STEM CELLS
the long march forward to the clinic

The area of cellular therapeutics has never been readily embraced by 'Big Pharma', with ethical and regulatory issues even more complex and troublesome than proteins. Can stem cell therapy provide the key to unlocking a rich vein of pharmaceutical activity?

By Dr Alan Colman

In 1956, it was reported that the lives of lethally irradiated mice could be saved by injection of bone marrow cells taken from healthy mice. Irradiation destroys the blood forming system – the survival of the transplanted mice was due to the restoration of this system by the transplanted bone marrow. Further investigation[1] revealed that the early phases of recovery were accompanied by the appearance of distinct colonies of donor cells in the spleens of the recipient mice. Each colony was the result of the capture and subsequent proliferation of a special type of donor cell – a stem cell. Current use of bone marrow transplantation in clinical practice is a direct consequence of this pioneering mouse work and attests to the extraordinary properties of stem cells. One objective of this article is to show how the extension of stem cell therapy to a broad range of human diseases, is now an imminent prospect. However, such are the crucial roles of stem cells in bodily functioning, that even this tantalising possibility may be dwarfed by future therapeutic strategies which recruit and stimulate the patient's own stem cells into effecting repair.

What are stem cells and how do they arise during development?

Stem cells (**Figure 1**) have the unique property of being able to provide exact replacements for themselves. They can also form the precursors of specialised cell types. In blood, these would include the various cell types that comprise the blood including erythrocytes, neutrophils and lymphocytes.

It is widely believed that most tissues of the body harbour stem cell populations that serve a maintenance function in replacing dead or damaged cells (**Figure 2a**). In blood, the short lifetime of the circulatory cells (eg red blood cells last about one month) ensures that the haemopoietic (ie blood-forming) stem cell population is very active. By contrast, in the brain where resident specialised cells like neurons survive for years, stem cell activity is extremely low. In organs like the liver, stem cell activity can be stimulated by tissue damage or disease. Because of the relative rarity of stem cells, they have proved very difficult to identify individually. Historically, their existence has been supported by bioassays like the spleen colony-forming assay referred to above. Only in recent times has it proved possible to purify and characterise adult stem cells.

The ultimate mammalian stem cell is the fertilised, one-cell embryo. This will produce all the diverse cell types that comprise the foetal and adult forms, and also tissues like the placenta, which serve to nurture the growing foetus. For this reason this cell is termed totipotent. As we shall see, no other stem cell has such broad properties. Development proceeds by a series of cell divisions, cell movements and differentiation into specialised cell types. Whether the appearance of new cell types is preceded by the formation of the stem cells of the corresponding lineage, is not clear. If stem cells do form in very early embryos, they certainly do not resemble their adult counterparts. Indeed, adult stem cells may be formed in later life by the de-differentiation of a specialised cell. For

example, liver regeneration is thought to be due to the activities of a stem cell called an oval cell. The oval cell is formed by the dedifferentiation of that most characteristic of liver cells, the hepatocyte. However, irrespective of the normal existence of stem cells in the early embryo, an important class of stem cells can certainly be experimentally derived from early embryos as shown in **Figure 2b**.

Methods first developed in mice, but now extended to a variety of mammalian species including hamsters, rats, cows, monkeys and humans, allow the production in the laboratory of embryonic stem (ES) or embryonic germ (EG) cells from early embryos or foetal germ cells respectively. For simplicity, we will refer to either cell type as an ES cell. Under certain circumstances (see below) ES cells can give rise to all (mice) or most (other species) of the cell types of the adult. They cannot form the extra-embryonic tissues like placenta, and for this reason are described as pluripotent. Adult-derived stem cells with more restricted potentialities are referred to as multipotent because they can only produce a restricted range of cell types.

