Realistic Prospects for Stem Cell Therapeutics

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

Hematologists appreciate the value of cell therapy. Having exploited cells as medicines for the better part of a century, hematologists are familiar with the principles and pitfalls of cellular replacement. Transfusion of red cells, platelets, lymphocytes, and hematopoietic stem cells (HSCs) are routine, and infusion of dendritic cells, granulocytes, and various other blood or bone marrow components are under clinical development. Growth factors and biomodulators that act on the hematopoietic and immune systems are widely used agents and among the greatest successes of modern biotechnology. The study of HSCs and their regenerative and therapeutic potential has provided a paradigm for the investigation of alternative stem cells.

In this review, we will survey the latest developments in the study of both embryonic and adult (somatic) stem cells, with an emphasis on translational aspects. We will attempt to provide a balanced critique of the prospects for stem cell therapeutics.

I. Therapeutic Potential of Embryonic Stem Cells

Most of the enthusiasm surrounding embryonic stem (ES) cells owes directly to the perceived need for cell replacement therapy for a host of degenerative diseases. Indeed, disorders of organ failure are not reversible, and organ transplantation cannot meet the needs of an ever-aging population. Primary pump failure in the heart, alcoholic or viral liver failure, beta-cell deficient type 1 diabetes, and Parkinson's disease (PD) are frequently cited as examples of monocellular deficiency states that might be amenable to cell replacement strategies, if a suitable and inexhaustible cell source could be found. Human ES cells might represent such a source, but the over-riding challenge is to achieve efficient directed differentiation of ES cells into therapeutically relevant cells, followed by proof-of-principle for

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effective restoration of tissue function in animal models. Once success in these areas of cell engineering is achieved, many challenges will remain, especially the immune barrier to tissue transplantation. Current strategies for confronting these challenges are outlined below.

ES cells are derived from the inner cell mass of the preimplantation embryo. When placed in culture, ES cells proliferate indefinitely and yet retain their potential to form all of the tissues of the developing organism. Murine ES cells have been intensively studied for over 20 years, yet the first derivation of ES cells from the human embryo was only reported in 1998.[1] Although advances in ES cell biology have revolutionized the creation of mouse models of disease, generating and breeding mice is time-consuming and costly. For addressing questions in cell and developmental biology, ES cells represent an excellent in vitro model system.

To maintain their undifferentiated, pristine state, ES cells are typically grown on feeder cell layers of mouse embryonic fibroblasts (MEFs), and cultures are supplemented by the antidifferentiation cytokine leukemia inhibitory factor (LIF). When ES cells are removed from these conditions, they undergo spontaneous differentiation, and initiate the diverse programs of tissue and cell specification (**Figure 1**; see Appendix, page 597).[2] ES cells recapitulate many of the developmental programs of the early embryo in culture, including generation of cells from all three classical embryonic germ layers, ectoderm, mesoderm, and endoderm.

Because the mouse embryo remains microscopic and inaccessible during most of the first week of gestation, a time when many central developmental programs are laid down, in vitro differentiation of ES cells provides a tractable model system for investigating the genetic and cell biological regulation of early development. Moreover, genes can be altered in ES cells through ectopic transgene expression or homologous recombination (HR), a process whereby specific sites in the genome can be altered directly. HR can be designed to produce gene deletion, gene mutation, or gene substitution. The genetic programs responsible for directing the development of blood, neurons, hepatocytes, cardiomyocytes, and a host of other tissues have been extensively explored using these techniques in ES cells.

Although ES cells have been touted as an inexhaustible resource for cell replacement therapies, they have already proven to be highly valuable as a research and discovery tool. By analyzing the effect of targeted gene deletions on the formation of specific lineages of cells, ES cells provide a tool for validating potential therapeutic targets for small molecule drug development. ES cells are emerging as a platform technology around which chemical screens can be built, providing for identification of compounds that promote or block cell differentiation. Schultz and colleagues[3] recently performed a chemical screen to identify agents that induce neurogenesis in ES cells, thereby establishing the proof-of-principle for using stem cell differentiation in assays for drug discovery. Kamp and colleagues[4] have convincingly shown that human ES cells differentiate into a number of cardiomyocyte classes, including embryonic atrial, ventricular, and nodal subtypes, each faithfully recapitulating their respective electrophysiologic properties and pharmacologic responses. Gauging the effects of compounds on differentiation of specific cell populations from ES cells would provide a screen for potential drug toxicities prior to clinical development. Assembly of a genetically diverse bank of human ES cells, together with detailed knowledge of human genetic variation emerging from the international haplotype mapping project ("the hap map"), could translate into a discovery platform for pharmacogenomics.

The Pathophysiology of Stem Cell Diseases

Tissues that actively regenerate from stem cell pools in adults maintain the appropriate stem cell niche, providing the signals for stem cell self-renewal, survival, and differentiation. The hematopoietic system, skin, gut, islets, liver, and parts of the central nervous system (CNS) fall into this category. Diseases or conditions that deplete stem cell pools while leaving the niche intact would represent the lowest hurdle for stem cell–based therapeutics, and there is clear proof-of-principle for such treatments in clinical settings. The successful transplantation of bone marrow, skin, pancreatic islets, liver, and to a lesser extent fetal mesencephalic dopaminergic tissue provides important confirmation that stem cell replacement therapies are a viable goal and an opportunity, given the paucity of donor organs. In this regard, stem cell–based therapeutics seek to emulate the objectives of organ transplantation on a micro scale.

In many instances of disease, however, we have a relatively poor understanding of the pathophysiology of cell degeneration. This is particularly true for the neurodegenerative and autoimmune diseases, making it difficult to predict whether stem cell–based replacement therapies can be successful without first interrupting the systemic disease process. In cases where stem cell depletion is a consequence of cell-extrinsic forces like autoimmune attack, stem cell replacement must be accompanied by immune suppression, as in the case of the "Edmonton protocol" for the treatment of diabetes.[5] Pathology that injures the stem cell niche itself may not be amenable to therapy with stem cell replacement alone. This might explain the poor results of bone

marrow transplantation for myelofibrosis, and will represent a limitation to cell therapy for burns, hepatic cirrhosis, and certain forms of neural injury. Indeed, cranial irradiation damages the stem cell niche of the hippocampus through inflammation and distortion of the vascular-progenitor relationship such that, even in this otherwise neurogenic structure, neither endogenous nor transplanted stem cells can differentiate into replacement neurons.[6]

Embryonic Stem Cells as a Source of Neurons for Neurodegenerative Diseases

Despite the inadequate knowledge about disease etiology and pathogenesis, neurodegenerative diseases like PD, Alzheimer's, Huntington's, amyotrophic lateral sclerosis, stroke, and anoxic brain injury, as well as a host of lysosomal storage diseases with CNS pathology, represent poorly managed diseases that are worthy targets for cell replacement therapy. The hippocampus and the olfactory bulb maintain self-renewing populations of neural stem/progenitor cells, but there is scant evidence for cell renewal beyond these limited regions of the CNS. Given the likelihood that many classes of highly specialized neurons develop only during critical periods of embryogenesis, ES cells might in principle be directed to differentiate into specialized neuronal subtypes for use in cell replacement therapy. Several groups have reported success in differentiating specific neuronal subtypes from mouse and human ES cells, and some groups have reported positive data from transplantation of such cells into animal models of disease. Among the most compelling reports of directed ES cell differentiation have come from the laboratories of Jessell[7] and McKay.[8] Both groups have exploited knowledge of the morphogens and transcription factors that program neuronal development during embryogenesis, and recapitulated the timing and sequence of exposure to direct neuronal patterning during in vitro ES cell differentiation. First using retinoic acid to program ectodermal commitment, followed by exposure to the morphogen sonic hedgehog, which acts to "ventralize" neuronal subtypes, Jessell and colleagues showed that they could pattern the formation of spinal motor neurons that successfully engrafted the embryonic spinal cord of the chick, extended axons, and formed synapses with target muscles. McKay's group exploited the instructive effects of sonic hedgehog and FGF8 to drive commitment of ES cells to ventral midbrain fates and ultimately to tyrosine hydroxylase-positive dopaminergic neurons. These cells functioned after transplantation into a rodent model of Parkinson's disease. Isacson and colleagues[9] have likewise demonstrated improvement in a rodent model of Parkinson's from transplantation of undifferentiated ES cells into the striatum, suggesting that the local environment is capable of inducing proper development of dopaminergic neurons. Introduction of neuronal populations of differentiated murine ES cells into a rat model of spinal cord injury has shown improved motor function,[10] though it is by no means clear that the mechanism was direct neuronal reconstitution as opposed to modulation of the repair process in the host through remyelination. A number of groups have demonstrated formation of neurons from human ES cells,[11-14] presaging future human applications.

Despite these apparent successes, it is sobering to note that there may be as many as 200 distinct neuronal subtypes in the adult brain, and that even within a given subtype, neurons show remarkable degrees of regional specificity. It requires a great leap of faith to believe that neurons produced from ES cells in culture will faithfully recapitulate the differentiated features of specific neuronal subtypes, and reestablish relevant neural networks established during formation of the embryonic brain. An alternative strategy is to differentiate ES cells into neural stem cells and progenitors in vitro, than coax local environments in the diseased region of the brain or spinal cord to direct further differentiation and accommodation of neural cells to their new niche. Whether this will occur is a matter of pure speculation, and subject to hyperbolic claims. The applications of somatically derived neural stem cells are covered in detail below.

Embryonic Stem Cells as an Inexhaustible Source for Islet Cell Replacement

Type 1 diabetes represents a major disease entity that has tremendous appeal as a target for cell replacement therapy. The disorder, which results from loss of mass of the insulin-producing beta cells of the pancreatic islets due to autoimmune attack, can be reversed by pancreatic or islet cell transplantation together with steroid-sparing immunosuppression.[5] The chief limitation to the wider application of this potentially curative therapy is the inadequate supply of islets from cadavers. Diabetes is particularly attractive because, unlike in Parkinson's disease where precise connections may be necessary, beta cells can function autonomously, even outside the pancreas (e.g., under the kidney capsule). Several groups have reported differentiation of murine and human embryonic stem cells into insulin-secreting cells,[15-18] with one group claiming normalization of hyperglycemia in a streptozotocin-treated diabetic model by transplanted cell clusters.[15] These reports are provocative, but much additional work remains

to characterize the functional nature of the cells as glucose regulators, and to document adequate, regulated production of insulin, which in one case was some 50-fold less than native beta cells.[16] Indeed, some of these reports have been called into question by subsequent studies showing that apoptotic cells can take up insulin from the culture medium and give the illusion of producing insulin without actually doing so.[19] Also, the specter that cell fusion rather than true cellular differentiation might account for detection of donor cells in regenerating tissues has muted some of the enthusiasm for genuine beta-cell differentiation.[20]

Some difficulties in generating beta cells from ES cells may stem from attempts to apply factors characteristic of late pancreatic development to essentially very early-stage cells. One may need to direct ES cells in a stepwise iterative fashion first toward endoderm, then toward anterior endoderm, and then to endocrine pancreas, essentially recapitulating pancreatic development. Although challenging, making endoderm is probably the most difficult lineage toward which to direct ES cells. Given the proof-of-principle that cadaveric islet cell transplantation can restore normoglycemia in diabetic patients, stem cell based therapies for type 1 diabetes represent one of the most compelling opportunities in regenerative medicine. Real clinical impact awaits the clear directed differentiation of appropriate cell populations. Present claims, many of which may be exaggerated, await further confirmation.

