

# stem cell PHARMACEUTICS

Recent research advances in the understanding of stem cell properties are inevitably leading to therapeutic strategies potentially applicable to many diseases. The pharmaceutical manipulation of multipotential cells such as stem cells is therefore now primed to evolve in its own right. This article focuses on known effects of introduced biologics and small molecules, and on the future of strategies enhancing the *ex vivo* or *in vivo* regenerative properties of these remarkable cells.

**T**herapies targeting multipotential cells offer hope to treat many degenerative diseases caused by the premature death or malfunction of specific cell types. With a lack of suitable pharmaceutical treatments and long waiting lists for transplantable organs, such cell oriented approaches are being advanced based on either the introduction of cells into patients (*ex vivo* cell therapies) or the use of agents to affect cells already within the patient (*in vivo* therapies). Cell pharmaceuticals are thus the application of biological or chemical molecules to enhance these therapeutic approaches and it is an emerging area of biotechnology taking advantage of research advances in fields such as cell signalling and the use of growth factors to repair damage caused by disease, trauma and processes such as ageing.

Therapies targeting multipotential cells address numerous large healthcare markets by promising novel therapies to treat debilitating diseases such as diabetes, Parkinson's, Huntington's, heart disease and stroke, as well as accidental damage such as spinal cord injury. They are emerging as a potentially revolutionary way to treat malignancies, blood disorders, as well as certain inborn errors of metabolism and immunodeficiencies. Replacement of the blood and immune systems with blood stem

cells, the use of neural stem cells to treat neurodegenerative systems, the use of mesenchymal stem cells to repair bones and joints and liver stem cells for liver failure are just a few examples of clinical applications of endogenous multipotential cell targeted therapies (Figure 1).

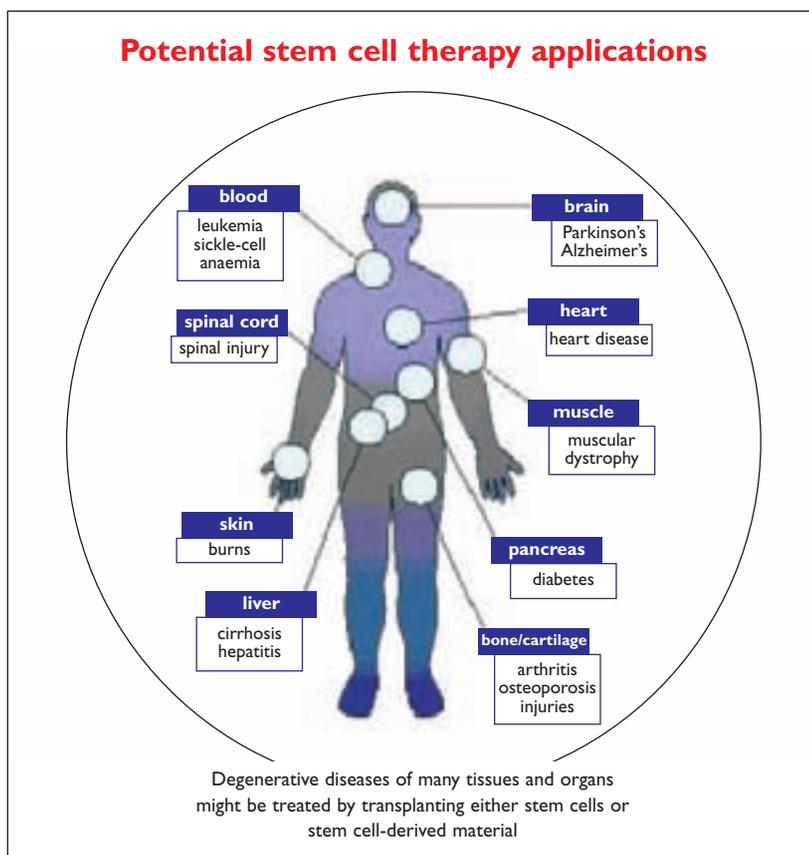
Taking into consideration the potential therapeutic benefits and thus market opportunities, drugs designed to enhance stem cell activity have a bright future. Like the development of most pharmaceuticals, the most effective ways to discover these agents will, however, need to encompass several disparate phenomena that will be briefly reviewed in this article: the natural distribution of such multipotential cells, molecules already known to affect them, technical opportunities to expand our knowledge of such cells and pharmaceuticals, and the regulatory environment which will greet such pharmaceutical products.