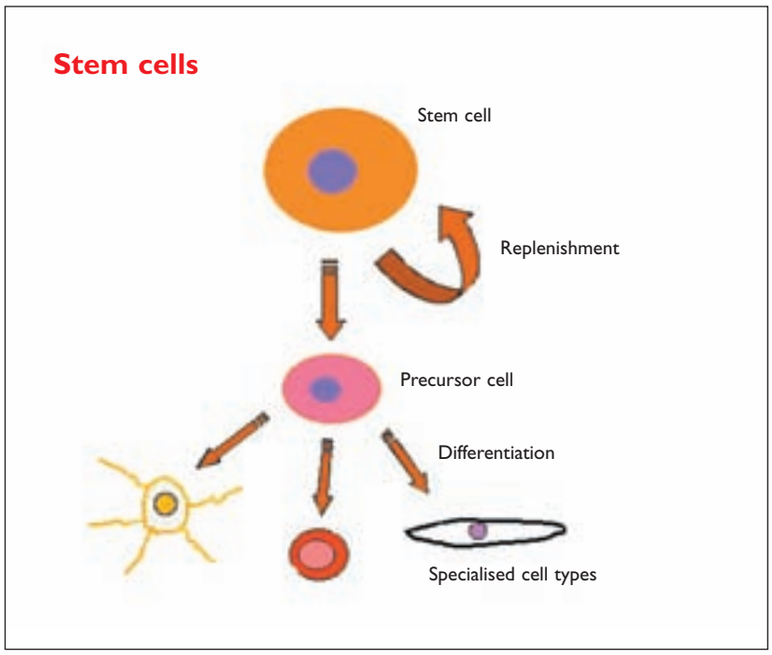


Figure 1
A generic stem cell: stem cells can divide to produce an identical stem cell or, alternatively, form a precursor cell. The precursor cell is capable of many cell divisions before differentiation into one or more specialised cell types

Why the current excitement?

The brain would not appear to provide a fertile experimental area to the inquisitive stem cell biologist. It has an amorphous appearance and its most defining cell type, the neuron, is a delicate cell whose complex and extensive axonal and dendritic protrusions are easily destroyed or disrupted by dissection. For many years, it was believed that regeneration of nerve tissue did not occur in adult mammals and that no resident stem cell population existed. Yet it is from this unlikely source that a revolution in the way we view stem cells has emerged.

In 1992, Reynolds and Weiss[2] isolated cells from a mouse brain and demonstrated that these cells could be cultured and multiplied as cellular aggregates called neurospheres. By adjustment of the growth conditions, with the removal and/or addition of specific growth factor molecules, these cells could be induced to differentiate into two different brain cell types, neurons and oligodendrocytes. This was a timely demonstration that at least one stem cell type could be propagated for long periods in culture, since cell multiplication in culture remains a major problem for most adult stem cell types. More importantly, this work served as a springboard for a plethora of recent reports that point to an unexpected plasticity in the properties of stem cells[3]. The most spectacular example of this was the demonstration by Clarke et al[4] that not only could the neurospheres produce neural cell types, but also form many unrelated cell types including kidney, liver, bone, etc. These dramatic findings have now been extended to a wide variety of enriched stem cell populations prepared from diverse tissue sources: haemopoietic stem cells can form liver and muscle; neural stem cells can form blood, muscle, kidney, skin, lung and other cell types; muscle stem cells can form blood; and finally, mesenchymal stem cells normally responsible for bone and cartilage, can form neurons and other brain cells. In some experiments, the cells have been transplanted into animal models of neurodegeneration or metabolic disease with resulting partial repair of the lesion. For example, Yandava et al[5] were able to promote reconstruction of damaged CNS regions by injecting murine neural stem cells into the brains of shiverer mice, so-called because of the trembling caused by incomplete myelination of axons. Lagasse et al[6] restored the biochemical function of the liver in mouse lacking a liver enzyme by injection of bone marrow cells.

There remains considerable uncertainty in the identity of the colonising stem cells present in non-neural preparations (these could be contaminating neural stem cells, for example). These and other findings hold great promise for the therapeutic application of stem cells in two areas, cell and gene therapy

