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Review Article:
Therapeutic Potential of Stem Cells

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INTRODUCTION

The use of hemopoietic stem cells derived from the bone marrow (BM) or from the peripheral blood after mobilization with growth factors, for the treatment of benign and malignant hematological disorders, is well established. This review is meant to cover the use of stem cells in regenerative therapy to repair or substitute other organs e.g. Liver, cardiac or skeletal muscles, neurological, pancreatic, renal tissue or others. In fact, it is the experience with BM transplantation (BMT) that opened the way for the potential use of stem cells to regenerate organs other than the BM. This was derived from an observation of hepatocytes carrying the Y chromosome in a female patient receiving a male BM allograft. Unless cell fusion has occurred, there would be no other explanation but the transformation of one or the other cell types of the BM graft into hepatocytes (vide infra).

Several diseases are characterized by depletion of the stem cell pool such as BM failure due to malignancy of the hematopoietic stem cell (HSC) or genetic defects in the HSC itself (e.g. Fanconi's Anemia); these diseases have been successfully treated by BMT. Other diseases involve destruction of tissues that may not be robustly replenished from stem cell pools e.g. liver failure due to cirrhosis; these diseases are dealt with by direct organ transplantation. However, organ transplantation has much limitation with the first and most prominent being inadequate availability of organs [1]. Besides, organ transplantation does not apply in other situations e.g. CNS diseases or muscular dystrophy.

It has to be clearly stated, however, that the results of stem cell therapy for regenerative medicine are still, largely, controversial. It has to be clearly stated, as well, that this should not hinder clinical trials as long as the fundamental criteria supporting ongoing trials have been met [2]. The declaration of Helsinki states. "In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent of the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering" [3].

Historically, it was only 40 years ago that two types of stem cells were identified, namely "hematopoietic" stem cells and BM "stromal" cells. In 1981 "embryonic" mouse stem cells (mESC) were isolated and described [4,5]. In the 90s, stem cells were discovered in the brain and more recently in the liver, heart, skin, GIT…etc. In 1998, human embryonic stem cells (hESC) were isolated [6].

By definition, stem cells should be capable of self-renewal, differentiation into at least one mature cell type as well as functionally repopulate the tissue of origin when transplanted in a damaged recipient. Stem cells are subclassified based on their species of origin, tissue of origin and their potential to differentiate into specific type(s) of mature cells. This latter character is referred to as stem cell potency. The hierarchies of stem cells include totipotent, pluripotent capable of producing the three germinal layer cells, multipotent giving rise to cells of close

family e.g. hemopoietic cells, oligopotent e.g. giving rise to a limited number of blood cell lineages, bipotent e.g. giving rise to B cells and macrophages and unipotent giving rise to one cell type. The only totipotent stem cell is the zygote and its immediate daughter cells. The embryonic stem cells are harvested from the inner cell mass of the blastocyst 7-10 days after fertilization and early differentiation; they are supposed to keep this totipotency. After birth, the stem cells are called adult stem cells. Adult (postnatal) stem cells, while still pluripotent, have been thought to have more limited differentiation ability and to be organ specific e.g. HSC that give rise to all types of blood cells, neural stem cells (NSC) that give rise to neurons, astrocytes, and oligodendrites, mesenchymal stem cells (MSC) that differentiate into fibroblasts, osteoblasts, chondrocytes and adipose tissue, endothelial progenitor cells (EPC) that give rise to endothelial cells of blood vessels, oval cells that can give rise to hepatocytes and biliary tract, pneumocytes that can give rise to lung cells….etc. [7]. However, this concept of specificity of adult stem cells has been recently challenged and adult stem cells proved to show some flexibility; this flexibility is termed "plasticity". A widely accepted definition of "plasticity" has yet to be established but, in general, it refers to the ability of adult stem cells to cross lineage barriers and adopt the expression profile and functional phenotype of cells that are unique to other tissues [7].