Lessons from Hematopoietic Development

Applications of HSC transplantation, which is curative for many genetic and malignant diseases of the bone marrow, is limited by a shortage of donors. In principle, this limitation might be relieved by deriving HSCs from human ES cells. Because transplantation of highly purified HSCs can induce immune tolerance,[21] directed differentiation of ES cells into HSCs represents one strategy for overcoming immune barriers to tissue transplantation. Cotransplantation of HSCs and any tissue of interest derived from the same ES cell line might enable tolerance induction and cell transplantation across histocompatibility barriers, as has been achieved for organ transplantation in mouse models.[22] Another method for enabling transplantation is to genetically customize ES cells by nuclear transfer cloning methods to match specific patients, as discussed below. Successful differentiation of ES cells into HSCs would greatly enable experimental models of blood transplantation, and thus in vitro hematopoietic differentiation of ES cells has been actively pursued. Mouse ES cells can be readily differentiated in vitro into myeloid, erythroid, and lymphoid lineages, but efforts at directed differentiation and transplantation of HSCs derived from ES cells in culture remain primitive. Indeed, given that we know so much about hematopoiesis and its clinical applications, the difficulty in generating in vivo repopulation from ES-derived blood derivatives should serve as a lesson: clinical development of ES cell therapies for less well-understood tissues is likely to present unforeseen challenges due to currently unanticipated biological principles.

Though blood formation from mouse ES cells differentiated into embryoid bodies (EBs) was reported nearly 2 decades ago,[2] achieving stable blood engraftment of irradiated mice with ES-derived HSCs remains challenging. Only limited hematopoietic reconstitution has been reported.[23-25] This failure has been blamed on the developmental immaturity of ES-derived blood cells, which most closely resemble primitive embryonic yolk sac hematopoietic progenitors and might therefore not engraft effectively in adults, but could also be explained by failure to capture and propagate adult-type HSCs in ES cell cultures. Lymphoid-myeloid HSCs do arise from ES cells differentiated in vitro, since they can be transformed by the chronic myeloid leukemia–associated oncoprotein BCR/ABL and shown to generate myeloid, lymphoid, and erythroid lineages in irradiated animals, albeit in the context of leukemia.[26] Using the homeobox gene *HoxB4* to stimulate HSC expansion, normal long-term multi-lineage hematopoiesis derived entirely from ES cells can also be demonstrated, but chimerism remains inefficient, suggesting engraftment with limiting numbers of true HSCs.[24]

Despite its inefficiency as a method for inducing engraftability, *HoxB4* modification of ES-derived blood progenitors does provide a means for modeling therapeutic blood cell transplantation. This strategy has proven successful in a mouse model of therapeutic cloning (nuclear transfer cloning combined with gene correction and cell therapy) for the treatment of a genetic form of immune deficiency.[27] In this experiment, nuclear transfer cloning from tail-tip cells of an immunodeficient $Rag2^{-/-}$ mouse was performed to generate a $Rag2^{-/-}$ ES cell line. One allele was then repaired by homologous recombination. The repaired $Rag2^{+/-}$ ntES line was differentiated in vitro, transduced with *HoxB4*, and engrafted into immunodeficient mice (**Figure 2;** see Appendix, page 597). The recipient mice showed partial reconstitution of B and T cell populations, Ig and TcR gene rearrangement, and IgM, IgG, and IgA in peripheral blood, thereby establishing that nuclear reprogramming and combined gene and cell therapy could be reduced to practice.

While theoretically appealing as a means of generating customized, patient-specific ES cells, nuclear transfer is cumbersome, inefficient, labor intensive, and expensive, and thus not likely to evolve into a commonplace therapy. However, studies of nuclear transfer will yield insights into reprogramming that might one day enable a more facile means of redirecting adult somatic cell fates toward therapeutic ends. Such a utopian state of stem cell biology will require a period of dependency on studies of nuclear transfer mechanisms, some of which must be done with human cells, with the attendant ethical difficulties involved in the creation and destruction of human embryos for research.

Clearly, more efficient methods for directed differentiation of ES cells into mature, definitive HSCs is needed. Initial efforts to harness embryonic morphogens like BMP-4 have shown encouraging results in tissue culture.[28-30] Further insights into the formation of hematopoietic tissues in the embryo are needed so that precise patterning can be reproduced in vitro.[31]

The challenges of translating methods for hematopoietic differentiation of ES cells into models of therapeutic hematopoietic engraftment highlight a major caveat in interpreting the promise of ES cell studies: simply identifying cells phenotypically in differentiating ES cell cultures does not ensure the cells will function as anticipated in vivo. Furthermore, residual undifferentiated ES cells carry the risk of teratoma formation if transplanted into patients, a fact that will necessitate incorporation of suicide gene strategies into ES cells prior to clinical application.[32] Extensive studies in animal models, analyzed with precision to discern their predictive scope and safety, are a prerequisite before subjecting human patients to unknown risks of stem cell–based therapies. The promise of ES cell–based therapies is considerable, but expectations of rapid progress toward clinical applications must be tempered by the enormous difficulties of harnessing these remarkably versatile cells for therapeutic ends.

II. Somatic (Adult) Stem Cell Therapies

The past 5 years have witnessed an explosion in interest in using somatic stem cells, particularly those derived from adults, for cell and gene therapy. This has been driven by a number of discoveries, but in particular, the possibility that some somatic stem cells can differentiate into nonautologous cell types, and also the discovery of multipotential stem cells in adult bone marrow. Stem cells are thought to be present in most adult tissues, and are responsible for replenishment of those tissues throughout life. By far the best known is the HSC that resides primarily in the bone marrow, and this will be the main focus of discussion below. However, lesser known but potentially of equal importance are stem cells from the CNS (discussed elsewhere in this chapter), the liver,[33] the skin,[34] the mammary gland,[35] the intestine,[36] and so on. With the resurgence of interest in stem cells in general, some researchers are turning attention to these less-studied systems, in order to determine how different stem cells compare with each other, and to exploit these stem cells for repopulation or reconstruction of their parent tissues after disease or surgery. Also, attention to the role of stem cells in malignancy has been renewed.[37]

Nonautochthonous Differentiation of Somatic Stem Cells

Unlike embryonic stem cells, somatic stem cells are thought to be fundamentally restricted in differentiation capacity, and only able to generate cells of the tissue (or germ layer) from which they are derived. This is one of their defining features, and is supported by the underlying tenets of developmental biology: the majority of commitment to specific differentiated lineages occurs during embryonic development. Tissue stem cells appear to be set aside during development within the tissue they serve. And, although they are not terminally differentiated, they are pre-programmed to generate, by default, cells of that tissue. For example, stem cells of the muscle, termed satellite cells, reside along the muscle fibers under the basal lamina. When activated by muscle injury, they proliferate extensively and activate a muscle-specific differentiation program, migrate to the repair site, and fuse to generate new muscle.[38] Likewise, HSCs generate, by default, hematopoietic cells. Therefore it came as a surprise when it was reported that some adult tissue–resident somatic stem cells could differentiate outside their tissue of origin.[39,40]

Generation of Hematopoietic Cells from Nonhematopoietic Stem Cells

Initial reports of hematopoietic differentiation from nonhematopoietic cells galvanized the stem cell community with ideas that somatic stem cells may be universally "plastic" and able to generate many new cell types. Neural stem cells and skeletal muscle cells were surprisingly reported to generate blood.[40-43] However, generation of blood from neural stem cells has been difficult to reproduce.[44] And skeletal muscle appears to harbor bone marrow–derived stem cells,[45,46] which arrive there via the circulation accounting for the apparent muscle-to-blood differentiation activity. While there are still some reports of adult-derived nonhematopoietic

cells generating hematopoietic cells, this mainly appears to occur after specific culture conditions[47,48] and may reflect a genetic reprogramming event, the consequences of which remain to be ascertained.

Generation of Nonhematopoietic Cells from Hematopoietic Stem Cells

Some of the earliest reports of so-called transdifferentiation described generation of non-hematopoietic cells after bone marrow transplantation (**Table 1**). All of these initial studies involved transplantation of whole bone marrow followed by examination of target tissues for rare cells that contained a marker of the donor cells. Diverse tissues ranging from cardiac muscle to neural cells were reported to harbor donor-derived cells, albeit generally at a very low level. The implication of these studies was that, if the efficiency of engraftment in nonhematopoietic tissues was sufficiently high, bone marrow transplantation could conceivably be used to treat a wide variety of non-hematopoietic diseases. This concept was particularly attractive when considering diseases such as muscular dystrophy where the affected tissues are distributed throughout the body, and hematopoietic stem cell transplantation offers the possibility to treat any tissue served by the circulation.

However, due to the heterogeneity of bone marrow, the possibility that nonhematopoietic stem cells contained within the transplanted marrow populations accounted for the nonhematopoietic reconstitution has plagued the field. While this might appear to be an academic question, the mechanism of engraftment is important to understand if one hopes to improve the efficiency prior to therapeutic use. Consequently, several groups attempted to transplant more highly purified populations of HSCs, in order to establish that HSCs or their progeny differentiated into these cell types (**Table 1**). Even in these carefully executed studies, impurities in the enriched stem cells could have accounted for nonhematopoietic regeneration. Nevertheless, data purporting differentiation of HSCs into skeletal muscle[39] and hepatocytes were compelling.[57] Relatively small numbers of highly enriched HSCs appeared to generate these

Table 1. Donor cell contribution to nonhematopoietic tissues after whole bone marrow or stem cell transplantation in the mouse.