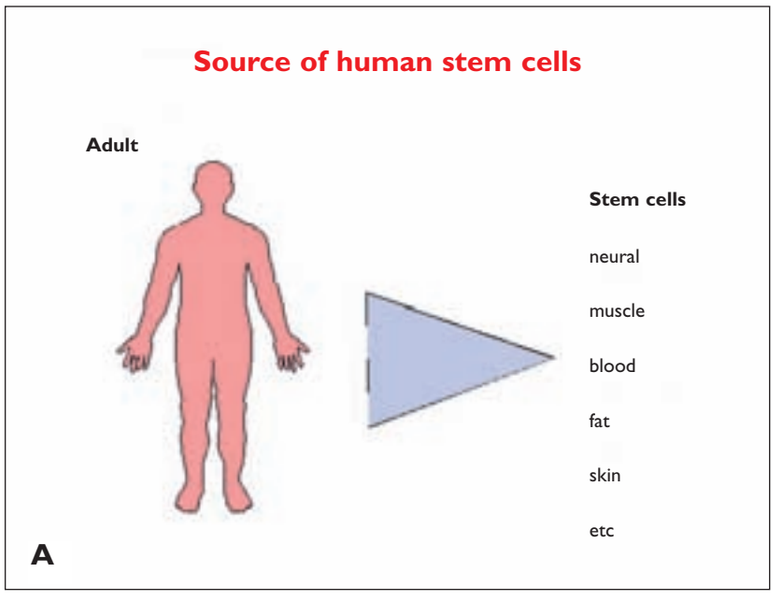
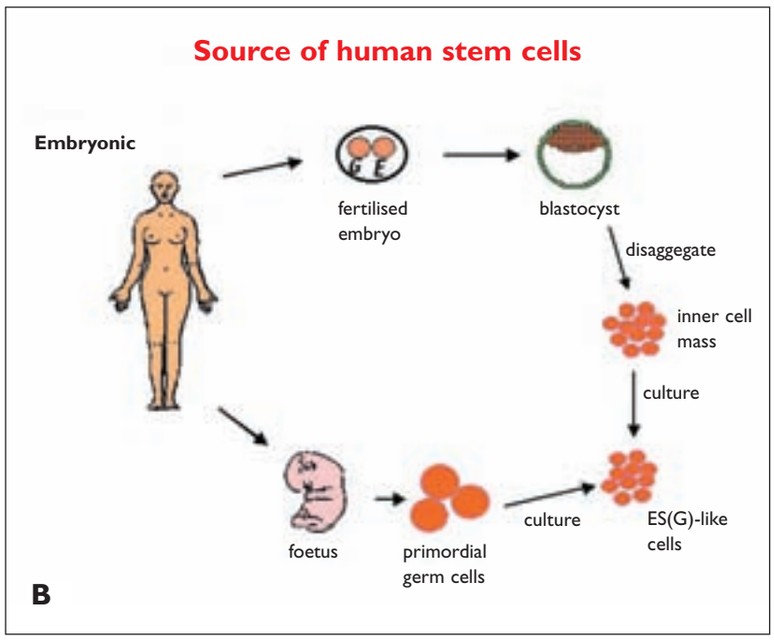


Figure 2
Sources of stem cells: stem cells occur naturally in adults **(A)** but are obtained experimentally from either embryos or foetal germ cells **(B)**



Cell therapy

In the US alone it has been estimated that up to 128 million people suffer from chronic, degenerative or acute diseases. In many cases, highly complex and poorly understood biological pathways have been disrupted and cannot be treated by conventional pharmaceutical (drugs) or biotechnological (proteins or peptides) approaches. Diabetes afflicts nearly 16 million US citizens with treatment costs approaching $140 billion annually (American Diabetes Association). Parkinson's and Alzheimer's affect 500,000 and five million respectively in the nervous system disorder category. Heart disease and stroke claim one million US lives per year and cost more than $285 billion per year (American Heart Association). A variety of other diseases and conditions could be mentioned including Huntingdon's, chronic intractable pain, muscular dystrophy, osteogenesis imperfecta and epilepsy. It is clear that as the population continues to age, the financial and human burden presented by these diseases will continue to rise. This scenario presents a commercial opportunity for cell therapy companies. **Table 1** shows the product development status in a variety of established companies.

Cell therapy is already a well-established therapeutic modality. Both blood transfusion and bone marrow transplantation are routinely used in clinical practice. Recently, liver hepatocytes were transplanted into a 10-year-old girl with Crigle-Najjor syndrome. The hepatocytes were infused via the portal vein and survived for more than 11 months, and partially corrected the metabolic disorder[7]. Advances in *in vitro* cell culture technology have now increased the scope of cell transplantation therapy. It is now possible to grow skin cells in culture and to cryopreserve structurally stabilised sheets of cells for subsequent use on burns patients. While the long-term survival of the transplanted cells remains uncertain, the transplants perform a valuable protective function and might also stimulate proliferation of host cells aligned around the margin of the treated wound area.

Table 2 displays other desirable cell candidates for transplantation. For most of these examples, the nature of the cell types precludes their removal from healthy donors and subsequent expansion of cell numbers in culture; most specialised cells simply will not grow in culture. Stem cells or their cultured progeny provide an attractive alternative. Two recent pre-clinical studies using murine cells provide examples of both strategies.