Possible mechanisms for plasticity:

Normal body homeostasis is kept by a balance between the rate of cell loss and regeneration. Under physiological conditions, this latter is secured by proliferation of progenitor cells; the tissue stem cells may be quiescent or undergo a very low rate of proliferation. In case of tissue injury, the stem cells may be called upon. If the injury is mild, the local stem cells will take care of the repair. But if the injury is too much, the local stem cells in the tissue may not cope; the cytokines released as a result of the injury will mobilize the BM stem cells, push them in circulation where ultimately they are going to home to the injured tissue through receptor ligand interaction. All the studies documenting stem cell plasticity used models of tissue injury to induce homing and differentiation of transplanted stem cells. Tissue damage likely creates a favorable environment for the crossing of lineage barriers. Probably tissue injury, through apoptosis and/or necrosis, creates a microenvironment (e.g. cytokine milieu or extracellular matrix characteristics) that enables efficient engraftment of circulating stem cells [7] Tissue injury may, probably create a situation comparable to the physiological situation necessitating continuous proliferation and production of new hemopoietic cells by the BM. Translineage differentiation of BM stem cells may occur either through differentiation or fusion. The proof of one mechanism does not exclude the others.

Direct and indirect differentiation:

Several mechanisms may be involved:

1- BM cells that differentiate into diverse cell types represent a previously unsuspected and unrecognized population of high pluripotent uncommitted stem cells located in the BM; in this case this is not true plasticity.

2- Committed HSCs undergo transdifferentiation. "Transdifferentiation" refers to ability of one committed cell type to change its gene expression pattern to that of a completely different cell type. This may be indirect through dedifferentiation going back to a stem cell phenotype and then redifferentiate along a different cell lineage. Alternatively it could be direct transdifferentiation involving direct transition in the gene expression pattern.

Fusion: An alternative mechanism would be the fusion of BM derived cell with an organ specific somatic cell to form a heterokaryon, thereby having the specific gene expression pattern of the organ in a cell capable of dividing and differentiation into new cells belonging to that organ. Cell fusion per-se is a known phenomenon e.g. in vitro fusion of fibroblasts with myoblasts is known to result in expression of muscle specific mRNA by the fibroblast nuclei [8]. However its contribution to plasticity is controversial.

Several studies have used chromosomal analysis to show that BM derived lung, muscle [9], and kidney [10] are 2N, suggesting but not proving that they do not result from fusion but rather transdifferentiation. Cell ploidy, however, is not an absolute proof as some tetraploid cells can be present in normal tissue and the other

way round a tetraploid cell resulting from fusion could subsequently be a 2N particularly if the 2 nuclei did not fuse. In contrast to the previous studies other workers reported the opposite in the case of severely injured liver **[11,12]**. In both studies, donor derived BMSC were transplanted into FAH–/– mice, and engraftment into hepatocytes was proved by weaning of the FAH–/– mice off NTBC, the drug which allows them to survive in the absence of the FAH enzyme. In those mice, the majority of the hepatocytes that were FAH+ (donor derived) also had markers of the recipient cells suggesting that fusion had occurred. Whether fusion occurs or not and the magnitude of its contribution to plasticity still awaits final answers. Fusion may be a naturally occurring phenomenon, or a response to intense tissue injury. If the resulting cells are healthy and functional, the phenomenon will have a great physiological significance. Of course, the concern that the resulting cells may carry high potential for malignant transformation will always be in the background.

Stem cell sources:

Currently the main source of stem cells for regenerative therapy is embryonic stem cells and BM derived Adult stem cells. However other types of stem cells are used mainly fetal stem cells, cord blood stem cells and placental stem cells. Also in special situation like CNS, specialized stem cells may be specifically collected.

EMBRYONIC STEM CELLS

Mouse ESCs were first isolated in 1981 **[4]**. Human ESCs were first reported in 1998 **[6]**. Although in its infancy, hESC is thought to represent a theoretically inexhaustible source for regenerative medicine, a research tool to study development, both normal and abnormal and also to provide a platform to develop and test new therapies. When ESC are removed from culture conditions that block differentiation, they aggregate and develop into cystic structures called embryoid bodies containing derivatives of all three embryonic germ layers **[13]**. However human development is a complex choreography of events, each taking place in a critical temporal and spatial pattern. Unraveling the developmental pathways that specify formation of specific tissue within the embryo, so the possibility that these pathways can be recapitulated in vitro, is one of the main challenges in hESC research **[1]**. Many cell types have been derived from hESC in vitro including neural tissue **[14-17]**, insulin secreting cells **[18]**, cardiomyocytes **[18-21]**, hematopoietic cells **[22-24]**, endothelial cells **[15]**, osteoblasts **[25]** and hepatocytes **[26]**.