Cells Transplanted	Target Tissue	Injury Induced	Approximate Frequency	Ref.
WBM	Macro- and microglia	TBI	0.5–2%	49
WBM	Skeletal muscle	TBI + intra-muscular cardiotoxin injury	Minimal	39
WBM	Skeletal, cardiac muscle	TBI	Not given	50
WBM	Skeletal muscle	Exercise	3.5%	51
WBM	Endothelial cells	TBI	Not given	52
WBM	Neurons	TBI	0.2–0.3%	53
WBM	Neurons	TBI	0.3–2.3%	54
WBM	Neurons	TBI and contusion	0	55
WBM	Hepatocytes	TBI	2.2%	56
SP	Skeletal Muscle	TBI + genetic deficiency (mdx mouse)	1–10	40
KTSL	Liver	TBI + genetic deficiency (FAH)	Rare	57
Lin^{-} Kit^{-hi}	Heart/vasculature	Coronary artery ligation	54% of new tissue	58
$CD34^{+}$	Vasculature	Coronary artery ligation	20-25	59
SP	Cardiac muscle, endothelial cells	TBI + coronary artery ligation	0.02 2-4	60
"Homed" HSC	Lung alveoli Lung bronchi Esophagus Stomach Small bowel Large bowel Bile duct Skin	TBI TBI TBI TBI TBI TBI TBI TBI	20 3.7 1.81 0.52 0.87 0.19 0.84 3.39	61
Single KTSL	Multiple	None	0	62

Abbreviations: WBM; TBI, total body irradiation; KTSL; SP; FAH, fumaryl acetoacetate hydrolase; HSC, hematopoietic stem cells

tissues after transplantation and tissue injury.

Still, the idea that an HSC, presumably committed to generate blood, could change its fate to make hepatocytes or muscle flies in the face of decades of equally careful work by developmental biologists. A number of other explanations have been put forward to explain this surprising behavior from HSC. One hypothesis is that the bone marrow (and possibly other tissues) continue to harbor primitive stem cells, akin to embryonic stem cells, throughout life.[63] These presumably rare cells could be capable of generating a variety of cell types, possibly after circulation to injured tissues (see section on multipotential stem cells, below). While an attractive hypothesis, we consider this unlikely: embryonic stem cells are known to form tumors readily when implanted in ectopic sites in the adult; indeed the ability to form teratomas is part of the operational definition of an embryonic stem cell. Many different layers of growth control exist to restrict the proliferation potential of cells in the adult, forcing commitment to differentiation and reducing the prevalence of tumorigenic transformation. Moreover, most tissues harbor a stem cell population that generally serves to replenish that tissue. So a "niche" or need for such totipotential stem cells in the adult is not evident.

Another possibility is that there is no true transdifferentiation of stem cells from one fate to another, but instead technical explanations for the perception of such events. Certainly, all methods of detection of donor cells are prone to artifacts, from β-galactosidase to fluorescent in situ hybridization (FISH) for the presence of a sex-mismatched chromosome (e.g., the Y chromosome of a donor cell in a female host). For example, the false-positive rate for FISH is ~3%. To be detected reliably, true events must occur significantly above that frequency, a level that has rarely if ever been reported (Table 1). When carefully performed, however, these detection techniques can be used with fidelity. Therefore, it would be disingenuous to attribute all reports to technical errors. Other biologically plausible but perhaps less interesting explanations may play a role. Among the most intriguing is the incontrovertible fact that two cell types can fuse. Two recent papers suggested that fusion of differentiated cells with embryonic stem cells in vitro could lead to functional cells with stem cell–like properties.[64,65] This led to the idea that transplanted HSCs or their progeny were fusing to cells, such as hepatocytes, resulting in cells with new phenotype and function. In some tissues such as muscle, where fusion is a natural step in the regeneration of the tissue, this hypothesis is difficult to test. However, in liver, fusion of hematopoietic cells and hepatocytes does appear to be at least part of the mechanism of generation of hepatocytes from bone marrow cells.[20,66] Similarly, in the brain, the initial perception that HSCs were giving rise to such complex neuronal cell types as Purkinje cells—even outside the temporal and spatial window for neurogenesis in the intact adult brain—have now been deemed attributable to fusion events (Helen Blau, personal communication).

Another fundamental scientific concern regarding the legitimacy of attributing extensive pluripotency to some tissue-derived somatic stem cells is the lack in virtually all studies of true clonal analysis. For a cell to be deemed as having multiple potencies, it *must* be affirmed that a single cell of that type, ideally isolated in a miniwell or marked by a unique retroviral insertion site, can give rise to progeny of multiple lineages. The fates of a single clone must shift simply based on changes in the milieu, either in vitro or in vivo following transplantation. That level of rigor has not been widely applied in the reports of transdifferentiation. Without this, the alternative explanation must be entertained that a polyclonal population of unipotent cells has simply been assayed. This level of proof—i.e., true clonal analysis—should be required in all areas of stem cell biology, particularly when transdifferentiation is being invoked, a condition that would challenge fundamental embryological concepts.

Despite these concerns, a number of groups have looked for evidence of non-hematopoietic cell generation after transplantation in the clinic, either after bone marrow transplantation, or after transplantation of donor tissue such as hearts. In the case of male bone marrow donated to female patients, Y chromosome positive cells have been found in a wide variety of tissues (**Table 2**). Because circulating donor-derived hematopoietic cells are also Y chromosome positive, attempts were made to demonstrate that some of the Y positive cells in host tissues had markers of the new tissue and had lost features of hematopoietic cells. In the case of hearts transplanted from female donors into male patients, biopsies showed that some cardiomyocytes and endothelial cells were Y chromosome positive, suggesting that circulating male host cells (presumably from the bone marrow) migrated to the transplanted heart and took part in regeneration of the tissue (**Table 2**). Again, there is a large range of reported frequencies that could be attributable to a number of different factors, including the disease state of the patients and/or the level of host/graft interaction.

It remains highly controversial whether there is true conversion of hematopoietic cells into non-hematopoietic cells—even via such a mechanism as nuclear reprogramming through cell fusion. Regardless of how the data ultimately resolve this controversy, there is little

Table 2. Circulating cell contribution to non-hematopoietic tissues in clinical specimens.*

Tissue Transplanted	Donor Cells Observed	Approximate Frequency, %	Ref.
Bone marrow	Osteoblasts	1.5–2	67
Bone marrow	Hepatocytes	2.2	68
Bone marrow	Gastro-intestinal (GI) tract epithelia	0–4.6	69
Bone marrow	Stroma	0	70
Mobilized peripheral blood	Keratinocytes	0	71
Mobilized peripheral blood	Hepatocytes GI tract and skin epithelia	0–7	72
Heart	Cardiomyocytes endothelium	20–15	73
Heart	Cardiomyocytes endothelium	0.04–25	74
Heart	Cardiomyocytes	0.2	75
Heart	Cardiomyocytes	0	76
Heart	Cardiomyocytes	0	77

*In the bone marrow or peripheral blood transplants, male donor cells were transplanted into female recipients. In the heart transplants, female hearts were transplanted into male recipients.

debate that a wide range of frequencies have been reported and, with few exceptions, the prevalence of such events is extremely low (**Table 1**), or under some experimental conditions, undetectable.[62] What accounts for the wide discrepancy in frequencies? Probably the use of different markers, different systems of injury of the target tissue, different donor cell populations, etc. What the discrepancies really indicate is that we understand very little about transdifferentiation, the process of commitment, and the cellular and molecular basis of plasticity, indicating that we have a great deal of basic research still to do.

Bone Marrow Transplantation for Nonhematopoietic Disease

Although clearly many questions remain that must be addressed by basic research, can we begin to think about using bone marrow transplantation (BMT) for therapy of nonhematopoietic disease? There has been a relatively long history of using BMT to treat certain inherited metabolic diseases, e.g., Hurler's disease and other mucopolysaccharidoses. In such storage diseases, blood-derived cells are not used for cell replacement but rather as "pumps" for lysosomal enzymes that are taken up by impaired host cells and thereby restore their normal metabolism. As a strategy for cell replacement, BMT has been used with some success for treatment of osteogenesis imperfecta, a brittle bone disease.[67] And clinical trials are underway in several countries to determine whether bone marrow stem cells could enhance cardiac repair after infarction, either by direct injection of bone marrow cells into the heart, or by mobilization through the circulation. Hopefully, good trial design will allow the outcomes of these trials to be rigorously evaluated. In some experimental settings, improvements in cardiac function following the use of bone marrow mesenchymal cells in myocardial infarction can be attributed *not* to transdifferentiation of such cells into cardiomyocytes, but rather to the non-cardiac cells occupying necrotic spaces that would otherwise have become scarred. Thus, improvement in cardiac contractility occurs via effects on Starling forces. In such cases, bone marrow–derived cells played an important therapeutic role, albeit *not* via cell replacement or transdifferentiation. In the brain, as discussed elsewhere in this chapter, non-neural cells (including those derived from bone marrow) seem to exert a therapeutic effect again *not* by cell replacement but by producing therapeutic molecules. These might include trophic factors that serve a protective, regenerative, or anti-apoptotic effect; angiogenic factors that promote revascularization of damaged areas; anti-inflammatory factors that can inhibit scar formation and cytokine-mediated secondary damage; and natiuretic factors that promote CNS-mediated diuresis and relief of cerebral edema. Similarly, BMT has been found to be beneficial in a diabetes model;[78] however, recent analyses have suggested that the impact might predominantly be exerted via cell *protection* and *rescue* rather than literal cell replacement.[79]

What about other diseases, such as those affecting the liver? One of the most impressive examples of cell conversion in the mouse has been the generation of hepatocytes from bone marrow stem cells.[57] These experiments were particularly effective because the host hepatocytes harbored a severe genetic defect (fumaryl acetoacetate hydrolase deficiency), enabling selection for wild-type hepatocytes after withdrawal of a supportive drug regimen. The selection occurred over a period of a few weeks, during which time the host hepatocytes experience a controlled demise, affording time for expansion of engrafting wild-type cells derived from bone marrow. The liver data are particularly impressive because approximately one third of the liver was repopulated by bone marrow–derived cells. Whether conversion occurs by transdifferentiation or, more likely, by fusion (as the authors' own re-interpretation sug-

gests[20]), one might be able to exploit this phenomenon to provide genetic repair, a different type of "gene" therapy. However, for such a conversion to work in humans, a strong selective pressure that does not put the host at risk of death has to be devised. Such conditions are exceedingly rare in humans, and the common forms of liver disease are not likely to be served by BMT in the near future.

