 61.9 +/- 10.3 (range 40 to 80) years with a male:female ratio of 27:5. Operative mortality was 6.3% (2 of 32). Twenty-two percent (7 of 32) had concomitant mitral valve procedures. Follow-up demonstrated a statistically significant improvement in left ventricular volumes and EF in survivors. Cox regression analysis identified the following to be significant predictors of mortality: pre-operative left ventricular end-systolic volume index >130 ml/m2; pre-operative diabetes; and intra-aortic balloon pump usage. Pre-operatively, all patients (32 of 32) were categorized as NYHA Class III/IV, with 69% (22 of 32) improving to NYHA Class I/II at follow-up (p < 0.01). Survival did not differ statistically between patients with an EF < or =20% and an EF >20% (n = 19).
CONCLUSIONS: Our results indicate that SVR improves left ventricular function and functional status for patients with advanced CHF and a pre-operative EF < or =20%. Therefore, SVR is a viable surgical alternative for patients with severe left ventricular dysfunction.

Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease.

Escolar ML, et al. N Engl J Med. 2005 May 19;352(20):2069-81.

BACKGROUND: Infantile Krabbe's disease produces progressive neurologic deterioration and death in early childhood. We hypothesized that transplantation of umbilical-cord blood from unrelated donors before the development of symptoms would favorably alter the natural history of the disease among newborns in whom the disease was diagnosed because of a family history. We compared the outcomes among these newborns with the outcomes among infants who underwent transplantation after the development of symptoms and with the outcomes in an untreated cohort of affected children.
METHODS: Eleven asymptomatic newborns (age range, 12 to 44 days) and 14 symptomatic infants (age range, 142 to 352 days) with infantile Krabbe's disease underwent transplantation of umbilical-cord blood from unrelated donors after myeloablative chemotherapy. Engraftment, survival, and neurodevelopmental function were evaluated longitudinally for four months to six years.
RESULTS: The rates of donor-cell engraftment and survival were 100 percent and 100 percent, respectively, among the asymptomatic newborns (median follow-up, 3.0 years) and 100 percent and 43 percent, respectively, among the symptomatic infants (median follow-up, 3.4 years). Surviving patients showed durable engraftment of donor-derived hematopoietic cells with restoration of normal blood galactocerebrosidase levels. Infants who underwent transplantation before the development of symptoms showed progressive central myelination and continued gains in developmental skills, and most had age-appropriate cognitive function and receptive language skills, but a few had mild-to-moderate delays in expressive language and mild-to-severe delays in gross motor function. Children who underwent transplantation after the onset of symptoms had minimal neurologic improvement.
CONCLUSIONS: Transplantation of umbilical-cord blood from unrelated donors in newborns with infantile Krabbe's disease favorably altered the natural history of the disease. Transplantation in babies after symptoms had developed did not result in substantive neurologic improvement. Copyright 2005 Massachusetts Medical Society.

Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells.

Sapir T, et al. Proc Natl Acad Sci U S A. 2005 May 31;102(22):7964-9. Epub 2005 May 17.

Abstract: Shortage in tissue availability from cadaver donors and the need for life-long immunosuppression severely restrict the large-scale application of cell-replacement therapy for diabetic patients. This study suggests the potential use of adult human liver as alternate tissue for autologous beta-cell-replacement therapy. By using pancreatic and duodenal homeobox gene 1 (PDX-1) and soluble factors, we induced a comprehensive developmental shift of adult human liver cells into functional insulin-producing cells. PDX-1-treated human liver cells express insulin, store it in defined granules, and secrete the hormone in a glucose-regulated manner. When transplanted under the renal capsule of diabetic, immunodeficient mice, the cells ameliorated hyperglycemia for prolonged periods of time. Inducing developmental redirection of adult liver offers the potential of a cell-replacement therapy for diabetics by allowing the patient to be the donor of his own insulin-producing tissue.

New Research On Adult Cells with Pluripotent Characteristics

The studies cited below are a sampling of the published and peer-reviewed material available on the subject. The list will be updated on a regular basis.

Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics

Zhao Y et al., Exp Cell Res. 2006 Aug 1;312(13):2454-64. Epub 2006 Apr 26.

Abstract: We identified stem cells from the umbilical cord blood, designated cord blood-stem cells (CB-SC). CB-SC displayed important embryonic stem (ES) cell characteristics including expression of ES-cell-specific molecular markers including transcription factors OCT-4 and Nanog, along with stage-specific embryonic antigen (SSEA)-3 and SSEA-4. CB-SC also expressed hematopoietic cell antigens including CD9, CD45 and CD117, but were negative for CD34. CB-SC displayed very low immunogenicity as indicated by expression of a very low level of major histocompatibility complex (MHC) antigens and failure to stimulate the proliferation of allogeneic lymphocytes. CB-SC could give rise to cells with endothelial-like and neuronal-like characteristics in vitro, as demonstrated by expression of lineage-associated markers. Notably, CB-SC could be stimulated to differentiate into functional insulin-producing cells in vivo and eliminated hyperglycemia after transplantation into a streptozotocin-induced diabetic mouse model. These findings may have significant potential to advance stem-cell-based therapeutics.

