

1 The Meritocracy of Stem Cells for Therapy

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Recent developments in stem cell biology have indicated a remarkable differentiation plasticity of adult stem cells in many tissues in the body. These findings have led to hopes that stem cell therapy can solve the problem of degenerative disorders for which organ transplantation is inappropriate or there is a shortage of organ donors. Stem cell transplantation for regenerative medicine is still in its infancy, but in many ways may be comparable to the early years of clinical bone marrow transplantation for haematological disease. Moreover, it can be anticipated that the progress of stem cell therapy in this broader context will gain from the experience in bone marrow transplantation. For example, bone marrow transplantation benefited from the dissemination of information via the transplant registries, much is known of the interactions between grafted cells and host tissue, there is considerable experience in the conditioning necessary for acceptance of the transplanted stem cells and processing technologies that are compatible with Good Manufacturing Practice requirements and regulatory authority approval have been developed.

Stem cells with the potential for the treatment of a wide range of degenerative disorders may be obtained from a variety of sources but, for practical reasons, some of them are more likely to find earlier clinical application than others. The main types that have been studied in the context of stem cell therapy are embryonic, foetal, and adult stem cells. The bone marrow is an easily available source of mesenchymal and haemopoietic stem cells that have been proposed for application in the treatment of tissue damage and degeneration like Alzheimer's and Parkinson's diseases, heart failure, myocardial infarction, diabetes, and liver insufficiency. Both the haemopoietic and mesenchymal stem cell pools contain, or generate, subpopulations of stem cells that have been variously claimed to have therapeutic potential. Already, the results of preliminary clinical trials evaluating bone marrow-derived stem cells in the treatment of heart disease and liver failure have been reported and it is timely to consider the relative merits of some of the candidate stem cell populations.

Adult stem cells exist in many tissues and organs and these stem cells have differentiation potentials beyond those required to regenerate the tissue or organ in

which they reside.¹ Clearly, some of these sources of adult stem cells, like brain stem cells, are less accessible than others, like bone marrow stem cells. The use of bone marrow-derived stem cells for tissue and organ repair has the additional advantage that there is considerable experience in the clinical application of bone marrow transplantation for the regeneration of the haemopoietic system, dating from the 1970s when relatively large clinical studies were initiated. By 2002, 20,207 haemopoietic stem cell transplants had been performed in Europe by 586 teams in 39 countries. This clinical experience has been accompanied by a wealth of laboratory studies on haemopoietic stem cells and transplantation biology.

In general, two distinct stem cell populations are thought to reside in haemopoietic tissue. These are the haemopoietic and the mesenchymal stem cells. Classically, the haemopoietic stem cells are the precursors of all of the blood cells,² and the mesenchymal stem cells are the source of the supporting stromal cells of the bone marrow, lineages³ including the osteogenic, chondrogenic, and adipogenic lineages.⁴ Multipotent adult progenitor cells (MAPC) are a sub-population of cells that arise in cultures of mesenchymal stem cells and seem to have a broader differentiation potential than the mesenchymal stem cells themselves.⁵ However, many cell population doublings are required before MAPC arise in mesenchymal cell cultures and this has been associated with a potential for genetic instability. Other consequences are that it is not known if MAPC exist *in vivo* or what their *in vivo* phenotype may be, MAPC cannot be prospectively isolated from a tissue like the bone marrow and there is no quantitative assay for MAPC so that it is not possible to predict with any accuracy how much tissue would be required to supply enough cells for a particular application. Similar considerations apply to other types of stem cell sub-populations with a mesenchymal cell origin that have been described.⁶⁻⁸

There are a number of key points that should be considered with regard to the clinical application of stem cells: ethical acceptability, ease of availability, numbers available, phenotypic identification for prospective isolation, physiological normality, and regulatory compliance. Ideal features of a stem cell population for therapy would be the identification of a homogeneous stem cell population from an ethically uncontroversial source that exists normally *in vivo* and which could be prospectively isolated from an easily available tissue like the blood or bone marrow. Ideally, prolonged tissue culture would not be necessary and the number of cells required would be attainable within a short period of time. Identification of a cell type in the bone marrow with these properties and with the ability to differentiate into multiple cell types would fulfil the immediate requirements for the early clinical application of stem cell therapy.⁹