In contrast to the liver, the repopulation of skeletal muscle by bone marrow derivatives is much less robust (< 0.1% on average), in part due to lack of strong selective pressure as discussed above. Strategies to increase the levels of engraftment therefore need to be developed. Potential bottlenecks being addressed are limitations on the number of stem cells getting to the target (potentially overcome by mobilization), and inefficient transdifferentiation. This latter issue is much more complex, and a number of strategies are being considered, including boosting the differentiation of hematopoietic cells down specific lineages by introduction of transcription factors which govern differentiation to that cell type. Finally, the relative risks of bone marrow transplantation will be a consideration. Continued advances in minimally myeloablative approaches to bone marrow transplantation would be a prerequisite to broad application of BMT as a therapy for nonhematopoietic disease.

Multipotential Somatic Stem Cells

The possibility that some somatic stem cells in the adult may have properties akin to embryonic stem cells has garnered enormous attention. While as yet there is no persuasive evidence that these cells exist in normal tissue, multipotential adult stem cells have been found after culture of bone marrow stromal elements for long periods of time under specific conditions.[48] These multipotential adult progenitor cells (MAPCs) have been reported to generate most cell types in vitro and in vivo after injection into mouse embryo blastocysts. MAPCs have been isolated from both human and mouse bone marrow, and conditions for differentiating them into specific cell types are being explored, with many groups interested in using them for repair and regeneration of a variety of cell types. A major constraint on the utility of these cells, however, is the highly specialized culture conditions they appear to require. If more laboratories succeed in deriving such cells, they might find broader use.

Somatic Versus Embryonic Stem Cells

In the weeks before Bush's decision to allow National Institutes of Health (NIH)–funded research on some human embryonic stem cell lines, the lay press were pitting somatic (adult) stem cell and embryonic stem cell researchers against each other, trying to force scientists to place bets on the therapeutic superiority of one or the other cell type. This was a political but not a scientific debate, as both stem cell types have advantages and disadvantages, some of which are listed in **Table 3**. Both classes of stem cells are going to require considerable development work to solve fundamental problems currently limiting their use. Whether somatic stem cells can transdifferentiate into diverse tissue types or not, they should in the future prove useful for regeneration of their host tissue, much as bone marrow stem cells are used in transplantation today. We are bound to see sound clinical use of both embryonic and somatic stem cells in the future, likely for complementary uses,

Table 3. Advantages and disadvantages of adult and embryonic stem cells.

	Advantages	Disadvantages
Human ES cells	Can make virtually any tissue (in theory)	Allogeneic only (currently)
	Some tissues "easy" to generate (e.g., cardiac)	Teratoma formation?
	Can be propagated indefinitely	Differentiation conditions to be established
	Amenable to genetic manipulation?	Some tissues difficult to generate (blood)
		Ethical issues
Adult stem cells	Autologous	Most have limited self-renewal
	Many types and sources	Differentiation outside lineage? (maybe)
	Some types have extensive self-renewal potential	Autologous (use more cumbersome and expensive)
	Not tumorigenic	
	Default differentiation	
	Amenable to gene transfer	
	Potential delivery methods attractive	
	No ethical issues	

although this may take a decade or more. The reader is referred to **Table 4** for some of the key recent papers that illustrate the points discussed above.

III. The Neural Stem Cell as a Model Somatic Stem Cell

Approximately 10½ years ago, a handful of investigators interested in fundamental neural development began to identify, within cultures obtained from the developing and mature CNS, cells with surprising plasticity, multipotency, and a propensity for dynamically shifting their fates.[86-89] The existence of such cells—if indeed they represented a population normally resident in the brain—challenged the prevailing dogma that the nervous system was rigidly and immutably constructed. Neural stem cells (NSCs), as these plastic cells came to be termed, began to garner the interest of not just the developmental community but also that of the neural repair, gene therapy, and transplant communities when it was recognized that they could be expanded in culture and reimplanted into the mammalian brain where they would reintegrate appropriately and stably express foreign genes.[87] Their abundance, multipotency, ease of manipulation, and engraftability made this strategy an attractive alternative for CNS gene therapy and repair.

In comparison to extant techniques, NSCs presented certain advantages: they were a homogeneous and relatively well-defined neural cell population that could be easily stored and expanded on demand, and, if necessary, genetically manipulated ex vivo to express a wide variety of foreign transgenes. These transduced genes, as well as their inherent genetic repertoire, could be effectively imported into the CNS "Trojan Horse"–style following transplantation almost anywhere into the developing and mature host brain. Furthermore, NSCs and their progeny possessed a capacity to integrate not only locally at their site of implantation, competing with and interdigitating seamlessly with endogenous cells,[87,90-94] but also more broadly.[95,96] They were quite migratory—particularly if implanted into germinal zones—permitting cell and gene therapy to be contemplated for disseminated, even global, CNS disease processes. In that sense, NSCs had a distinct advantage over fetal tissue and non-neural cells for cell replacement and over most viral vectors for gene delivery. Even such alternative cellular vectors as hematopoietic cells, when used for protein delivery in bone marrow transplantation paradigms, could not circumvent the restrictions of the blood-brain barrier and integrate throughout the CNS as effectively as NSCs. A single bona fide NSC clone could take up residence in, and accommodate to, any nervous system region, permitting an economy of resources.

In addition, NSCs were attracted by degenerating neural tissue,[90,97,98] effectively replacing dead or dysfunctional cells in those regions. In these pathological niches, these multipotent cells, in response to signals still poorly understood, would shift their progeny's fate toward that of neural lineages most in need of repletion—even if beyond the classical developmental window for genesis of that cell type. Indeed, these observations gave birth to the hypothesis that certain neurodegenerative environments recapitulate developmental cues because NSCs responded to neurogenic cues not only during their normal embryological expression, but also when recreated by particular types of cell death. NSCs, in other words, were sufficiently sensitive to "sense" niches of neurogenesis and/or small nidi of pathology in the brain.[91,92,98]

These observations in the CNS stimulated investigators in other solid organ systems to search for stem-like cells even within tissues generally held to be more regenerative, more forgiving, and/or more redundant than the CNS. Hence, the neural stem cell—in effect the first solid organ stem cell isolated and exploited—served as a model for most other somatic stem cells.

Importantly, despite the spotlight of therapeutic promise the NSC has thrown upon itself (and other stem cells), it is critical to remember that its existence was unveiled in the course of understanding *development* and that, in the end, it is simply one player in a broad and exceedingly complex, interdependent, finely tuned developmental system, one that requires fundamental developmental understanding. In this endeavor, the CNS continues to serve as an instructive model for the stem cell field in general.

The Biological and Therapeutic Role of the Neural Stem Cell

Although the degree to which the mammalian CNS supports the birth of neurons and other cell types outside of their classical spatial or temporal developmental windows has become an area of intense investigation and debate,[95,97-99] most agree that one of the repositories of whatever plasticity exists is the NSC, residing lifelong within various secondary germinal zones of the brain. Indeed, it was the observation that exogenous multipotent NSCs could respond to the prevailing cues of normal and abnormal microenvironments that first suggested the existence of spontaneous compensatory mechanisms for genetic[91,98,100] or acquired deficiencies,[90,92] including neurogenesis beyond its normal confines. It is acknowledged, however, that these compensations alone are *not* sufficient to redress neurological deficits in the most devastating of cases. The degree to which these natural processes might be augmented by

Table 4. Selected additional reading on adult stem cell therapy and stem cell "plasticity."*

Topic		Ref.
"Transdifferentiation"	Generation of skeletal muscle from bone marrow and stem cells	39,40
	Generation of hepatocytes from small numbers of hematopoietic stem cells	57
	Generation of neurons from bone marrow	53,54
	Multipotential adult progenitor cells	48
Lack of "transdifferentiation"	No generation of neurons from hematopoietic stem cells	55
	Lack of generation of many cell types from hematopoietic stem cells	62
Fusion as transdifferentiation	Cell fusion as the mechanism for transdifferentiation	20
Recent reviews	Covers current controversies and recent published data	84
	Detailed review including technical caveats	85

* This table is not meant to be comprehensive, but to highlight some of the best and/or most illustrative and readable papers in the field.

supplying exogenous NSCs with or without exogenous stimulants and/or molecular prompting and priming is the primary focus today of regenerative neurobiology.[90,98,99,101-105]

Although important differences exist between rodent and human NSCs—principally attributable to issues of cell cycle (protracted in human cells with a strong predilection to exit the cycle and differentiate, or to cease cycling entirely after ~124 doublings)—many of the important biological principles gleaned from examining rodent cells have been conserved in the human CNS.[97,98] Lines of engraftable human NSCs (hNSCs) have been isolated from fetal and adult specimens that, in many ways emulate their rodent counterparts.[88] For example, hNSCs can participate in CNS development (including of subhuman primates), respond to local cues, migrate to widely disseminated CNS regions[88-90,97,98,106] including homing to areas of intracranial pathology, express transgenes, replace missing neural cells, and, in some cases, help promote functional improvement in some disease models.[97,98] For example, in pilot studies on the contused adult rat spinal cord, hNSCs yield neurons that make long distance connections both rostral and caudal to the lesion that appear to facilitate the conduction of cortico-spinal impulses and concomitant behavioral improvement.[91] In other pilot experiments in which hNSCs were implanted in key regions along the spinal cord of the SOD1 transgenic mouse model of amyotrophic lateral sclerosis (ALS), a significant proportion of the recipients experienced a remarkable preservation of motor function and, in some animals, nearly a doubling of lifespan.[92]

Transplantation into lesioned monkeys not only assays the hNSC's response to a neurodegenerative milieu that closely mimics that of humans, but also begins to lay the groundwork for clinical translation by requiring practical protocols for the administration of cells to large recipients (e.g., number of cells to inject, placement and number of injections, rate of delivery) while establishing safety and efficacy. One recent set of experiments has analyzed the fate and impact of hNSCs in the MPTP-induced model of dopamine (DA) depletion in African Green Monkeys, an authentic animal model of Parkinson's disease (PD). In pilot studies, hNSCs appeared to colonize the mesostriatum, with some spontaneously converting to tyrosine hydroxylase (TH)-expressing cells. Given that hNSCs, like murine NSCs, intrinsically produce many neurotrophic and neuroprotective factors, the improvement in DA activity observed in some recipients is likely the combined effect of not only DA cell replacement but also the provision by hNSCs of factors promoting the survival and enhanced function of *host* DA neurons and their nigrostriatal connections.[93] These dual mechanisms will likely be therapeutically significant.