Identification of pulmonary Oct-4+ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection in vitro

Ling T-Y et al., Proc Natl Acad Sci U S A. 2006 Jun 20;103(25):9530-5. Epub 2006 Jun 13

Abstract: In this study, we report a serum-free culture system for primary neonatal pulmonary cells that can support the growth of octamer-binding transcription factor 4+ (Oct-4+) epithelial colonies with a surrounding mesenchymal stroma. In addition to Oct-4, these cells also express other stem cell markers such as stage-specific embryonic antigen 1 (SSEA-1), stem cell antigen 1 (Sca-1), and Clara cell secretion protein (CCSP) but not c-Kit, CD34, and p63, indicating that they represent a subpopulation of Clara cells that have been implicated as lung stem/progenitor cells in lung injury models. These colony cells can be kept for weeks in primary cultures and undergo terminal differentiation to alveolar type-2- and type-1-like pneumocytes sequentially when removed from the stroma. In addition, we have demonstrated the presence of Oct-4+ long-term BrdU label-retaining cells at the bronchoalveolar junction of neonatal lung, providing a link between the Oct-4+ cells in vivo and in vitro and strengthening their identity as putative neonatal lung stem/progenitor cells. Lastly, these Oct-4+ epithelial colony cells, which also express angiotensin-converting enzyme 2, are the target cells for severe acute respiratory syndrome coronavirus infection in primary cultures and support active virus replication leading to their own destruction. These observations imply the possible involvement of lung stem/progenitor cells, in addition to pneumocytes, in severe acute respiratory syndrome coronavirus infection, accounting for the continued deterioration of lung tissues and apparent loss of capacity for lung repair.

Derivation of male germ cells from bone marrow stem cells

Nayernial K et al., Lab Invest. 2006 Jul;86(7):654-63. Epub 2006 May 1

Abstract: Recent studies have demonstrated that somatic stem cells have a more flexible potential than expected, whether put into tissue or cultured under different conditions. Bone marrow (BM)-derived stem cells can transdifferentiate into multilineage cells, such as muscle of mesoderm, lung and liver of endoderm, and brain and skin of ectoderm origin. Here we show that BM stem cells are able to transdifferentiate into male germ cells. For derivation of male germ cells from adult BM stem (BMS) cells, we used the Stra8-enhanced green fluoresence protein (EGFP) transgenic mouse line expressing EGFP specifically in male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells, such as fragilis, stella, Rnf17, Mvh and Oct4; as well as molecular markers of spermatogonial stem cells and spermatogonia including Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90alpha, beta1- and alpha6-integrins. Our ability to derive male germ cells from BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine.

A population of very small embryonic-like (VSEL) CXCR4+SSEA-1+Oct-4+ stem cells identified in adult bone marrow

Kucia M et al., Leukemia. 2006 May;20(5):857-69

http://www.nature.com/leu/journal/v20/n5/abs/2404171a.html

Abstract: By employing multiparameter sorting, we identified in murine bone marrow (BM) a homogenous population of rare (approximately 0.02% of BMMNC) Sca-1(+)lin(-)CD45- cells that express by RQ-PCR and immunohistochemistry markers of pluripotent stem cells (PSC) such as SSEA-1, Oct-4, Nanog and Rex-1. The direct electronmicroscopical analysis revealed that these cells are small (approximately 2-4 microm), posses large nuclei surrounded by a narrow rim of cytoplasm, and contain open-type chromatin (euchromatin) that is typical for embryonic stem cells. In vitro cultures these cells are able to differentiate into all three germ-layer lineages. The number of these cells is highest in BM from young (approximately 1-month-old) mice and decreases with age. It is also significantly diminished in short living DBA/2J mice as compared to long living B6 animals. These cells in vitro respond strongly to SDF-1, HGF/SF and LIF and express CXCR4, c-met and LIF-R, respectively, and since they adhere to fibroblasts they may be coisolated with BM adherent cells. We hypothesize that this population of Sca-1(+)lin(-)CD45- very small embryonic-like (VSEL) stem cells is

deposited early during development in BM and could be a source of pluripotent stem cells for tissue/organ regeneration.

Pluripotency of spermatogonial stem cells from adult mouse testis

Guan K et al. , Nature. 2006 Apr 27;440(7088):1199-203. Epub 2006 Mar 24

Abstract: Embryonic germ cells as well as germline stem cells from neonatal mouse testis are pluripotent and have differentiation potential similar to embryonic stem cells, suggesting that the germline lineage may retain the ability to generate pluripotent cells. However, until now there has been no evidence for the pluripotency and plasticity of adult spermatogonial stem cells (SSCs), which are responsible for maintaining spermatogenesis throughout life in the male. Here we show the isolation of SSCs from adult mouse testis using genetic selection, with a success rate of 27%. These isolated SSCs respond to culture conditions and acquire embryonic stem cell properties. We name these cells multipotent adult germline stem cells (maGSCs). They are able to spontaneously differentiate into derivatives of the three embryonic germ layers in vitro and generate teratomas in immunodeficient mice. When injected into an early blastocyst, SSCs contribute to the development of various organs and show germline transmission. Thus, the capacity to form multipotent cells persists in adult mouse testis. Establishment of human maGSCs from testicular biopsies may allow individual cell-based therapy without the ethical and immunological problems associated with human embryonic stem cells. Furthermore, these cells may provide new opportunities to study genetic diseases in various cell lineages.