The strategy to produce these specialized cells from hESC involves 3 factors:

1- A combination of culture conditions that favors differentiation towards one or the other cell type.

2- Transgenic approaches that exploit factors known to direct differentiation.

3- Reporter systems to identify and allow isolation of that cell type.

The transgenic approach is helpful in defining developmental pathways, and may allow development of culture conditions that would enrich for a specific cell type without reliance on transgenes. However, it would be preferable to develop methods to purify the cells of interest relying on their endogenous and surface marker characteristics without introducing a reporter gene. If transgenes have to be introduced, safety measures are of absolute necessity including for instance the introduction of a suicide gene that would specifically and selectively destroy the transplanted cells if necessary **[27]**.

BONE MARROW DERIVED STEM CELLS

Types of BM derived stem cells:

Hematopoietic stem cells (HSC):

The only true assay for the presence of HSCs is their ability to reconstitute the hematopoietic system of a myeloablated host. If BM is depleted from cells bearing lineage specific markers, the resultant populations called "lin-" is 10-100 times enriched for HSC. HSC are characterized by a number of markers; in human they are generally CD34+. A special subset deserves mentioning that is the side population (SP). They are so called because they have a unique ability to extrude Hoechst dye, and when examined by FACS analysis they fall within a separate population that is to the "side" of the rest of the cells on a dot plot of emission data in the blue Vs. red spectrum. This ability to extrude the dye is attributed to the expression of ABCG2 transporter. SP cells are also present in other tissues. There is controversy whether these SP

cells are tissue specific stem cells within these organs [28] or if they are actually BM derived SP cells lodged within these tissues [29-31].

Marrow stromal cells or Mesenchymal stem cells (MSCs):

MSCs are multipotent adult stem cells that reside within the bone marrow microenvironment [32,33]. These cells are characterized by growing and expanding in culture as an adherent layer with finite life span. They have the ability to differentiate not only into osteoblasts, chondrocytes, neurons, skeletal muscle cells, but also into vascular endothelial cells [34] and cardiomyocytes [35-38]. MSCs are present as a rare population of cells in BM, representing perhaps 0.001% to 0.01% of the nucleated cells; ~ 10 fold less abundant than HSC. In contrast to HSCs which are elusive, difficult to isolate and grow in culture, MSCs can be readily grown in culture. They can be cloned and expanded in vitro ≥ one million fold and retain the ability to differentiate to several mesenchymal lineages [33,39]. Although perhaps quiescent, bone marrow MSCs can divide rapidly once cell division begins; the cell population becomes very homogenous with time in culture and remains so for many passages [40]. A rapidly dividing population termed recycling stem (RS) cells was isolated from the BM as a subpopulation of MSC. RS cells were termed RS1 for small agranular rapidly proliferating cells and RS2 for small granular cells whereas the more typical fibroblastic MSCs were considered mature MSCs in culture [41,42]. In a series of articles, Verfiaille and collaeuges [43-46] described marrow progenitor cells or multipotent adult progenitor cells (MAPKs). The cultured cells have many of the attributes of MSCs; they lack MHC class I and II on their surface, so presumably, they may be used allogeneically. MSCs have been isolated from adipose tissue [47-50], a source that is readily available and easily accessible in many patients (vide infra); their characteristics and behavior are virtually indistinguishable from BM-derived MSCs [48]. Although MSCs express a number of surface molecules that have cognate legends on T cells as well as they can be induced to express class II MHC by incubating the cells with INFγ, yet they lack the B7 costimulatory molecules CD80 and CD86 and these are not induced by INFγ treatment [51,52]. MSCs have been shown to inhibit T cell proliferation; when cultured with responder T cells in mixed lymphocyte reaction, they do not generally cause T cell proliferation but usually reduce the response of T cells to other stimulators. This effect is mediated via secretion of hepatocyte growth factor and transforming growth factor β [52]. The inhibition is dose dependent, independent of MSC source [53] and affects as well memory T cells [54]. Whereas autologous cell-base therapy poses no risk of rejection, an "off shelf" allogeneic cell product would be much more cost effective and much easier to administer and could potentially allow delivery of greater number of cells than autologous cell therapy [2]. MSCs appear to avoid the problem of rejection by being hypoimmunogenic (vide supra). As such, MSCs may allow allogeneic cell therapy while avoiding rejection. The advantages of all MSCs are many. Besides being readily accessible, easy to handle, can be expanded in culture and maintain their multipotentiality, the donor can be chosen ahead, qualified and tested for the absence of different disease organisms. Allogeneic MSCs can be ready in advance so they are immediately available when needed by a patient.