What About Somatic Stem Cells of Fetal Origin—Umbilical Cord Cells?

Although, as noted above, debates abound concerning whether truly pluripotent somatic stem cells exist, there is a growing interest in whether cells derived from the fetal milieu, for example, umbilical cord cells (UCCs) and placenta, have interesting properties and can be exploited clinically. UCCs include a rich source of hematopoietic and possibly nonhematopoietic stem cells and can be used in place of bone marrow or mobilized peripheral blood as a source of hematopoietic reconstitution following myeloablative chemotherapy.[58,59] Beyond their applications in the treatment of bone marrow disorders, some reports have suggested their util-

ity for treatment of neurologic disease.[60,61] Indeed, it has been observed in preliminary studies that children with some neurodegenerative processes—Krabbe leukodystrophy (galactocerebrosidase [GalC] deficiency) being the most successful—evince a blunting of disease progression if UCC transplantation is performed within the first 2 weeks of life (J Kurtzberg, unpublished data). The mechanism of this effect is unknown. In a preliminary study, the brain of a 21-month-old girl with Krabbe's disease treated by UCC transplantation was examined (EY Snyder, unpublished data). This patient provided an opportunity to ask whether UCCs can transdifferentiate into neural cells in a favorable therapeutic setting: the recipient brain was immature and developing but plagued by a global neurodegenerative process; the patient received effective pretransplantation induction/ablation and posttransplantation immunosuppression; donor and host were well matched; engraftment and chimerism were long term (1 year); donor cells could be unambiguously discriminated from host by sex mismatch. Misinterpretation from cell/nuclear fusion events could be excluded by ascertaining only one nucleus-per-cell and only 2 sex chromosomes-per-nucleus. In this case, UCCs appeared to be present throughout the host brain, both in white and gray matter and juxtaposed with host cells where they presumably produced missing GalC; they were also present within blood vessels and integrated within ventricular ependyma. Donor cells could be identified as microglia but UCC cells did not contribute to neurons, oligodendrocytes, astrocytes, or immature progenitors. Levels of GalC in this GalC-deficient brain were detectable. Hence, this patient illustrated the feasibility of using UCCs for *molecular* therapies—but provided no evidence that the mechanism of disease amelioration involved neural cell replacement.

Lessons to Be Learned—A Roadmap to the Clinic

Given how radically the stem cell concept has altered our formerly deterministic view of the CNS, it is not unexpected that the public and even the scientific community have become eager to apply this newfound knowledge to clinical situations. With results of laboratory research so enticing, what is preventing us from imminent translation to the clinic, particularly for some of the most devastating diseases? The answer, most simply put, is that we still do not know the safest, most practical, most efficacious methods for exploiting stem cell biology. If we proceed prematurely, we *can* do harm even in the most dismal of diseases. Ill-conceived and untutored clinical trials could make the suffering of the patient worse; shorten an already limited lifespan; compromise residual function; add additional symptoms, deficits, pain, and side effects; precipitate an even greater decline in an already marginal quality of life. And such adverse outcomes impact not only the patient's own life but that of family and caretakers.

Therefore, for the remainder of this section, we propose a roadmap to the clinic, with a particular emphasis on flagging the potholes and speed bumps through which we must navigate.

1. Know your disease

In order to reasonably predict success using stem cells in the treatment of a disease, one must have an understanding of its pathobiology beyond simply that a particular cell type *seems* to be dysfunctional and might benefit from replacement therapy. It's important to know how *disease* biology itself impinges on *stem cell* biology. Without this knowledge, one is simply "dumping" cells into a defect with the vapid hope that the cells will survive and function. While one might argue that many accepted treatment practices emerged in medicine through this empiric approach, the evolving standards of mechanism-based medicine make simple trial and error unacceptable, particularly in view of the potential harm that could be rendered. To predict the success of cell therapy, one must understand whether a given pathophysiological process, or the milieu it creates, inhibits or promotes the unfolding of developmental programs that direct the survival, differentiation, and integration of a replacement cell type. For many diseases, we might be unduly presumptuous in assuming that we know what cell type is needed, that we know the true locus of the disease, and that we know what is required to reconstitute a given region and restore function.

As fundamental as these questions are, the answers are actually quite elusive in the majority of neurological diseases—Alzheimer's, mental retardation, autism, stroke, Huntington's disease, ALS. These diseases may actually need multiple cell types to reconstruct a milieu, and there is no guarantee that we know the signals to mold the cells. Furthermore, the answer for one disease does not necessarily extrapolate to another even ostensibly similar disease, and any given disease will have different needs at different times within the same patient. Diseases are dynamic. The acute phase after an injury or after the onset of a degenerative process is different from the subacute phase, and those are different from the chronic phase. In the NSC field, for example, we have observed that extensive migration and shifts in differentiation fate will occur in rodents only during certain windows following ischemic injury (e.g., 3–14 days).[90] That behavior is no longer apparent even a few weeks later. Coincident with this observation, an entirely different set of genes are upregulated in the

NSC confronted with an acutely asphyxiated brain than when confronted with the same brain following the passage of time. Furthermore, the needs for one brain region (e.g., striatum) may be inappropriate for another (e.g., cortex),[90,92,99,107] a challenge for a pathological process that is extensive and multiregional in its manifestations, such as stroke or Huntington's disease or Alzheimer's disease. Such are the sobering challenges to NSC-based therapies.

Cellular therapies alone might be ill suited for some cell *non*-autonomous disease processes (i.e., problems extrinsic to the stem cell), e.g., aspects of radiation-, ischemia-, inflammatory-, and metabolic-induced encephalopathies. In such cases, defects in the microenvironment might first need to be rectified before initiating stem cell therapy. This realization was highlighted by a recent examination of the impact of cranial irradiation on the neurogenic zones of the adult rat hippocampus,[6] a region plausibly implicated in the cognitive deficiencies frequently encountered as a side effect following this common medical intervention. The pathology appeared to reside less within the neural stem/progenitor cell itself than within the *microenvironment* in which that cell had to function. Irradiation altered some of the regulators of NSC survival, number, and fate. These defects included a striking increase in the number of activated microglia (promoting inflammation and scarring) and a 3-fold increase in the distance between NSCs and their microvascular supply (disrupting a complex vascular-progenitor relationship). Such defects are not compatible with ready neuronal replacement, whether by endogenous neurogenesis or transplanted NSCs. This recognition does not preclude *changing* the milieu, e.g., via anti-inflammatory and pro-angiogenic maneuvers. However, indiscriminate transplantation of exogenous NSCs or stimulation of endogenous NSCs here would likely prove futile—and potentially *harmful*. An example of the latter possibly might be found in the work of Parent et al[108] who reported that, while status epilepticus promoted increased hippocampal neurogenesis, those newborn neurons were often ectopic, made aberrant connections, and provided new epileptic foci.

Therefore for certain cell extrinsic pathologies, both the donor and host NSC might need protection—either conferred by genetic engineering or provided via chaperone cells (see below) or molecules.

For even some cell-*autonomous*/cell *intrinsic* defects, the use of *endogenous* NSCs alone might be problematic. For example, in such genetically based neurodegenerative diseases as Huntington's, Alzheimer's, Parkinson's, ALS, and multiple sclerosis (MS), endogenous NSCs may *not* be optimal given that they likely already harbor a genetic flaw or disease-predisposition. Indeed, there has been growing speculation that the initiating locus of some neurodegenerative diseases may actually reside within the NSC population itself.

Such observations highlight the necessity first to understand the actual pathophysiological process to be redressed and rule out the milieu it creates as an *obstacle* to unfettered dependence on NSCs (endogenous or exogenous).

2. Know your cell

Is the regenerative task at hand within the biological repertoire of the stem cell? In other words, is disease pathobiology consistent with stem cell biology? This question takes on prominence when asking whether a stem cell from a non-neural system (e.g., muscle, blood, mesenchyme) can become a neural cell. It becomes no less vexing, however, when dealing with stem cells of direct neuroectodermal origin. To direct NSCs (whether endogenous or exogenous) to different CNS regions to yield cells of the right type(s) and number, in the right ratio, in the right location, making the right connections with the right partners without making any wrong connections, and to shield non-targeted cells and regions from such influences, presupposes a level of understanding we may not have for years. The complexity of these questions is compounded by two emerging realizations. First, a disease may actually need replacement of multiple cell types and multiple proteins in order to entirely reconstruct the damaged milieu—not only the neurons that have died, but also the support cells that detoxify the environment—myelinate the axons and dendrites, provide ongoing trophic and matrix support, and provide reservoirs for ongoing cell replenishment. Second, the interaction between transplanted NSC and recipient host is a dynamic, complex, ongoing reciprocal interaction where both entities are constantly in flux. The implications of this complexity have practical ramifications, as detailed below.

3. Know what your stem cell is doing; things aren't always what they seem

Most work to date in stem cell biology has focused on the concept that the *host* CNS environment—as it changes over the course of development and aging, or as it is altered by injury or degeneration—influences or instructs the transplanted stem cell. Therefore, when recovery has been observed, it has always been assumed that the stem cell must have replaced what was missing. However, an unanticipated phenomenon has begun to emerge: transplanted stem cells also influence the host. We have postulated that this ability to pro-

mote a regenerative response in the host is actually a fortuitous byproduct of fundamental stem cell biology. The emerging recognition of a *dynamic reciprocal stem cell–host interaction*[109] adds not only a powerful new avenue for stem cell–mediated recovery but also another level of complexity—and yet another way for stem cell biologists to be fooled. Nevertheless, that stem cells may change and/or protect the *host* CNS is a dynamic that can be exploited and augmented.[90-93]

The NSC inherently expresses genes that we have learned are capable of signaling, instructing, remodeling, and protecting the host CNS. This speculation derives in part from 2 recent studies in 2 different regions of the nervous system at 2 different ages. In the first, murine NSCs were implanted into the substantia nigra of aged mice that, 1 month previously, had received repetitive systemic administrations of high-dose MPTP, the neurotoxin described above that produces a persistent impairment of mesencephalic DA neurons and their striatal projections.[96] *Unilaterally* implanted NSCs not only migrated and integrated extensively within *both* hemispheres but were associated with dramatic reconstitution of DA function throughout the mesostriatal system in a manner that mirrored the spatiotemporal distribution of donor-derived cells. While there was spontaneous conversion of NSCs to DA neurons in DA-depleted areas, and while cells of donor origin contributed to nigral reconstitution, the *majority* (~138%) of DA neurons in the "reconstituted" mesostriatal system were actually *host* cells "rescued" by constitutively-produced NSC-derived factors. Although the mechanism underlying this inherent NSC-mediated protection or activation of a host regenerative capacity remains uncertain, one mode is likely to be the production by NSCs of trophic and tropic agents. NSCs constitutively produce a broad range of peptide neurotrophic factors, adhesion and extracellular matrix molecules, and lysosomal enzymes. In the example above, intermixed among NSC-derived DA neurons was a larger subpopulation of clonally-related *undifferentiated* or *glial-differentiated* NSC-derived cells that spontaneously expressed GDNF (among other peptides), a molecule known to be neuroprotective of DA neurons.