Diabetes results from abnormal function of

beta cells present in the pancreatic islets of Langerhans. Clinical trials using cadaveric sources of islets are proving a very successful substitute for insulin therapy[8]. However, supply of suitable human material will be severely limiting since 2-3 pancreases are needed per patient, so another source of material is needed. Lumelsky et al[9] report that with judicious use of various growth conditions, mouse ES cells can be converted into three-dimensional clusters resembling pancreatic islets and containing insulin producing cells, although the insulin content of the cells is much lower than in beta cells. Diabetic mice injected subcutaneously with these cells maintain their body weight better than diabetic controls but their hyperglycaemia was not cured probably because of the as yet sub-optimal availability of insulin.

Coronary heart disease accounts for 50% of all cardiovascular deaths and nearly 40% of the incidence of heart failure. Orlic et al[10] find that injection of enriched murine bone marrow stem cells into infarcted mouse hearts effected repair of the myocardium in 40% of the mice. Meanwhile, Kocher et al[11] used human bone marrow stem cells to improve heart function in 30-40% infarcted rats; in this case, the transplanted cells were injected into the rats via the tail vein, found their way to the damaged heart and participated in and stimulated the production of new blood vessels from host heart tissue. These results reveal the exciting potential of stem cells to contribute specialised cell replacement components to the repair of damaged tissue. Kocher et al[11] also reveals two other facets of stem cells – their ability to home in on the site of damage and their ability to recruit host cells into an orchestrated repair processes. These properties could offer important therapeutic advantages. First, the homing ability of stem cells could be exploited to effect repair in inaccessible areas of the body after simple intravenous injection. Second, the central role stem cells normally play in tissue maintenance and the self-regulating nature of many stem cell-mediated processes may offer safety and other advantages over the use of other cell types and other therapeutic modalities. Third, stem cells may be used to target gene constructs to desired tissues with the objective of stimulating tissue remodelling (by expression of specific differentiation factors) or to destroy tissue-specific tumours. For example, Aboody et al[12] were able to effect an 80% reduction in a mouse malignant brain tumour by intravenous injection of genetically engineered neural stem cells.

What are the challenges?

The challenges facing future stem cell therapy strategies are legion. Some of the issues would apply to any cell therapeutic. For example, if the cells are not derived from the patient, all preparations will have to be validated to exclude infectivity and any tendency to become cancerous. Validation will have to be performed on large batches leading to concerns about methods of storage and distribution with the consequent loss of some cell viability. A further issue that greatly constrains bone marrow transplantation is the immunological mismatch posed by donor to patient transplants. Unless strategies can be designed to modify the immune response (eg tolerance induction) or to procure stem cells from the patient, chemical immunosuppression may be necessary with all the attendant long-term side effects. Other problems will depend on whether stem cells or their derivatives are used in therapy.

Adult stem cells

Adult stem cells are either difficult or impossible to access in sufficient numbers. The most favoured adult stem cell is the haemopoietic stem cell. These cells are present in umbilical cord blood and companies have formed to freeze down and store the tissue as insurance against life threatening disease in the donor. Haemopoietic stem cells will not proliferate or survive for long in culture. While it is possible to rescue an irradiated mouse with a single stem cell, this is not possible in humans where residual host (and diseased) stem cells would outcompete the transplanted cells. Unless culture techniques improve or are supplemented for example, by addition to the cells of an active telomerase gene, this need for numbers may prevent therapeutic use of cord blood taken and stored at the patient's birth.

Given the ease with which different stem cells seem to lose their lineage restrictions, adult neural stem cells would seem to offer a compromise since expansion of cell numbers in culture is not a problem. However, long-term culture may affect the ability of the cells to differentiate as expected[13]. The plasticity of adult stem cells has been attributed to the effects of the different microenvironments to which they are exposed during transplantation. If uncontrolled changes of this nature occur to some of the cells during therapeutic application, concerns will arise regarding the appearance of unexpected structures during tissue repair.

Finally, most cell therapy will probably need

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Table 1: Cellular therapies in development