## Multipotential cell distribution

Human cells that have the potential to give rise to many differentiated cell types, such as stem cells, can be found in the inner cell mass of the early embryo, in some tissues of the foetus, the umbilical cord and placenta, and in several adult organs. Sources of adult stem cells that have the potential

By Dr Gregory G. Lennon

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**Figure 1**

Degenerative diseases of different tissues/organs amenable to cell therapies

Source: MRC, London: stem cell therapy

manipulate them *in vitro*<sup>2</sup>. These methods suffer from both qualitative inadequacies, since the process may itself change the intrinsic properties of the cells and may entirely miss many types of multipotential cells, and quantitative shortcomings, in that it remains to be seen how reflective numbers estimated from such methods will compare to numbers enumerated from actual adult tissues.

For example, the stem cells of the pancreas, which can give rise to islet cells, are usually thought to be in the pancreatic ducts, but methods of isolating them so that they can be expanded in the laboratory have not been found. Similarly, stem cells do exist in the brain and spinal cord, but are rare and tend to be in regions that are difficult to access. If insufficient numbers of stem cells turn out to limit some potential therapeutic applications it may become important to find ways to increase the numbers of multipotential cells prior to other therapeutic interventions.

Transdifferentiation or reprogramming may be one means to augment multipotential cell numbers. Several cell types, even including cells that appear to have terminally differentiated, may turn out to actually have the ability to 'transdifferentiate' into cells of completely different types under appropriate stimuli. For example, blood cells could be converted into neuronal cell types of use in treating Parkinson's disease (Figure 2).

### Molecules affecting stem cells

Once we identify the molecules that act on stem cells, we might be able to use similar molecules to recruit more stem cells to an area of damage in the body. Based upon the type and chemical composition, molecules acting on stem cells can be classified as:

#### Proteins

Proteins which are known to act on stem cells and are used clinically include G-CSF (granulocyte colony stimulating factor<sup>3,4</sup>; GM-CSF (granulocyte-macrophage colony stimulating factor<sup>5</sup>; and Albugranin™ (Albumin Granulocyte Colony Stimulating Factor).

#### Drugs

AMD 3100 is an example of a drug having specific actions and effects on stem cells. Many drugs,

to become specialised cell types include bone marrow, blood, the eye, brain, skeletal muscle, dental pulp, liver, skin, the lining of the gastrointestinal tract and pancreas.

Certain stem cells are rather rare, or at the least inaccessible, and thus difficult to identify and purify. When grown in culture, stem cells are difficult to maintain in an undifferentiated state and may be subject to undetected changes occurring over time. Though there is now a widespread consensus that many adult mammalian organs do contain stem cells, there is no consensus about how many populations of stem cells exist. (For that matter, the total number of cell types in the body as previously estimated based on such criteria as morphology is in flux due to more detailed data available from techniques such as molecular profiling.) One reasonably well known estimate<sup>1</sup> is that there is one hematopoietic stem cell present in every 10,000 to 15,000 nucleated bone marrow cells. However, a lack of consensus on stem cell numbers can be readily seen from publications pointing out that there are no markers currently available to identify *in vivo* several stem cell types, and that the only method for testing whether a given population of cells contains stem cells is to isolate the cells and

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including clozapine, cerebral vasodilators like vinpocetine, neuroleptics, anti-thyroid medications, analgesics, phenothiazine derivatives and anti-inflammatory drugs are known to induce agranulocytosis and bone marrow suppression at the committed stem cell level.

Table 1 summarises the positive effects, adverse effects and primary indications for some prominent protein and drug molecules acting on stem cells.