In the second study—this time in the adult rat spinal cord—implantation of NSCs (supported by a biodegradable scaffold) into a large hemi-resection cavity significantly improved functional recovery.[96,105] The reconstituted parenchyma and neuronal fibers bridging the lesion were *not*, however, derived from the NSCs—although they persisted in abundance—but rather from the *host*. Indeed, the NSCs did not simply differentiate into astrocytes that might have contributed to glial scar formation; in fact, scarring, necrosis and secondary injury, host cell death, and inflammation were diminished. *Host* tissue preservation and regeneration—as stimulated or instructed by the many intermingled undifferentiated NSC-derived progenitors—were responsible for the functional improvement.

The broader implications from these observations are 2-fold. First, that the dogma of an immutable, irreparable postdevelopmental mammalian CNS devoid of inherent plasticity must be revised. And, second, that the host CNS may benefit not only from stem cell–derived replacement of lost neurons but also from the chaperone effect of other stem cell–derived progeny; even undifferentiated/glial-differentiated progeny appear to be equally necessary for promoting optimal reconstitution. (Indeed, developmental studies have suggested that such homeostasis-maintaining cellular pools are established from stem cells at the earliest stages of organogenesis[100] expressing factors in a differentiation state–dependent manner.) While NSCs have been touted most vociferously for cell and gene therapy,[105] these findings suggest yet another mechanism by which therapeutic outcomes might be achieved: an inherent capacity of stem cells (without genetic engineering) to create host environments sufficiently rich in trophic and/or neuroprotective support to promote the recovery of damaged *endogenous* cells, to mobilize *host* progenitors, to remodel the impaired milieu such that latent regenerative responses, intrinsic protective mechanisms, and/or inherent neurogenetic programs within the host are favored, triggered, and/or amplified. The cocktail of stem cell–derived factors—that includes not only neurotrophic and neuroprotective but also proangiogenic, anti-inflammatory, and anti-apoptotic factors—might help shift the balance between permissive and nonpermissive microenvironments in a manner that favors the reacquisition of CNS integrity and function. This phenomenon has now been replicated in other systems using other stem cells: embryonic germ cells,[102] hematopoietic stem cells,[79] and bone marrow mesenchymal cells.[82,83] It is, in fact, this function—and not cell replacement—that may well account for some of the reported beneficial effects of stem cells from other systems in other animal models, including those outside the CNS. This possibility warrants scrutiny not as an admonition but as an opportunity: unravelling the molecular mechanisms underlying graft-host interactions will allow them to be exploited more efficiently and predictably. Given the complexities of CNS development, *preserving established CNS circuitry* is as important—and probably safer and more tractable—than attempting to reconstruct proper new connections.

4. Exploit endogenous and exogenous repair

The question of whether to mobilize a patient's endogenous stem cells or implant exogenous stem cells has become pertinent to most organ systems. Most studies to date that have examined the behavior of endogenous progenitor cells in non-neurogenic regions in the intact and injured adult mammalian brain find neuron replacement to be meager,[95,99,103,107] if present at all,[97] very restricted, short-lived, and functionally insignificant. The small number and low survival of incipient neurons might reflect unfavorable microenvironmental conditions for neurogenesis and/or survival due perhaps to a lack of appropriate trophic support, or exposure to toxic factors emanating from damaged tissue, or simply the absence of appropriate developmental cues. Therefore, strategies have been proffered to expand the pool of neuron-yielding progenitors with mitogens, differentiation factors, antiapoptotic factors, or to bias neuronal differentiation by inhibiting glial differentiation. How successful these techniques will be remains to be determined. However, even if stem cells in situ are recruited, beckoned to reenter the cell cycle and coaxed to yield neurons, the challenge still exists of generating adequate numbers (without creating deformations or tumors) of proper phenotypes in correct distributions with sufficient integrative capacity. The most effective therapies will likely entail mobilized endogenous cells *supplemented* by exogenous cells in particular developmental states, perhaps engineered ex vivo to express particular neurotrophic, neuroprotective, or detoxifying molecules.[90,104]

5. Suit the type of stem cell to the purpose

Of the various sources of somatic stem cells, which one is best—bone marrow, blood, muscle, brain, etc? All things being equal, a disease of a particular organ system is probably most efficiently treated with stem cells *from* that organ—for example, for the CNS, a neural stem cell or an ES cell directed to become a neural stem cell. For a given tissue, how does one decide between using an ES cell directed toward that organ's cell type or using a somatic progenitor/stem cell derived directly from that organ?

Starting with an ES cell and directing it toward a particular lineage requires a knowledge of developmental mechanisms that might not be required of stem cells derived from the tissue of interest. For example, if one starts with NSCs, much of the developmental instruction has already taken place; NSCs have already learned that their address is within the nervous system. Therefore, there are a number of steps that will not be required to exploit these stem cells. Not only is there less of a need to direct these cells, there is probably also a diminished risk of obtaining cells that are *inappropriate* to a given tissue. Shamblott et al[110] found that, even among embryonic germ cell lines thought to be experimentally differentiated toward a particular lineage, genes consistent with other organ types were simultaneously expressed—for example, in "neuralized" cultures, gene patterns consistent with bone marrow–derived cell types were also found. ES cell cultures do not contain cells of *purely* one differentiated tissue type. Reassuringly, inappropriate or undesired cells have never been observed in intracranial transplantation paradigms using *NSCs*, despite their extensive multipotency and their possession of all the stem cell attributes that make them appealing for repair.

In dealing with ES cells, however, one must be certain to direct them *invariantly* down a given lineage and create safeguards against the appearance of inappropriate cells (e.g., muscle or bone in brain), conversion to teratocarcinomas (an ability that actually constitutes part of the operational definition of an ES cell), or the emergence of autonomous organs within the larger organ (e.g., independent neural tubes within the brain, heart, or pancreas). While predifferentiation ex vivo might preclude this (once we can do so reliably), the comfort of invariant commitment to one cell type must be weighed against the loss of plasticity wherein a degenerating microenvironment directs a somatic stem cell to reconstitute a region by prompting it to yield multiple interacting cell types.

The notion, however, that somatic stem cells (particularly from an adult) are equivalent to and may replace ES cells is also unfounded. Somatic stem cells, too, have their limitations: they are often hard to identify and isolate from a specific organ (although this limitation does not seem to apply to the CNS). They often are slow to expand from the human and, unless genetically augmented, typically senesce. ES cells, on the other hand, are naturally immortalized and hence provide an unlimited supply of rapidly dividing reagents. Furthermore, there are limitations to the use of adult stem cells, particularly in autograft paradigms, that transcend the technical hurdles of isolation, expansion, and differentiation and would compromise our ability to combat disease if they were our only weapon in the stem cell armamentarium. While autografts of immunologically compatible and "ethically neutral" adult stem cells are often touted as being useful for such diseases as Huntington's, Alzheimer's, Parkinson's, ALS, and MS, it is probably *not* optimal to employ such cells for genetically based diseases: one will be reimplanting cells that *may already be flawed* in that they harbor a genetic defect and perhaps a predisposition to the disease in question. Indeed, since there has been growing speculation that the locus of some neurodegenerative

diseases may actually reside *within* the progenitor cell population—for example, a failure of progenitors to repopulate adequately, to resist or metabolize certain toxins, or to have a shortened lifespan—this could make for a truly disappointing outcome. To correct these somatic (including adult) stem cells ex vivo (or endogenous progenitors in situ for that matter) presumes a level of genetic understanding that we do not possess and will not likely have for decades.

This potential limitation for genetically based diseases would still seem to allow for the possibility that adult stem cells might be useful for trauma-based or acquired deficits. There, too, circumspection is warranted. While grafting a patient's own stem cells in such circumstances might circumvent ethical and immunological concerns, one now confronts practical hurdles that may be daunting. When a trauma or stroke patient, for example, presents to a health care facility, the team is confronted theoretically with the prospective isolation, expansion, decontamination, characterization, and directed differentiation of cellular reagents for *each* new patient with its attendant costs in time, resources, and manpower, with potential interpreparation, interpatient, and interinstitutional variability. There will certainly be issues of quality control. (It is unlikely that we would want most hospitals doing this in an unregulated on site fashion.) If one is to use *non*-neural adult stem cells for neural purposes, for example, it also presumes the ability to scale up a low efficiency transdifferentiation event to a clinically relevant level, which, in turn, presupposes a knowledge of the signals involved and an ability to provide them controllably—a goal far from realized. Also significant is the recognition that a finite amount of time is needed to isolate, characterize, and ensure the quantity, identity, quality, and safety of the cellular population to be implanted. It is becoming increasingly recognized that the optimal time for dealing with CNS injury using stem cells is during the acute or subacute period. It is possible that, by the time adequately characterized cells are optimized for use, the window of opportunity for using them may have passed. While future studies will be needed to define the obligate time windows for both preparation and implantation, this consideration may also limit the use of adult stem cells for a significant subset of cases.

An alternative to the use of autografts in transplantation-based strategies is the use of stable, established somatic stem cell lines that might serve as "universal donor cells" for most patients. These have the appeal of being off-the-shelf reagents, prepared and/or additionally engineered under good manufacturing practices, readily available in limitless quantities for the acute phases of an injury or disease. Its downside, of course, is the possibility of immune incapability (possibly addressed through some additional genetic engineering) and the fact that the best source for such universal lines may, in fact, be the embryo or the fetus, not the adult, where various immunogenic markers are less prevalent. (Indeed, we have determined that such NSCs lack major histocompatibility complex (MHC) class II and, when used for intraspecies transplants, do not provoke immunorejection or require immunosuppression.)

So, the answer to "Which cell for which disease?" might, in fact, vary from disease to disease, and organ to organ, to be determined empirically over the next decade.