COMPANY	PRODUCT	INDICATION	STAGE OF DEVELOPMENT	MARKET SIZE $M (APPROX)
Aastrom Biosciences	Bone Marrow Therapy	Chemotherapy induced injury	Phase III	0-500
Aastrom Biosciences	Cord Blood Therapy	Chemotherapy induced injury	Phase III	0-500
Aastrom Biosciences	Dendritic cell therapy	Cancer	Preclinical	2,001-5,000
Advanced Tissue Sciences	Dermagraft	Diabetic ulcers	Launched	501-2,000
Advanced Tissue Sciences	Transcyte/dermagraft TC	Burns	Launched	501-2,000
Aegera	Stem Cell Technologies	Unspecified	Preclinical	10,000+
Alexion	UniGraft	Parkinson's, Spinal cord injury	Preclinical	10,000+
AVAX technologies	B-Vax	Cancer	Phase II	2,001-5,000
AVAX Technologies	Chondrocyte Therapy	Regeneration	Preclinical	5,001-10,000
BioTransplant	AlloMune System	Transplant rejection	Phase II	501-2,000
BioTransplant	XenoMune System	Transplant rejection	Preclinical	501-2,000
Biovector Therapeutics	CMV cell therapy	Unspecified	Preclinical	2,001-5,000
BresaGen	Stem Cell Therapies	Parkinson's	Preclinical	501-2,000
Cell Based Delivery	BioArtificial Muscle	N/A	Preclinical	N/A
Cerus	allogeneic cell immune therapy	Transplant rejection, bone marrow	Phase I	2,001-5,000
Cerus	Stem Cell Therapy	Cancer	Preclinical	2,001-5,000
Chimeric Therapies	Allomax LK	Transplant rejection	Phase III	501-2,000
Curis	Cell Based therapies	Diabetes, Type I and II	Preclinical	2,001-5,000
Cypress Bioscience	Cyplex	Surgery adjunct	Phase II	501-2,000
Dendreon	Mylovenge	Cancer	Phase II	2,001-5,000
Dendreon	APC-8015	Cancer	Phase III	2,001-5,000
Dendreon	Cancer Immunotherapy	Cancer	Preclinical	2,001-5,000
Diacrin	Human liver cells	Cirrhosis	Phase I	501-2,000
Diacrin	Human muscle cells	Unspecified	Phase I	10,000+
Diacrin	NeuroCell-FE	Epilepsy	Phase I	501-2,000
Diacrin	NeuroCell-HD	Huntington's chorea	Phase I	501-2,000
Diacrin	NeuroCell-PD	Parkinson's	Phase II	501-2,000
Diacrin	Porcine Cell Technology	Intractable human disease	Phase II	N/A
Diacrin	HepatoCell	Hepatic Dysfunction	Preclinical	501-2,000
Diacrin	Porcine RPE cells	Macular degeneration	Preclinical	2,001-5,000
Diacrin	Porcine spinal cells	Spinal cord injury	Preclinical	10,000+
EnCelle	Encellin XP	Diabetes, Type I	Preclinical	2,001-5,000
EntreMed	Theramed	Unspecified	Preclinical	N/A
Genzyme	DC-tumour fusion	Cancer	Phase II	2,001-5,000
Geron	Human Pluripotent cells	Unspecified	Preclinical	501-2,000
Geron	Telomerase Vaccine	Cancer	Preclinical	2,001-5,000
Hemosol	HML-115	Cancer	Preclinical	2,001-5,000
Igeneon	IGN-201	Cancer	Preclinical	2,001-5,000
Immuno-Designed Molecules	Dendritophages	Cancer	Phase II	2,001-5,000
Immuno-Designed Molecules	MAK therapy	Cancer	Phase II	2,001-5,000
Immuno-Designed Molecules	IDM-1	Cancer	Phase III	2,001-5,000
Incara Pharmaceuticals	Progenitor cells	Hepatic dysfunction	Preclinical	501-2,000
Inflammatics	LeukoVAX	Arthritis	Phase II	5,001-10,000
Innogenetics	AutoDerm	Burns	Launched	501-2,000
Intracel	OncoVAX-CL	Cancer	Launched	2,001-5,000
Layton Bioscience	LBS-Neurons	Ischaemia	Phase II	10,000+
Modex	EpiDex	Diabetic ulcers	Phase II	501-2,000
Modex	Insulin cell therapy	Diabetes, type I	Preclinical	2,001-5,000
Modex	Encapsulated cell technology	Cancer	Preclinical	2,001-5,000
NeuroNova	Stem Cells	Unspecified	Preclinical	0-500
Neurotech	Encapsulated cell technology	N/A	Phase II	N/A
Neurotech	Anaemia therapy	Anaemia	Preclinical	2,001-5,000
Neurotech	NTC-200	Macular degeneration	Preclinical	2,001-5,000
Neurotech	NTC-201	Retinitis	Preclinical	2,001-5,000
Northwest Biotherapeutics	Dendritic cell vaccine	Cancer	Preclinical	2,001-5,000
Onyvax	Onyvax-CR	Cancer	Phase II	2,001-5,000
Organogenesis	Apligraf	Ulcers	Launched	501-2,000
Organogenesis	Vitrix	Wound Healing	Phase I	501-2,000
Organogenesis	Tissue Engineered products	N/A	Registered	N/A
Ortec	Composite cultured skin	Burns	Registered	501-2,000
Osiris Therapeutics	Allogen	Chemo injury	Phase II	0-500
Proneuron Biotechnologies	Autologous Macrophage therapy	Spinal cord injury	Phase I	10,000+
Research Corporation Technologie	Sertoli Cells	Diabetes, Type I	Preclinical	2,001-5,000
Stem Cells	Cell Based Therapies	Liver disease	Preclinical	501-2,000
Stem Cells	Neural stem cells	Alzheimer's, Parkinson's	Preclinical	2,001-5,000
Takara Shuzo	Dendritic vaccine	Cancer	Phase I	2,001-5,000
Targeted genetics	CellExSys	AIDS cell therapy	Phase I	0-500
Titan	Spheramine	Parkinson's	Phase II	501-2,000
Transgene	Cellular vectors	N/A	Preclinical	N/A
Vasogen	VAS-981	Transplant rejection	Phase I	501-2,000
Vasogen	VAS-972 PST	Psoriasis	Phase II	0-500
Vasogen	CLL Therapy	Cancer	Preclinical	2,001-5,000
Vasogen	VAS-971	Reperfusion injury	Preclinical	10,000+
Vasogen	VasoCare PST	Peripheral Vascular Disease	Registered	10,000+
Xcyte Therapies	Xcellerate	Cancer, renal infection	Phase I	2,001-5,000