Many aspects of cell behaviour, such as growth, motility, differentiation and apoptosis, are regulated by signals cells receive from their environment. Such signals are important during embryonal development, wound healing, hematopoiesis and in the regulation of the immune response. These signals come from the binding of soluble signalling molecules like G-CSF and GM-CSF to specific receptors at the cell membrane<sup>6</sup>. Many growth factors bind to receptors which contain an intrinsic tyrosine kinase domain<sup>7</sup>, whereas many cytokines bind to receptors devoid of kinase domains but which bind to intracellular kinases of the Jak family<sup>8</sup>. In these cases, ligand binding induces receptor dimerisation or oligomerisation resulting in tyrosine phosphorylation of the receptors and activation of specific signalling pathways leading to cell growth, migration or the prevention of apoptosis<sup>9</sup>. There is a network of cytokine interactions and a cytokine cascade that allows considerable flexibility

and ready amplification of response to a particular molecular stimulus. Extensive profiling of the signalling pathways underlying the redirection of cell fate is clearly needed to drive the next generation of 'stem cell drugs'.

### Stem cell monitoring

As high-throughput screens to identify molecules acting on multipotential cells yield more leads, and as we begin to understand more aspects of cell specificity, it will become important to be able to monitor the effect of such agents on cells within relevant animal models as well as ultimately within human subjects. Monitoring cells as they are driven towards different cell fates will also involve the development of new techniques useful for both research and pharmaceutical development purposes.

The two methods most commonly used to measure changes in at least hematopoietic stem cell numbers, the colony-forming unit (CFU) assay and the CD34+ assay<sup>10</sup>, are effectively single measurement assays. The CFU assay involves growing a small portion of the stem cell collection in a culture. A single stem cell in the sample can form groups of mature blood cells, which are then microscopically counted. While the CFU assay is the best indicator of the ability of the stem cells to grow in a patient after transplantation, its major

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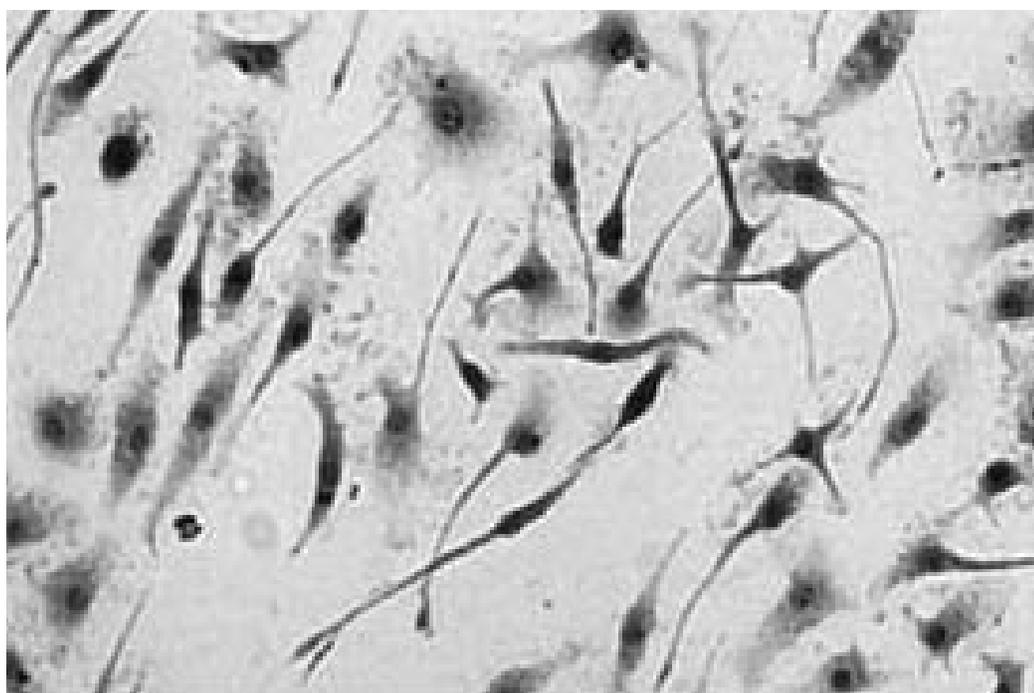
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**Figure 2**