6. Complex diseases require complex solutions

The complexity of most disorders—particularly in the CNS—calls for multifaceted solutions. Restitution of function likely requires more than the replacement of a single cell type (even if a neuron), but rather reconstitution of the entire milieu, including glia,[106,111] angioblasts, etc—i.e., cells that might provide trophic support, guidance cues, myelination for new neurons, refine differentiation,[96,105,109,111] detoxify the environment, and maintain homeostasis. Indeed, one may want to take advantage of the ability of the neural stem cell itself to give rise to multiple types of both neurons and glia in the appropriate ratio. Furthermore, cell-mediated interventions must go hand-in-glove with adjunctive pharmacological and molecular interventions, orchestrated to synergistically overcome restrictions imposed by the milieu, e.g., proangiogenic and/or anti-excitotoxic, anti-inflammatory, anti-apoptotic manipulations. And, of the cell-based therapies, combinations of stem cells may be required; various types at perhaps different developmental or differentiation stages for different phases of a given disease.

Therefore, *combined therapies* will likely be the key to most neurological diseases. While stem cells are just one weapon in an armamentarium, they may be glue that bonds these multiple approaches. The question then becomes how to orchestrate these interventions such that they work synergistically and not at cross-purposes. Manipulating one parameter is bound to affect another, often in unanticipated ways. We learned recently, for example, that engineering NSCs to over-express neurotrophin-3 to optimize neuronal differentiation did so at the expense of subverting the NSC's production of GDNF, the very factor needed to promote axonal outgrowth from some neurons.[104] Anti-apoptotic therapies are quite useful in a number of pathological conditions.[96,105] Interestingly, however, it is becoming recognized that apoptotic signals are pivotal for directing NSCs toward particular cell replace-

ment fates.[92,107] How might one combine anti-apoptotic therapy with stem cell therapy such that they do not abrogate the efficacy of each other?

A similar conundrum may pertain to inflammation. Indeed, stem cells themselves, at least in the CNS, appear to produce anti-inflammatory factors.[105,109] In fact, certain inflammatory molecules (e.g., interleukin-6) are inimical to stem cell function and survival. However, we are also beginning to realize that some of the signals used by stem cells to home to degenerating CNS areas are likely to be chemokines released at certain phases of the inflammatory reaction—both from activated microglia and from damaged CNS parenchyma. How might this "yin and yang" of inflammation be accommodated to best effect?

7. The devil is in the details—practical issues

Even after the philosophical and scientific issues have been resolved, one is still left with the logistical issues that ultimately determine whether clinical translation is feasible and successful.

1. Given that, in every organ system, the stem cell population to be abstracted is small, what is the most efficacious yet safest method for expanding it ex vivo such that the cells maintain genetic fidelity from passage to passage (i.e., faithful self-renewal with few mutations), do not experience phenotypic drift, and do not senesce? Is it with growth factors, with certain genes interacting with cell cycle regulatory proteins (e.g., *myc*) or with telomeres (e.g., telomerase), or a combination of the above?[87]
2. Prior to transplantation, what is the optimal degree of differentiation of a somatic stem cell for a particular disease—a *predifferentiated*, rigidly committed state *or* actually less differentiated and more plastic, *letting the cells mature in situ?* In the case of cells for the nervous system, implanting cells precommitted ex vivo to yield a uniform mature neural cell type (e.g., a motor neuron or an oligodendrocyte or DA neuron) would certainly maximize numbers (and, in the case of ES cells, safety); yet their ability to engraft, to respond and accommodate to varying environmental cues, to migrate, to integrate, to provide other needed neural cell types may be compromised. Also it presumes a knowledge of exactly what cell types are desired for a given disease. In other words, providing solely 1 neural cell type may construe the pathophysiology of a given disease so simplistically and narrowly that recovery may actually *not* be realized. For example, while conventional wisdom would dictate that spinal motor neurons need to be replaced in ALS, there is growing evidence that glial cells (in particular, astrocytes) may be the initiating cell in the disease process.[86,100,105] Therefore, predifferentiating cells to yield solely motor neurons may actually preclude optimal preservation of function or inhibition of degeneration. The best approach for this disease may be a *minimally* committed cell that yields both neurons *and* glia simultaneously and in a proportion dictated by the host environment itself.
3. How and where does one deliver cells, particularly for a disease process that is expansive in the CNS into the parenchyma? the cerebrospinal fluid? the vasculature? How does one make sure that the cells do not go to inappropriate tissue beds, for example, stem cells engineered to express a growth factor, or antiangiogenic factor, or cytolytic factor designed for the brain but also lodged and integrated into the liver or lung? When in the course of a given disease should the stem cells be delivered and how often?
4. How much of an obstacle will immune barriers actually be in transplantation paradigms? This is critical because it influences how much emphasis is placed on (1) using autologous cells vs. universal donor cell lines; or (2) obtaining stem cells from more accessible but less optimal tissues in an adult (e.g., skin for brain diseases) versus from more abundant but less accessible locations (e.g., NSCs from periventricular regions for CNS diseases); or (3) the need for somatic cell nuclear transfer; or (4) the need for potentially toxic immunosuppressive pharmacology.
5. How much, in fact, do human stem cells differ from nonhuman stem cells? How quickly can insights from rodents and primates be imported directly to clinical applications?

"Low Hanging Fruit" in the Neural Stem Cell Field

Despite the admonitions to be cautious and circumspect, are there disease processes that are within the grasp of proven stem cell properties and might be approachable in the relatively near future? There are some in our view:

1. *Brain tumors.* It has been observed that NSCs are rapidly drawn to such intracranial pathology and can effectively express antitumoral genes.[97] In such conditions, differentiation into a particular cell type is unimportant. The NSCs simply need to serve a tracking function to effectively deliver an oncolytic product while creating no "mischief." If the NSCs are armed with a gene that kills dividing cells, they will self-eliminate should they become problematic, a built-in safety bonus. A potential immune reaction against NSCs intermixed with a tumor

engenders little concern since that serves only to enhance their tumor-killing action.

2. *Metabolic neurogenetic diseases of childhood.* For these typically incurable neurodegenerative diseases, one often simply needs to express, in a disseminated manner, a very small amount of a diffusible lysosomal enzyme to restore normal CNS metabolism. Cell replacement is a bonus but not a requirement. In going into a young CNS, however, one may be able to harness developmental processes that optimize benefit. Because their monogenetic basis and pathobiology have been identified, many of these childhood diseases can serve as excellent models for certain adult diseases that are their phenocopies but are complicated by other factors.
3. *Diseases where rescue/protection of cells and circuits or blunting inflammation/scarring is therapeutic.* As described above, these actions—via the regulated local release of multiple identified and as-yet-to-be identified molecules—are attainable via stem cells in a manner unlikely to be achieved by drugs, pumps, or viral vectors. If in the course of these studies, cell replacement and/or the promotion of host regeneration is observed, that is a bonus. However, as previously noted, for stroke, spinal cord injury, ALS, Parkinson's disease, and MS, the protection of pre-existing circuits may be as important and more tractable than attempting to establish proper, functional new ones. It would be optimal to approach these pathologies early in their onset *before* muscle atrophy, contractures, scarring, and various *non*neurobiological complications present insurmountable barriers and when the signals directing stem cell behavior appear to be at their peak. Ultimately, we will learn to deal with chronic situations by recreating the acute milieu in that setting, fooling the stem cells into thinking they are confronting a recently injured system. Finally, attempting to preserve function one spinal segment at a time—rather than aiming to rescue the entire cord—is not only more realistic but can nevertheless powerfully impact a patient's quality of life if, for example, that one segment preserves respiratory independence or movement of a finger.

In the course of approaching these more modest but achievable goals, we as investigators will not only establish a track record of safety and efficacy, but will be able to learn enormously, including devising protocols for addressing some of the practical issues outlined above.

Cell Replacement Is Translational Developmental Biology

In thinking about the practical application of stem cell biology to clinical situations, it is instructive to remember that the stem cell field emerged as the unanticipated byproduct of investigations by developmental biologists into fundamental aspects of cell fate determination and commitment. Stem cell behavior is ultimately an expression of developmental principles, an alluring vestige from the more plastic and regenerative stages of organogenesis. In attempting to apply stem cell biology therapeutically, it is instructive always to bear in mind what role the stem cell plays in *development* and to what cues it was designed to respond in trying to understand the logic behind its behavior.

While the state-of-the-art of the stem cell field is sobering, we should be left not feeling that the obstacles are insurmountable, but simply that much work remains, principally in understanding both fundamental developmental principles *and* basic pathophysiological processes. We must understand how these 2 forces interact before cell replacement and circuit reconstruction will become tractable for most diseases (whether by exogenous cells transplanted in or endogenous cells drawn out). Circumspection is not a retreat from the promise of stem cells, simply an acknowledgement of the sophistication that will be required to exploit them properly.

References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145-1147.
2. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol. 1985;87:27-45.
3. Ding S, Wu TY, Brinker A, et al. Synthetic small molecules that control stem cell fate. Proc Natl Acad Sci U S A. 2003;100:7632-7637.
4. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes. action potential characterization. Circ Res. 2003;5:5.
5. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343:230-238.
6. Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. Nat Med. 2002;8:955-962.
7. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. Cell. 2002;110:385-397.
8. Kim JH, Auerbach JM, Rodriguez-Gomez JA, et al. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature. 2002;418:50-56.
9. Bjorklund LM, Sanchez-Pernaute R, Chung S, et al. Embryonic stem cells develop into functional dopaminergic neurons