Expression of early transcription factors Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells

Carlin R et al. , Reprod Biol Endocrinol. 2006 Feb 6;4(1):8 [Epub ahead of print]

Abstract: Three transcription factors that are expressed at high levels in embryonic stem cells (ESCs) are Nanog, Oct-4 and Sox-2. These transcription factors regulate the expression of other genes during development and are found at high levels in the pluripotent cells of the inner cell mass. The downregulation of these three transcription factors correlates with the loss of pluripotency and self-renewal, and the beginning of subsequent differentiation steps. The roles of Nanog, Oct-4 and Sox-2 have not been fully elucidated. They are important in embryonic development and maintenance of pluripotency in ESCs. We studied the expression of these transcription factors in porcine umbilical cord (PUC) matrix cells. METHODS: Cells were isolated from Wharton's jelly of porcine umbilical cords (PUC) and histochemically assayed for the presence of alkaline phosphatase and the presence of Nanog, Oct-4 and Sox-2 mRNA and protein. PCR amplicons were sequenced and compared with known sequences. The synthesis of Oct-4 and Nanog protein was analyzed using immunocytochemistry. FACS analysis was utilized to evaluate Hoechst 33342 dye-stained cells. RESULTS: PUC isolates were maintained in culture and formed colonies that express alkaline phosphatase. FACS analysis revealed a side population of Hoechst dye-excluding cells, the Hoechst exclusion was verapamil sensitive. Quantitative and non-quantitative RT-PCR reactions revealed expression of Nanog, Oct-4 and Sox-2 in day 15 embryonic discs, PUC cell isolates and porcine fibroblasts. Immunocytochemical analysis detected Nanog immunoreactivity in PUC cell nuclei, and faint labeling in fibroblasts. Oct-4 immunoreactivity was detected in the nuclei of some PUC cells, but not in fibroblasts. CONCLUSIONS: Cells isolated from PUC matrix express three transcription factors found in pluripotent stem cell markers both at the mRNA and protein level. The presence of these transcription factors, along with the other characteristics of PUC cells such as their colony-forming ability, Hoechst dye-excluding side population and alkaline phosphatase expression, suggests that PUC cells have properties of primitive pluripotent stem cells. Furthermore, PUC cells are an easily and inexpensively obtained source of stem cells that are not hampered by the ethical or legal issues associated with ESCs. In addition, these cells can be cryogenically stored and expanded.

Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury

Xiao J et al., Stem Cells Dev. 2005 Dec;14(6):722-33

Abstract: Umbilical cord blood (UCB) is a rich source of hematopoetic stem cells (HSCs). We have isolated a novel cell line population of stem cells from human UCB that exhibit properties of self-renewal, but do not have cell-surface markers that are typically found on HSCs. Analysis of transcripts revealed that these cells express transcription factors Oct-4, Rex-1, and Sox-2 that are typically expressed by stem cells. We refer to these novel cells as nonhematopoietic umbilical cord blood stem cells (nh-UCBSCs). Previous studies have shown that the intravenous infusion of UCBCs can ameliorate neurological deficits arising from ischemic brain injury. The identity of the cells that mediate this restorative effect, however, has yet to be determined. We postulate that nh-UCBSCs may be a source of the UCB cells that can mediate these effects. To test this hypothesis, we intravenously injected one million human nh-UCBSCs into rats 48 h after transient unilateral middle cerebral artery occlusion. Animals in other experimental groups received either saline injections or injections of RN33b neural stem cells. Animals were tested for neurological function before the infusion of nh-UCBSCs and at various time periods afterwards using a battery of behavioral tests. In limb placement tests, animals treated with nh-UCBSCs exhibited mean scores that were significantly better than animals treated with RN33b neural stem cells or saline. Similarly, in stepping tests, nh-UCBSC-treated animals again exhibited significantly better performance than the other experimental groups of animals. Analysis of infarct volume revealed that ischemic animals treated with nh-UCBSCs exhibited a 50% reduction in lesion volume in comparison to saline-treated controls. Histological analysis of brain tissue further revealed the presence of cells that stained for human nuclei. Some human nuclei-positive cells were also co-labeled for NeuN, indicating that the transplanted cells

expressed markers of a neuronal phenotype. Cells expressing the human nuclei marker within the brain, however, were rather scant, suggesting that the restorative effects of nh-UCBSCs may be mediated by mechanisms other than cell replacement. To test this hypothesis, nh-UCBSCs were directly transplanted into the brain parenchyma after ischemic brain injury. Sprouting of nerve fibers from the nondamaged hemisphere into the ischemically damaged side of the brain was assessed by anterograde tracing using biotinylated dextran amine (BDA). Animals with nh-UCBSC transplants exhibited significantly greater densities of BDA-positive cells in the damaged side of the brain compared to animals with intraparenchymal saline injections. These results suggest that restorative effects observed with nh-UCBSC treatment following ischemic brain injury may be mediated by trophic actions that result in the reorganization of host nerve fiber connections within the injured brain.