Neuroblasts differentiated from bone marrow stromal cells: augmenting stem cell numbers via transdifferentiation  
Source: Laboratory Virola, Ukraine

**Table I: Molecules acting on stem cells**

		<b>POSITIVE EFFECTS</b>	<b>ADVERSE EFFECTS</b>	<b>CLINICAL INDICATIONS</b>
<b>PROTEINS</b>	G-CSF (Filgrastim)	Stimulates neutrophil granulopoiesis  Prevents infectious complications  Decreases the morbidity of cancer chemotherapy	Well tolerated  Bone pain-major adverse effect, occurring in 15-20% of patients  Headache & Rash  Asymptomatic increase in spleen size	Cancer chemotherapy  Bone marrow transplantation  Severe chronic neutropenia
	GM-CSF (Sargramostim)	Stimulates the growth & differentiation of a broad range of cell lineages, including granulocytes, macrophages, erythrocytes & eosinophils  Induces glucose transport, ion fluxes & expression of variety of genes	Not as well tolerated as G-CSF and has a broader range of side effects  Anaphylactic reactions reported on a limited basis & are poorly characterised  Rashes and local reaction at injection sites	Acceleration of myeloid engraftment after cancer chemotherapy, radiotherapy, and/or hemopoietic stem cell transplantation  Treatment of Juvenile Myelo Monocytic Leukemia (JMML)
	AG-CSF (Albugranin)	Significantly increases neutrophil counts for a longer time due to longer half-life  Reduces the number of life-threatening infections in cancer patients who can be at high risk during chemotherapy	Under investigation to evaluate its adverse effects if any	Treatment for chemotherapy-induced neutropenia
	PDGF-BB (Platelet derived growth factor)	Enhances ligament fibroblast proliferation <i>in vitro</i>  Improves the structural properties of a healing femur-MCL-tibia complexes (FMTCs) <i>in vivo</i>	Under investigation	Ligaments and tendons healing <i>in vivo</i> where a mid-substance tear generally cannot heal spontaneously
	BDNF (Brain-Derived Neurotrophic Factor)	Supports the growth and survivability of nerve and/or glial cells  Protects striatal GABAergic neurons against cell death	Under investigation	Potential for the neuroprotective treatment of Huntington's disease  Working directly on the serotonin system, it can be used for treating behaviour-related disorders
	GDNF (Glial Derived Neurotrophic Factor)	Neuroprotective effects on dopaminergic neurons following lesions of the nigro-striatal pathway  Rescue septal cholinergic cells from death following fimbria/fornix transections	Under investigation	To accelerate functional recovery following traumatic brain injury  Significantly reduce the infarct size and brain edema after ischemic brain injury

drawback is the need to wait two weeks before results can be obtained. This limits its usefulness in determining adequacy of stem cell collection for transplants. The CD34+ assay, measuring stem cell content by counting cells displaying CD34 surface protein with a flow cytometer, can give a readout in two hours. This can help prevent a patient from needlessly undergoing a second stem cell collection when the first collection was adequate.

Tracking the *in vivo* biodistribution and movement of either transplanted cells or endogenous cells of interest requires techniques that can monitor their fate non-invasively and repeatedly, such as by taking numerous 'snapshot' assessments of the cellular biodistribution at a particular given time point. One such technique uses tiny inorganic spheres known as Magnetic Dendrimers to magnetically label cells and then track them via Magnetic Resonance Imaging (MRI)<sup>11</sup> (Figure 3). This technique and other non-invasive, real-time approaches are allowing researchers to watch, for example, the movement and ultimately differentiation of stem cells after they are injected into the circulatory system.

As more information is gained about cell movement, knowledge of the microenvironments that impinge on those cells becomes equally important. Microenvironments have been implicated as critical for stem cell homeostasis and may be of equal importance as the differentiation state itself in determining a cell's ultimate fate. With the bone marrow cavity, surrounding stromal cells are clearly important mediators and provide a solid support upon which stem cell hematopoiesis occurs. The physical anchoring of the stem cell in its niche plays a vital role in the ability to respond to differentiation factors, and thus in the potential to become a cell type needed for regenerative purposes. For example, recent research<sup>12</sup> implicates D-cadherin-mediated cell adhesion, or 'cell glue', as a critical factor anchoring stem cells in a position to receive differentiation signals. Once the cell glue is lost, the stem cells are also lost and can no longer receive the instructions that control their actions. Thus, some of the signals instructing the stem cell to be primitive or definitive reside in the microenvironment and searches for pharmaceutically active molecules have and will continue to target such sources<sup>13-15</sup>.