after transplantation in a Parkinson rat model. Proc Natl Acad Sci U S A. 2002;99:2344-2349.
10. McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med. 1999;5:1410-1412.
11. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol. 2001;19:1129-1133.
12. Schuldiner M, Eiges R, Eden A, et al. Induced neuronal differentiation of human embryonic stem cells. Brain Res. 2001;913:201-205.
13. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. Nat Biotechnol. 2001;19:1134-1140.
14. Carpenter MK, Inokuma MS, Denham J, Mujtaba T, Chiu CP, Rao MS. Enrichment of neurons and neural precursors from human embryonic stem cells. Exp Neurol. 2001;172:383-397.
15. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes. 2000;49:157-162.
16. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science. 2001;292:1389-1394.
17. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. Diabetes. 2001;50:1691-1697.
18. Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. Proc Natl Acad Sci U S A. 2002;99:16105-16110.
19. Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. Science. 2003;299:363.
20. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature. 2003;422:897-901.
21. Shizuru JA, Weissman IL, Kernoff R, Masek M, Scheffold YC. Purified hematopoietic stem cell grafts induce tolerance to alloantigens and can mediate positive and negative T cell selection. Proc Natl Acad Sci U S A. 2000;97:9555-9560.
22. Shizuru JA, Jerabek L, Edwards CT, Weissman IL. Transplantation of purified hematopoietic stem cells: requirements for overcoming the barriers of allogeneic engraftment. Biol Blood Marrow Transplant. 1996;2:3-14.
23. Hole N, Graham GJ, Menzel U, Ansell JD. A limited temporal window for the derivation of multilineage repopulating hematopoietic progenitors during embryonal stem cell differentiation in vitro. Blood. 1996;88:1266-1276.
24. Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell. 2002;109:29-37.
25. Muller AM, Dzierzak EA. ES cells have only a limited lymphopoietic potential after adoptive transfer into mouse recipients. Development. 1993;118:1343-1351.
26. Perlingeiro RC, Kyba M, Daley GQ. Clonal analysis of differentiating embryonic stem cells reveals a hematopoietic progenitor with primitive erythroid and adult lymphoid-myeloid potential. Development. 2001;128:4597-4604.
27. Rideout WM, 3rd, Hochedlinger K, Kyba M, Daley GQ, Jaenisch R. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. Cell. 2002;109:17-27.
28. Nakayama N, Lee J, Chiu L. Vascular endothelial growth factor synergistically enhances bone morphogenetic protein-4-dependent lymphohematopoietic cell generation from embryonic stem cells in vitro. Blood. 2000;95:2275-2283.
29. Li F, Lu S, Vida L, Thomson JA, Honig GR. Bone morphogenetic protein 4 induces efficient hematopoietic differentiation of rhesus monkey embryonic stem cells in vitro. Blood. 2001;98:335-342.
30. Chadwick K, Wang L, Li L, et al. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. Blood. 2003;17:17.
31. Dyer MA, Farrington SM, Mohn D, Munday JR, Baron MH. Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neurectodermal cell fate in the mouse embryo. Development. 2001;128:1717-1730.
32. Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a "Suicide" gene. Stem Cells. 2003;21:257-265.
33. Alison M, Sarraf C. Hepatic stem cells. J Hepatol. 1998;29:676-682.
34. Watt FM. Epidermal stem cells: markers, patterning and the control of stem cell fate. Philos Trans R Soc Lond B Biol Sci. 1998;353:831-837.
35. Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA. Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. Dev Biol. 2002;245:42-56.
36. Gordon JI, Schmidt GH, Roth KA. Studies of intestinal stem cells using normal, chimeric, and transgenic mice. FASEB J. 1992;6:3039-3050.
37. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414:105-111.
38. Goldring K, Partridge T, Watt D. Muscle stem cells. J Pathol. 2002;197:457-467.
39. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science. 1998;279:1528-1530.
40. Gussoni E, Soneoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature. 1999;401:390-394.
41. Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science. 1999;283:534-537.
42. Jackson KA, Mi T, Goodell MA. Hematopoietic potential of stem cells isolated from murine skeletal muscle [see comments]. Proc Natl Acad Sci U S A. 1999;96:14482-14486.
43. Pang W. Role of muscle-derived cells in hematopoietic reconstitution of irradiated mice. Blood. 2000;95:1106-1108.
44. Morshead CM, Benveniste P, Iscove NN, van der Kooy D. Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations. Nat Med. 2002;8:268-273.
45. McKinney-Freeman SL, Jackson KA, Camargo FD, Ferrari G, Mavilio F, Goodell MA. Muscle-derived hematopoietic stem cells are hematopoietic in origin. Proc Natl Acad Sci U S A. 2002;99:1341-1346.
46. Kawada H, Ogawa M. Bone marrow origin of hematopoietic progenitors and stem cells in murine muscle. Blood. 2001;98:2008-2013.
47. Jay KE, Gallacher L, Bhatia M. Emergence of muscle and neural hematopoiesis in humans. Blood. 2002;100:3193-3202.
48. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41-49.

49. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A. 1997;94:4080-4085.
50. Bittner RE, Schofer C, Weipoltshammer K, et al. Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Anat Embryol (Berl). 1999;199:391-396.
51. LaBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. Cell. 2002;111:589-601.
52. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999;85:221-228.
53. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science. 2000;290:1775-1779.
54. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science. 2000;290:1779-1782.
55. Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells in vivo. Science. 2002;297:1299.
56. Theise ND, Badve S, Saxena R, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology. 2000;31:235-240.
57. Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med. 2000;6:1229-1234.
58. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001;410:701-705.
59. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nature Med. 2001;7:430-436.
60. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001;107:1395-1402.
61. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell. 2001;105:369-377.
62. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science. 2002;297:2256-2259.
63. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell. 2001;105:829-841.
64. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature. 2002;416:545-548.
65. Terada N, Hamazaki T, Oka M, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature. 2002;416:542-545.
66. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature. 2003;422:901-904.
67. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta [see comments]. Nat Med. 1999;5:309-313.
68. Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. Hepatology. 2000;32:11-16.
69. Okamoto R, Yajima T, Yamazaki M, et al. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med. 2002;8:1011-1017.
70. Awaya N, Rupert K, Bryant E, Torok-Storb B. Failure of adult marrow-derived stem cells to generate marrow stroma after successful hematopoietic stem cell transplantation. Exp Hematol. 2002;30:937-942.
71. Hematti P, Sloand EM, Carvallo CA, et al. Absence of donor-derived keratinocyte stem cells in skin tissues cultured from patients after mobilized peripheral blood hematopoietic stem cell transplantation. Exp Hematol. 2002;30:943-949.
72. Korbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med. 2002;346:738-746.
73. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. N Engl J Med. 2002;346:5-15.
74. Laflamme MA, Myerson D, Saffitz JE, Murry CE. Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. Circ Res. 2002;90:634-640.
75. Muller P, Pfeiffer P, Koglin J, et al. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. Circulation. 2002;106:31-35.
76. Hruban RH, Long PP, Perlman EJ, et al. Fluorescence in situ hybridization for the Y-chromosome can be used to detect cells of recipient origin in allografted hearts following cardiac transplantation. Am J Pathol. 1993;142:975-980.
77. Glaser R, Lu MM, Narula N, Epstein JA. Smooth muscle cells, but not myocytes, of host origin in transplanted human hearts. Circulation. 2002;106:17-19.
78. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest. 2003;111:843-850.
79. Zorina TD, Subbotin VM, Bertera S, et al. Recovery of the endogenous beta cell function in the NOD model of autoimmune diabetes. Stem Cells. 2003;21:377-388.
80. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. Blood. 2001;97:2957-2961.
81. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med. 2001;344:1815-1822.
82. Buzanska L, Machaj EK, Zablocka B, et al. Human cord blood-derived cells attain neuronal and glial features in vitro expression of neural markers in human umbilical cord blood. J Cell Sci. 2002;115:2131-2138.
83. Chen J, Sanberg PR, Li Y, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke. 2001;32:2682-2688.
84. Goodell MA. Stem-cell "plasticity": befuddled by the muddle. Curr Opin Hematol. 2003;10:208-213.
85. Wulf GG, Jackson KA, Goodell MA. Somatic stem cell plasticity: current evidence and emerging concepts. Exp Hematol. 2001;29:1361-1370.
86. Rao SD, Yin HZ, Weiss JH. Disruption of glial glutamate transport by reactive oxygen species produced in motor neurons. J Neurosci. 2003;23:2627-2633.
87. Gritti A, Parati EA, Cova L, et al. Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J Neurosci. 1996;16:1091-1100.
88. Doering LC, Snyder EY. Cholinergic expression by a neural stem cell line grafted to the adult medial septum/diagonal band complex. J Neurosci Res. 2000;61:597-604.

89. Yang M, Stull ND, Berk MA, Snyder EY, Iacovitti L. Neural stem cells spontaneously express dopaminergic traits after transplantation into the intact or 6-hydroxydopamine-lesioned rat. Exp Neurol. 2002;177:50-60.
90. Akerud P, Canals JM, Snyder EY, Arenas E. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. J Neurosci. 2001;21:8108-8118.
91. Liu Y, Himes BT, Solowska J, et al. Intraspinal delivery of neurotrophin-3 using neural stem cells genetically modified by recombinant retrovirus. Exp Neurol. 1999;158:9-26.
92. Rubio F, Kokaia Z, Arco A, et al. BDNF gene transfer to the mammalian brain using CNS-derived neural precursors. Gene Ther. 1999;6:1851-1866.
93. Himes BT, Liu Y, Solowska JM, Snyder EY, Fischer I, Tessler A. Transplants of cells genetically modified to express neurotrophin-3 rescue axotomized Clarke's nucleus neurons after spinal cord hemisection in adult rats. J Neurosci Res. 2001;65:549-564.
94. Kitchens DL, Snyder EY, Gottlieb DI. FGF and EGF are mitogens for immortalized neural progenitors. J Neurobiol. 1994;25:797-807.
95. Gould E, Reeves AJ, Graziano MS, Gross CG. Neurogenesis in the neocortex of adult primates. Science. 1999;286:548-552.
96. Li M, Ona VO, Chen M, et al. Functional role and therapeutic implications of neuronal caspase-1 and -3 in a mouse model of traumatic spinal cord injury. Neuroscience. 2000;99:333-342.
97. Kornack DR, Rakic P. Cell proliferation without neurogenesis in adult primate neocortex. Science. 2001;294:2127-2130.
98. Eriksson PS, Perfilieva E, Bjork-Eriksson T, et al. Neurogenesis in the adult human hippocampus. Nat Med. 1998;4:1313-1317.
99. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc Natl Acad Sci U S A. 1993;90:2074-2077.
100. Bruijn LI, Becher MW, Lee MK, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron. 1997;18:327-338.
101. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol. 2002;174:11-20.
102. Kerr DA, Llado J, Shamblott MJ, et al. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. J Neurosci. 2003;23:5131-5140.
103. Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol. 2002;52:802-813.
104. Lu P, Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp Neurol. 2003;181:115-129.
105. Zhu S, Stavrovskaya IG, Drozda M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature. 2002;417:74-78.
106. Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. Nature. 2002;417:39-44.
107. Zhao M, Momma S, Delfani K, et al. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A. 2003;100:7925-7930.
108. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997;17:3727-3738.
109. Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat Biotechnol. 2002;20:1111-1117.
110. Shamblott MJ, Axelman J, Littlefield JW, et al. Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. Proc Natl Acad Sci U S A. 2001;98:113-118.
111. Wagner J, Akerud P, Castro DS, et al. Induction of a midbrain dopaminergic phenotype in Nurr1-overexpressing neural stem cells by type 1 astrocytes. Nat Biotechnol. 1999;17:653-659.