Human fibroblast-derived cell lines have characteristics of embryonic stem cells and cells of neuro-ectodermal origin

Rieske P et al., Differentiation. 2005 Dec;73(9-10):474-83

Abstract: Fibroblasts are the most ubiquitous cells in complex organisms. They are the main cells of stromal tissue and play an important role in repair and healing of damaged organs. Here we report new data-initially serendipitous findings-that fibroblast-derived cell line (human fetal lung derived cells, MRC-5) have the morphology, growth rate and gene expression pattern characteristic of embryonic stem cells and cells of neuro-ectodermal origin. We have developed a serum-free culture system to maintain these cells in proliferative state. We discovered that, at proliferative state, these cells express transcription factors of pluripotent cells, OCT-3/4 and REX-1, and embryonic cell surface antigens SSEA-1, SSEA-3, and SSEA-4, as well as TRA-1-60 and TRA-1-81. In addition to embryonic cell markers, the fibroblasts expressed neuroectodermal genes: Musashi-1, nestin, medium neurofilament, and beta-III tubulin. RT-PCR data revealed that mesencephalic transcription factors, Nurr-1 and PTX-3, were also expressed in MRC-5 cells, and that these cells could be induced to express tyrosine hydroxylase (TH). Expression of TH followed down-regulation of genes associated with cell proliferation, OCT-3/4, REX-1, and beta-catenin. These data indicate that the cells commonly known as fibroblasts have some of the characteristics of stem cells, and can be induced to become neuroectodermal cells and perhaps even mature neurons.

Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity

Tondreau T et al., Stem Cells. 2005 Sep;23(8):1105-12. Epub 2005 Jun 13

Abstract: In this study, we used a common procedure to assess the potential of mobilized peripheral blood (MPB) and umbilical cord blood (UCB) as sources of mesenchymal stem cells (MSCs) in comparison with bone marrow (BM). We tested three methods: plastic adhesion supplemented with 5% of BM-MSC conditioned medium, unsupplemented plastic adhesion, and selection of CD133-positive cells. MSCs derived from MPB or UCB are identified by their positive expression of mesenchymal (SH2, SH3) and negative expression of hematopoietic markers (CD14, CD34, CD45, HLA-DR). We observed that the CD133-positive cell fraction contains more MSCs with high proliferative potential. Placed in appropriate conditions, these cells proved their capacity to differentiate into adipocytes, osteocytes, chondrocytes, and neuronal/glial cells. MPB- and UCB-MSCs express Oct4, a transcriptional binding factor present in undifferentiated cells with high proliferative capacity. The selection of CD133-positive cells enabled us to obtain a homogeneous population of MSCs from UCB and MPB. These sources may have a major clinical importance thanks to their easy accessibility.

Stem cell characteristics of amniotic epithelial cells

Miki T et al., Stem Cells. 2005 Nov-Dec;23(10):1549-59. Epub 2005 Aug 4

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

Production of stem cells with embryonic characteristics from human umbilical cord blood

McGuckin CP et al., Cell Prolif. 2005 Aug;38(4):245-55.

A**bstract:** When will embryonic stem cells reach the clinic? The answer is simple -- not soon! To produce large quantities of homogeneous tissue for transplantation, without feeder layers, and with the appropriate recipient's immunological phenotype, is a significant scientific hindrance, although adult stem (ADS) cells provide an alternative, more ethically acceptable, source. The annual global 100 million human birth rate proposes umbilical cord blood (UCB) as the largest untouched stem cell source, with advantages of naive immune status and relatively unshortened telomere length. Here, we report the world's first reproducible production of cells expressing embryonic stem

cell markers, - cord-blood-derived embryonic-like stem cells (CBEs). UCB, after elective birth by Caesarean section, has been separated by sequential immunomagnetic removal of nucleate granulocytes, erythrocytes and haemopoietic myeloid/lymphoid progenitors. After 7 days of high density culture in microflasks, (10(5) cells/ml, IMDM, FCS 10%, thrombopoietin 10 ng/ml, flt3-ligand 50 ng/ml, c-kit ligand 20 ng/ml). CBE colonies formed adherent to the substrata; these were maintained for 6 weeks, then were subcultured and continued for a minimum 13 weeks. CBEs were positive for TRA-1-60, TRA-1-81, SSEA-4, SSEA-3 and Oct-4, but not SSEA-1, indicative of restriction in the human stem cell compartment. The CBEs were also microgravity--bioreactor cultured with hepatocyte growth medium (IMDM, FCS 10%, HGF 20 ng/ml, bFGF 10 ng/ml, EGF 10 ng/ml, c-kit ligand 10 ng/ml). After 4 weeks the cells were found to express characteristic hepatic markers, cytokeratin-18, alpha-foetoprotein and albumin. Thus, such CBEs are a viable human alternative from embryonic stem cells for stem cell research, without ethical constraint and with potential for clinical applications.