The origin of embryonic stem cells is ethically unacceptable to many individuals since it requires the manipulation of pre-implantation embryos and compromises embryonic survival. Unacceptability is based on the belief that an egg fertilised *in vitro* has the same rights as a post-natal human being and has resulted in limitations of federal funding in the United States. Considerable efforts are being made to circumvent the ethical hurdles involved in the clinical application of embryonic stem

cells.^{10–11} In contrast, adult stem cells for clinical trials in regenerative medicine are obtained without severe intervention from sources like bone marrow and blood, and with informed consent.

The stem cells need to be obtained from an accessible source. Bone marrow and blood are obvious choices with the advantage that there is considerable experience in bone marrow harvesting and leukapheresis of G-CSF mobilised blood in the haematological community. Moreover, the identification of the stem cells in the source tissue is desirable since it allows prospective enumeration of the cells and also confirms that they are a normal physiological component. This requires knowledge of their phenotypic properties. Mesenchymal stem cells remain poorly characterised and stem cell populations like the MAPC⁵ and USSCs⁸ are only derived after long periods in culture. In contrast, the VSEL,¹² side population and Hoechst low rhodamine low cells¹³ are identifiable by multiparameter flow cytometry and can be purified by FACS and the small lymphocyte-like stem cells we have described express CD34 and are separated from the bulk of the CD34-positive cells using their adherence properties. A stem cell population that is purified to homogeneity may be desirable because this permits a high concentration of the relevant cells to be transplanted locally into injured tissue. Regulatory compliance includes method of isolation and flow cytometry, for example, does not fulfil this objective. However, isolation of CD34+ cells from haemopoietic tissue has been used in stem cell transplantation and some devices for this manipulation are accepted by the regulatory authorities.

The number of stem cells available for clinical use can be a limiting factor. These numbers may be available immediately or require a period in culture to amplify them. The difficulties associated with amplifying haemopoietic stem cell numbers are notorious because stem cells tend to divide asymmetrically and this is incompatible with an increase in stem cell numbers.^{14–17} Also, large numbers of cell divisions introduce the risk of genetic instability.¹⁸ Some of these obstacles may be overcome by so-called therapeutic cloning although it attracts some of the controversies associated with embryonic stem cell research. Amplification in cell numbers can be achieved by genetic manipulation of the stem cells but this step seems unwarranted, except perhaps in extreme cases, in view of the inherent risks attached to transplanting permanently, or conditionally, self-renewing cells. Embryonic stem cells and, to a lesser extent, foetal stem cells have the potential to repair many types of tissues because they are totipotent.¹⁹ Embryonic stem cells can be greatly increased in number in culture as cell lines *in vitro* and may be immuno-privileged. These attributes mean that they can be used to treat multiple patients. However, their use has been confounded by the very real likelihood that, being immortal, they will form tumours after they have been transplanted into patients.²⁰ Undoubtedly, however, these barriers to widespread application will be overcome in the future.

In spite of the limited knowledge about the best source and type of stem cells to use for clinical applications, stem cell therapy for degenerative conditions is being applied in several settings. For example, stem cell-induced cardiac regeneration

in patients with ischaemic heart failure has now been investigated by many groups with encouraging results.²¹ The cells administered were obtained from bone marrow and injected by intramyocardial, intracoronary, and transendocardial routes. We have performed a phase I clinical trial of stem cell transplantation in patients with liver insufficiency. For this, autologous mobilised stem cells were procured and purified before they were injected into the portal vein or hepatic artery for local delivery into the damaged tissue.⁹ This experience has demonstrated the safety and lack of toxicity of the procedure and has led to the initiation of a phase II clinical trial. These examples demonstrate that the revolution of using stem cells for regenerative medicine has begun. It is to be anticipated that the future will see ever expanding applications of this novel approach to conditions involving tissue damage and degeneration.

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