Pharmaceutical therapies targeting multipotential cells may be best applied using drug delivery systems to ensure delivery to only certain sites within the body, mimicking microenvironments in their ability to act locally. Cell pharmaceuticals

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may thus be able to minimise systemic adverse effects while maintaining the efficacy benefits of high local drug tissue levels. The capability to direct drugs to specific multipotential cell populations, to confirm their presence at the site-of-interest, and to quantify the adequacy of the local drug concentration at the time of treatment through rational targeted drug delivery holds great promise for changing clinical treatment paradigms in the foreseeable future.

**Regulatory issues**

The clinical use of both *in vivo* and *ex vivo* cell targeting therapies must satisfy rigorous regulatory criteria. For *ex vivo* cell therapies, even if one can set aside ethical considerations surrounding the use of cells derived from embryos, there are still significant regulatory hurdles in the form of a variety of FDA regulations. Certain existing stem cell lines may not be used in clinical trials due to their propagation using mouse cell feeder layers, and thus possible non-human contamination. Furthermore, trials involving the injection of large numbers of multipotential cells will require long-term studies to estimate the frequency with which such cells can either themselves become cancerous or increase the frequency at which endogenous cells do. Therapies using drugs or proteins to act on endogenous cells already present with the patient may face issues of

target selectivity as discussed earlier but, on the other hand, will have a decided regulatory advantage in that the clinical trials and approval procedures will be consistent with well established pharmaceutical regulatory processes.

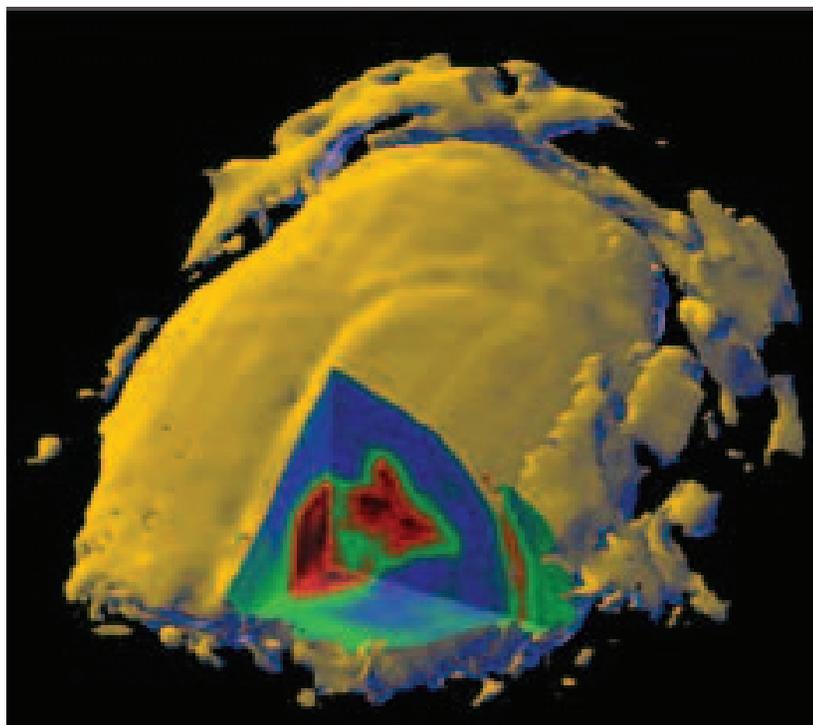
More specifically, in October 1993, the FDA published a notice stating that somatic cell therapy products are biological products under the PHS Act as well as drugs under the FD&C Act and are thus subject to investigational new drug (IND) application requirements as regulated by the CBER section of the FDA<sup>16</sup>. While this remains true for these *ex vivo* cell therapies, an announcement made in 2002 by the FDA indicates that in the future proteins (such as G-CSF or other biologics acting on multipotential cells as part of the *in vivo* therapies discussed) will be regulated by the CDER section of the FDA, as small molecule drugs have been.