Characterization of multipotent mesenchymal stem cells from the bone marrow of rhesus macaques

Izadpanah R et al., Stem Cells Dev. 2005 Aug;14(4):440-51

Abstract: The isolation and characterization of embryonic and adult stem cells from higher-order mammalian species will enhance the understanding of the biology and therapeutic application of stem cells. The aim of this study was to purify rhesus mesenchymal stem cells (MSCs) from adult bone marrow and to characterize functionally their abilities to differentiate along diverse lineages. Adherent cells from adult rhesus macaque bone marrow were characterized for their growth characteristics, lineage differentiation, cell-surface antigen expression, telomere length, chromosome content, and transcription factor gene expression. Rhesus bone marrow MSCs (BMSCs) are very heterogeneous, composed of primarily long, thin cells and some smaller, round cells. The cells are capable of differentiating along osteogenic, chondrogenic, and adipogenic lineages in vitro. The cell morphology and multipotential differentiation capabilities are maintained throughout extended culture. They express CD59, CD90 (Thy-1), CD105, and HLA-1 and were negative for hematopoietic markers such as CD3, CD4, CD8, CD11b, CD13, CD34, and platelet endothelial cell adhesion molecule-1 (CD31). BMSCs were also demonstrated to express the mRNA for important stem cell-related transcription factors such as Oct-4, Sox-2, Rex-1, and Nanog. Rhesus BMSCs have a normal chromosome content, and the shortening of telomeres is minimal during early passages. These data demonstrate that BMSCs isolated from rhesus macaques have a high degree of commonality with MSCs isolated from other species. Therefore, isolation of these cells provides an effective and convenient method for rapid expansion of pluripotent rhesus MSCs.

Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood

Johnson J et al., Cell. 2005 Jul 29;122(2):303-15

Abstract: It has been suggested that germline stem cells maintain oogenesis in postnatal mouse ovaries. Here we show that adult mouse ovaries rapidly generate hundreds of oocytes, despite a small premeiotic germ cell pool. In considering the possibility of an extragonadal source of germ cells, we show expression of germline markers in bone marrow (BM). Further, BM transplantation restores oocyte production in wild-type mice sterilized by chemotherapy, as well as in ataxia telangiectasia-mutated gene-deficient mice, which are otherwise incapable of making oocytes. Donor-derived oocytes are also observed in female mice following peripheral blood transplantation. Although the fertilizability and developmental competency of the BM and peripheral blood-derived oocytes remain to be established, their morphology, enclosure within follicles, and expression of germ-cell- and oocyte-specific markers collectively support that these cells are bona fide oocytes. These results identify BM as a potential source of germ cells that could sustain oocyte production in adulthood.

Multipotent stem cells from adult olfactory mucosa

Murrell W et al., Dev Dyn. 2005 Jun;233(2):496-515

Abstract: Multipotent stem cells are thought to be responsible for the generation of new neurons in the adult brain. Neurogenesis also occurs in an accessible part of the nervous system, the olfactory mucosa. We show here that cells from human olfactory mucosa generate neurospheres that are multipotent in vitro and when transplanted into the chicken embryo. Cloned neurosphere cells show this multipotency. Multipotency was evident without prior culture in vitro: cells dissociated from adult rat olfactory mucosa generate leukocytes when transplanted into bone marrow-irradiated hosts, and cells dissociated from adult mouse olfactory epithelium generated numerous cell types when transplanted into the chicken embryo. It is unlikely that these results can be attributed to hematopoietic precursor contamination or cell fusion. These results demonstrate the existence of a multipotent stem-like cell in the olfactory mucosa useful for autologous transplantation therapies and for cellular studies of disease. Copyright 2005 Wiley-Liss, Inc

Transplanted human bone marrow cells generate new brain cells

Crain BJ et al., Proc Natl Acad Sci U S A. 2003 Feb 4;100(3):1364-9. Epub 2003 Jan 21