Source: Pharmaprojects version 4; May 2001. PJB Publications LTD.

prior conversion of stem cells into the desired cell type. The production of large numbers of specialised cell types from stem cells will present a major challenge because of a tendency of stem cells in culture to produce many different cell types simultaneously. Quantitative conversion into one cell type has never been achieved leading to a need for sophisticated cell selection and purification technology in order to enrich for the desired cell type.

ES cells

Lee et al[14] reported that whereas cultured adult neural stem cells could not differentiate into dopaminergic neurons, those derived from ES cells could. This emphasises a principal virtue of the ES cell, its unlimited growth potential and pluripotency. However, as with adult stem cells, indeed more so, defining the exact conditions for inducing production of the correct cell type in culture remains a challenge while the dangers of contaminating undifferentiated ES cells (they can form teratocarcinomas) are clear. Since ES cells cannot be found in the adult, the immunological difficulties raised above, will probably be unavoidable. In principle, the use of therapeutic cloning could avoid this issue[15,16]. Here, a healthy cell from a patient can be used in somatic nuclear transfer (the 'Dolly' procedure) to prepare a customised ES cell immunologically identical to the patient's own cells. However, at present this technique seems too costly and inefficient to ever be a commercial proposition. In addition, this and the use of ES cells in general are still subject to considerable debate and controversy worldwide[17], principally due to the issue of the embryo destruction needed to make ES cells in the first place.

Conclusion

The promise revealed above will not be transferred into clinical practice for several years, and even this timeline could be extended by a premature move to the clinic. The recent less than spectacular success of a Parkinson's disease trial where foetal neurons were transplanted into 15 patients[18] has been cited as a warning even though no stem cells were involved in this trial. The ethical debate about human ES cell use rumbles on with the UK seemingly the most progressive of all countries. Sceptics will point out that many of the claimed functional demonstrations of successful stem cell therapy may be attributed to a stimulation of host cell recovery but herein lies one of the most interesting and commercially relevant messages: Big Pharma does not embrace cellular therapeutics readily. The regulatory issues are even more complex and troublesome than those associated with proteins. The ability of stem cells to recruit and stimulate local remedial activity probably reflects the activities of molecules secreted from these cells. Identification of these factors and their targets should open up a rich vein of pharmaceutical activity. It also suggests the biblical revision of St Luke's 'physician heal thyself' to 'body heal thyself'. DDW

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Table 2
Specialised cell types required for cell therapy

CELL TYPE	USE
Cardiomyocytes	Heart repair after myocardial infarction
Islet cells	Types 1 and 2 diabetes
Endothelial cells	Blood vessel repair for atheroschlerosis or ischemia
Chondrocytes	Replacement purposes in osteoarthritis, Osteoporosis or rheumatoid arthritis
Fibroblasts and keratinocytes	Wound healing or burns
Hepatic cells	Hepatitis and cirrhosis
Respiratory epithelial cells	Lung cancer, emphysema, cystic fibrosis
Retinal pigment epithelial cells	Macular degeneration