**The future of stem cell pharmaceuticals**

Since it is now clear that the adult human body contains numerous types of multipotential cells, future research must increasingly address the mechanisms by which these cells choose – and change – their cellular fates in order to advance the translation of this research into clinically relevant therapies. Such research will need to focus both on the development of positive effectors and on the minimisation of adverse consequences, with both

**Table I (continued): Molecules acting on stem cells**

		<b>POSITIVE EFFECTS</b>	<b>ADVERSE EFFECTS</b>	<b>CLINICAL INDICATIONS</b>
<b>DRUGS</b>	AMD 3100	Mobilises pluripotent hematopoietic progenitor cells to the peripheral blood  When used in combination with G-CSF, hematopoietic stem (HSC) and progenitor cells (HPC) mobilising capacity of AMD 3100 is greatly enhanced	Well tolerated. Mild adverse effects reported, which include: Nausea, Headache, Transient perioral paresthesias	Mobilisation and collection of CD34+ cells for clinical hematopoietic stem cell transplantation
	Clozapine	Novel pharmacological profile, lack of extra pyramidal effects and therapeutic benefit in tardive dyskinesia  Impressive symptom resolution and improvement in the quality of life	Induces immunologic destruction of myeloid progenitors  Prolonged agranulocytosis with significant morbidity, mortality and high incidence (1% – 2%)	Schizo-affective and other affective disorders like psychotic depression  Psychosis in patients with dementia  Parkinson's disease



**Figure 3**

Non-invasive monitoring of stem cells: MRI detects the magnetically labelled stem cells (red and green) moving away from the injection site in a living rat's brain. Computer software enables the scientists to construct 3-dimensional images and 'peel off' layers of information until only the brain remains

requiring the concomitant development of increasingly sophisticated techniques to non-invasively monitor and image specific cell populations.

In terms of positive effectors, there are most likely at least three classes to be further developed. First, agents capable of 'simply' increasing the abundance (and perhaps distribution) of multipotential cells will be developed. Second, *ex vivo* therapeutic approaches are likely to drive the development of agents enhancing the survival of implanted stem cells. And of perhaps most significance, the development of classes of agents able to influence the fate of specific endogenous multipotential cell populations will be advantageous.

Adverse consequences of cell differentiation

must be faced as well, both in the context of spontaneous excessive cell conversion (such as to precancerous cells, which themselves may therefore be targets for inhibitors of multipotentiality) and in the context of undesired differentiation of injected multipotential cells. Safety concerns must be addressed in order to garner FDA approval of biologics and drugs capable of altering the fate of multipotential cells, and extensive studies are likely to be needed in the case of *ex vivo* cell therapies involving large numbers of stem cells.

The presence of numerous multipotential cell types in adults makes it clear that the cells of an adult are not as static as previously thought. Hence, the repair of organs damaged by either disease or injury will be facilitated by cell-based therapies such as the directed activation of endogenous cells to accelerate organ regeneration. Already, several families of proteins and drugs are known to be involved in regulating endogenous signals that direct cell migration and differentiation, with most acting as partners in complex pathways. For pharmaceutical development, one promising emphasis in the future will be the development of single agents able to dramatically alter cell fate, both positively and negatively, to achieve long-lasting and highly effective medical treatments. **DDW**

*Gregory G. Lennon obtained a PhD in Genetics from the University of Pennsylvania, after which he created the first arrayed cDNA library while a post-doc in the lab of Hans Lehrach. While a senior scientist at Lawrence Livermore National Laboratory's Human Genome Center, he founded the public cDNA effort known as the IMAGE Consortium, and then developed the extensive gene expression database at Gene Logic Inc where he was Chief Scientific Officer. He is currently the CEO and founder of Endogeny Bio Corporation.*

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