Abstract: Adult bone marrow stem cells seem to differentiate into muscle, skin, liver, lung, and neuronal cells in rodents and have been shown to regenerate myocardium, hepatocytes, and skin and gastrointestinal epithelium in humans. Because we have demonstrated previously that transplanted bone marrow cells can enter the brain of mice and differentiate into neurons there, we decided to examine postmortem brain samples

from females who had received bone marrow transplants from male donors. The underlying diseases of the patients were lymphocytic leukemia and genetic deficiency of the immune system, and they survived between 1 and 9 months after transplant. We used a combination of immunocytochemistry (utilizing neuron-specific antibodies) and fluorescent in situ hybridization histochemistry to search for Y chromosome-positive cells. In all four patients studied we found cells containing Y chromosomes in several brain regions. Most of them were nonneuronal (endothelial cells and cells in the white matter), but neurons were certainly labeled, especially in the hippocampus and cerebral cortex. The youngest patient (2 years old), who also lived the longest time after transplantation, had the greatest number of donor-derived neurons (7 in 10,000). The distribution of the labeled cells was not homogeneous. There were clusters of Y-positive cells, suggesting that single progenitor cells underwent clonal expansion and differentiation. We conclude that adult human bone marrow cells can enter the brain and generate neurons just as rodent cells do. Perhaps this phenomenon could be exploited to prevent the development or progression of neurodegenerative diseases or to repair tissue damaged by infarction or trauma.

Clonal multilineage differentiation of murine common pluripotent stem cells isolated from skeletal muscle and adipose stromal cells

Case J et al., Ann N Y Acad Sci. 2005 Jun;1044:183-200

Abstract: Pluripotent stem cells (PSCs) with transdifferentiation capacity may provide useful therapeutic modalities in the areas of cellular restoration and regenerative medicine. The utility of PSCs depends on their ability to respond to different stimuli and to adapt to tissue-specific differentiation conditions. Given that a number of cells possessing characteristics of PSCs have been identified and isolated from several adult murine tissues, we hypothesized that a common PSC may exist in multiple murine tissues and that these cells may either reside permanently in specific sites or continue to circulate and colonize tissues as needed. Previous data from our laboratory suggest that PSCs exhibiting an immunophenotype of CD45(-)Sca-1(+)c-kit(-)Thy-1(+) can be isolated from multiple murine tissues and may represent putative common PSCs (CoPSCs). To investigate whether the multiple tissue differentiation potential observed with these cells resulted from the presence of different tissue-restricted progenitors within CD45(-)Sca-1(+)c-kit(-)Thy-1(+) cells or was the product of clonal differentiation of CoPSCs, clonality studies were performed. Single skeletal muscle (SM)-derived CoPSCs were expanded for 10 days, and progeny cells were split into three culture conditions designed to stimulate myogenic, adipogenic, and neurogenic differentiation. Analysis of 600 clones indicated that 2.16%, 0.83%, and 0.33% of the total number of plated single cells were capable of unipotent, bipotent, and tripotent differentiation, respectively, into combinations of myocytes, adipocytes, and neuronal cells. Given that SM-derived CoPSCs represent 4.78% of the total cells analyzed, tripotent CoPSCs made up 0.016% of the total muscle cells. Similar results were obtained in clonal analyses using adipose stromal cell (ASC)-derived CoPSCs, suggesting that both SM- and ASC-derived CoPSCs may be phenotypically and functionally identical. Taken together, these data demonstrate that a common PSC can be identified in different murine tissues and suggest that a small fraction of these cells are capable of clonal differentiation into multiple cell types.

Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction

Yoon Y-s et al., J Clin Invest. 2005 Feb;115(2):326-38

Abstract: We have identified a subpopulation of stem cells within adult human BM, isolated at the single-cell level, that self-renew without loss of multipotency for more than 140 population doublings and exhibit the capacity for differentiation into cells of all 3 germ layers. Based on surface marker expression, these clonally expanded human BM-derived multipotent stem cells (hBMSCs) do not appear to belong to any previously described BM-derived stem cell population. Intramyocardial transplantation of hBMSCs after myocardial infarction resulted in robust engraftment of transplanted cells, which exhibited colocalization with markers of cardiomyocyte (CMC), EC, and smooth muscle cell (SMC) identity, consistent with differentiation of hBMSCs into multiple lineages in vivo. Furthermore, upregulation of paracrine factors including angiogenic cytokines and antiapoptotic factors, and proliferation of host ECs and CMCs, were observed in the hBMSC-transplanted hearts. Coculture of hBMSCs with CMCs, ECs, or SMCs revealed that phenotypic changes of hBMSCs result from both differentiation and fusion. Collectively, the favorable effect of hBMSC transplantation after myocardial infarction appears to be due to augmentation of proliferation and preservation of host myocardial tissues as well as differentiation of hBMSCs for tissue regeneration and repair. To our knowledge, this is the first demonstration that a specific population of multipotent human BM-derived stem cells can induce both therapeutic neovascularization and endogenous and exogenous cardiomyogenesis.

Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation in vitro

Moriscot C et al., Stem Cells. 2005 Apr;23(4):594-603

Abstract: Multipotential stem cells can be selected from the bone marrow by plastic adhesion, expanded, and cultured. They are able to differentiate not only into multiple cell types, including cartilage, bone, adipose and fibrous tissues, and myelosupportive stroma, but also into mesodermal (endothelium), neuroectodermal, or endodermal (hepatocytes) lineages. Our goal was to characterize the multipotential capacities of human mesenchymal stem cells (hMSCs) and to evaluate their ability to differentiate into insulin-secreting cells in vitro. hMSCs were obtained from healthy donors, selected by plastic adhesion, and phenotyped by fluorescence-activated cell sorter and reverse

transcription-polymerase chain reaction analysis before and after infection with adenoviruses coding for mouse IPF1, HLXB9, and FOXA2 transcription factors involved early in the endocrine developmental pathway. We found that native hMSCs have a pluripotent phenotype (OCT4 expression and high telomere length) and constitutively express NKX6-1 at a low level but lack all other transcription factors implicated in beta-cell differentiation. In all hMSCs, we detected mRNA of cytokeratin 18 and 19, epithelial markers present in pancreatic ductal cells, whereas proconvertase 1/3 mRNA expression was detected only in some hMSCs. Ectopic expression of IPF1, HLXB9, and FOXA2 with or without islet coculture or islet-conditioned medium results in insulin gene expression. In conclusion, our results demonstrated that in vitro human bone marrow stem cells are able to differentiate into insulin-expressing cells by a mechanism involving several transcription factors of the beta-cell developmental pathway when cultured in an appropriate microenvironment.

A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential

Kögler G et al., J Exp Med. 2004 Jul 19;200(2):123-35

Abstract: Here a new, intrinsically pluripotent, CD45-negative population from human cord blood, termed unrestricted somatic stem cells (USSCs) is described. This rare population grows adherently and can be expanded to 10(15) cells without losing pluripotency. In vitro USSCs showed homogeneous differentiation into osteoblasts, chondroblasts, adipocytes, and hematopoietic and neural cells including astrocytes and neurons that express neurofilament, sodium channel protein, and various neurotransmitter phenotypes. Stereotactic implantation of USSCs into intact adult rat brain revealed that human Tau-positive cells persisted for up to 3 mo and showed migratory activity and a typical neuron-like morphology. In vivo differentiation of USSCs along mesodermal and endodermal pathways was demonstrated in animal models. Bony reconstitution was observed after transplantation of USSC-loaded calcium phosphate cylinders in nude rat femurs. Chondrogenesis occurred after transplanting cell-loaded gelfoam sponges into nude mice. Transplantation of USSCs in a noninjury model, the preimmune fetal sheep, resulted in up to 5% human hematopoietic engraftment. More than 20% albumin-producing human parenchymal hepatic cells with absence of cell fusion and substantial numbers of human cardiomyocytes in both atria and ventricles of the sheep heart were detected many months after USSC transplantation. No tumor formation was observed in any of these animals.

New Research on Alternative Sources of Pluripotent Stem Cells Since May 2005

The material below was presented to the Council at its November 2006 meeting by Richard Roblin, Ph.D., the Council's Scientific Director.

A. Pluripotent stem cells derived from Organismically Dead Embryos (Landry-Zucker proposal).

Council preliminary evaluations in 2005: Not yet tested, even in animals. Natural history studies proposed could be undertaken forthwith and in an ethical manner. “Ethically acceptable for basic investigation in humans”

Update: Recently demonstrated for “arrested” human embryos. Zhang, X., P. Stojkovic, et al., *Derivation of human embryonic stem cells from developing and arrested embryos,* Stem Cells Express, published online September 21, 2006.

Abstract: Human embryonic stem cells (hESC) hold huge promise in modern regenerative medicine, drug discovery, and as a model for studying early human development. However, usage of embryos and derivation of hESC for research and potential medical application has resulted in polarised ethical debates since the process involves destruction of viable developing human embryos. Here we describe that not only developing embryos (morulae and blastocysts) of both good and poor quality but also arrested embryos could be used for the derivation of hESC. Analysis of arrested embryos demonstrated that these embryos express pluripotency marker genes such OCT4, NANOG and REX1. Derived hESC lines also expressed specific pluripotency markers (TRA-1-60, TRA-1-81, SSEA4, alkaline phosphatase, OCT4, NANOG, TERT and REX1) and differentiated under in vitro and in vivo conditions into derivates of all three germ layers. All the new lines including line derived from late arrested embryo have normal karyotype. These results demonstrate that arrested embryos are additional valuable resources to surplus and donated developing embryos and should be used to study early human development or derive pluripotent hESC.

B. Pluripotent Stem Cells via Blastomere Extraction from Living Embryos

Council preliminary evaluations in 2005: Now technically feasible, though large ethical difficulties remain. Ethically unacceptable in humans.

Update: Demonstration that hESC cultures could be derived from single blastomeres, even though in these experiments, all the human embryos were destroyed. Klimanskya, I., Y. Chung, S. Becker, et al., *Human embryonic stem cell lines derived from single blastomeres,* Nature. 2006 Nov 23; 444 (7118): 481-5. Epub 2006 Aug 23.

Abstract: The derivation of human embryonic stem (hES) cells currently requires the destruction of ex utero embryos. A previous study in mice indicates that it might be possible to generate embryonic stem (ES) cells using a single-cell biopsy similar to that used in preimplantation genetic diagnosis (PGD), which does not interfere with the embryo's developmental potential. By growing the single blastomere overnight, the resulting cells could be used for both genetic testing and stem cell derivation without affecting the clinical outcome of the procedure. Here we report a series of ten separate experiments demonstrating that hES cells can be derived from single blastomeres. Nineteen ES-cell-like outgrowths and two stable hES cell lines were obtained. The latter hES cell lines maintained undifferentiated proliferation for more than eight months, and showed normal karyotype and expression of markers of pluripotency, including Oct-4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, nanog and alkaline phosphatase. These cells retained the potential to form derivatives of all three embryonic germ layers both in vitro and in teratomas. The ability to create new stem cell lines and therapies without destroying embryos would address the ethical concerns of many, and allow the generation of matched tissue for children and siblings born from transferred PGD embryos.

C. Pluripotent Stem Cells derived from Biological Artifacts

Council preliminary evaluations in 2005: Technically the most demanding, and ethically the most complex and puzzling. It would need to be carefully tested in animals before any thought of human trials could be countenanced. Not at this time ethically acceptable for trials with human material.

Update: One possible approach appears to work in a first test in the mouse. Meissner, A. and R. Jaenisch, *Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocyst,* Nature, 239: 212-5 (2006)

Abstract: The derivation of embryonic stem (ES) cells by nuclear transfer holds great promise for research and therapy but involves the destruction of cloned human blastocysts. Proof of principle experiments have shown that 'customized' ES cells derived by nuclear transfer (NT-ESCs) can be used to correct immunodeficiency in mice. Importantly, the feasibility of the approach has been demonstrated recently in humans, bringing the clinical application of NT-ESCs within reach. Altered nuclear transfer (ANT) has been proposed as a variation of nuclear transfer because it would create abnormal nuclear transfer blastocysts that are inherently unable to implant into the uterus but would be capable of generating customized ES cells. To assess the experimental validity of this concept we have used nuclear transfer to derive mouse blastocysts from donor fibroblasts that carried a short hairpin RNA construct targeting Cdx2. Cloned blastocysts were morphologically abnormal, lacked functional trophoblast and failed to implant into the uterus. However, they efficiently generated pluripotent embryonic stem cells when explanted into culture.

D. Pluripotent Stem Cells via Somatic Cell Dedifferentiation

Council preliminary evaluations in 2005: ethically the most unobjectionable, but for now scientifically and technologically uncertain. Ethically unproblematic and acceptable for use in humans.

Update: Adding four genetic factors to mouse fibroblasts changes them into pluripotent stem cells. Takahashi, K. and S. Yamanaka, *Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors* , Cell, 126: 1-14 (2006) [Note that what was produced in these experiments were "genetically engineered" mouse pluripotent stem cells, which may or may not be the functional equivalent of mouse ESCs isolated from the inner cell mass of early mouse embryos.]

Abstract: Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

Initial work with human materials using cell fusion rather than genetic factors: Cowan, C.A., et al., *Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells* , Science, 309: 1369-1373 (2005)

Also noteworthy is the following publication reporting isolation of rare cells with embryonic stem cell-like characteristics from the bone marrow of young mice .

Kucia, M., et al., *A population of very small embryonic-like (VESL) CDCR+ SSEA-1+ Oct-4+ stem cells identified in adult* [mouse] *bone marrow,* Leukemia, 20: 857-869 (2006). When I inquired of Professor Mariusz Ratajczak (University of Louisville) whether his lab or anyone else that he knew about had found similar cells in human material he replied:

"Recently we isolated a similar population of cells from the human cord blood".

Another recent publication points to the existence of "embryonic-like", multipotent stem cells in human cord blood.
Zhao, Y., et al., *Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics,* Experimental Cell Research, 312: 2454-2464 (2006)

Abstract: We identified stem cells from the umbilical cord blood, designated cord blood-stem cells (CB-SC). CB-SC displayed important embryonic stem (ES) cell characteristics including expression of ES-cell-specific molecular markers including transcription factors OCT-4 and Nanog, along with stage-specific embryonic antigen (SSEA)-3 and SSEA-4. CB-SC also expressed hematopoietic cell antigens including CD9, CD45 and CD117, but were negative for CD34. CB-SC displayed very low immunogenicity as indicated by expression of a very low level of major histocompatibility complex (MHC) antigens and failure to stimulate the proliferation of allogeneic lymphocytes. CB-SC could give rise to cells with endothelial-like and neuronal-like characteristics in vitro, as demonstrated by expression of lineage-associated markers. Notably, CB-SC could be stimulated to differentiate into functional insulin-producing cells in vivo and eliminated hyperglycemia after transplantation into a streptozotocin-induced diabetic mouse model. These findings may have significant potential to advance stem-cell-based therapeutics.

Thus, small numbers of multipotent, if not pluripotent, human stem cells may exist in a readily available, ethically unproblematic form without the need to do any exotic genetic engineering or cell biology.