Endothelial progenitor cells (EPCs):

Cells with phenotypic and functional characteristics similar to the fetal angioblast are also present in adult human bone marrow. 6 These cells, known as EPCs, express some, but not all, cell surface markers characteristic of mature endothelium, certain surface markers of hematopoietic cells, and transcription factors that identify them as precursor cells [55,56-58]. In addition to endothelial cell surface markers, EPCs also express markers of immature cells, including AC133, a novel hematopoietic stem cell marker [59] not expressed on mature endothelial cells [60].

UMBILICAL CORD BLOOD STEM CELLS

Umbilical cord blood (UCB) contains both hematopoietic stem cells and mesenchymal precursor cells [61]. Because stem cells in UCB exist in higher numbers than in adult human blood or bone marrow [62], several populations of cells derived from UCB are possible sources of stem cells for tissue repair. Kogler and colleagues [63] have described a population of cells from human UCB called unrestricted somatic stem cells. These cells, which are fibroblast like in appearance, adhere to culture dishes; are negative for c-kit, CD34, and CD45; and are

capable of differentiating, both in vitro and in vivo, into a variety of tissues [2].

STEM CELL APPLICATIONS IN REGENERATIVE THERAPY

Stem cell therapy for cardiac repair:

Coronary heart disease and heart failure continue to be significant burdens to health care systems all over the world. It is also one of the leading causes of death even if the standard therapeutic measures are followed especially in cases with congestive heart failure. Therefore any new treatment modality that benefits heart failure patients has the potential to result in dramatic improvement in health outcomes and substantial cost savings for communities.

Animal studies for regenerative cardiac repair dates back to early 90s [64,65]. Menasche et al. [66] described the first group of patients to receive skeletal myoblasts for cardiac repair. An elegant review of the current situation has been published by Boyle et al. [2]. Currently, results on more than 400 patients have been published. Though most of them are small pilot studies that lack randomization or control groups, yet all of them have proved that cell therapy is safe and feasible as well as they provide encouraging, albeit preliminary, signs of efficacy.

Administered stem cells may improve cardiac functions through different mechanisms:

1- Active myocardial regeneration resulting from transdifferentiation of the stem cells [67].

2- Development of new blood vessels of donor origin due to transdifferentiation of the stem cells into endothelial cells [68] or of host origin due to growth factor-mediated paracrine effect [69].

3- Production of cytokines and other factors that promote myogenic repair and prevent fibrosis [70].

4- Cellular therapy contributes to the restoration of stem cell niches, facilitating the ability of the heart to regenerate itself [71].

Of course understanding the mechanism is essential and would lead to improvement in the approach to therapy and hence the outcome. Yet the more important is that the patient will benefit whatever the mechanism is.

Various types of stem cells have been used including embryonic stem cells, resident cardiac stem cells, skeletal myoblasts, adult BM-derived stem cells, mesenchymal stem cells, endothelial stem cells and umbilical cord stem cells.

When transplanted into infracted myocardium, embryonic stem cell-derived cardiomyocytes engraft and improve cardiac function in rodent models [72]. Embryonic stem cells have the advantage of being capable to differentiate, as well, into vascular endothelium thus improving blood supply. Directed differentiation of hESC is based on protocols used in mESC [73,74]. However, these hESC-derived cardiomyocytes are immature, and have structural and functional properties consistent with fetal cardiomyocytes [75]. ESC-derived cardiomyocytes constitute a mixed population; they were transplanted as such in animal experiments. In a clinical setting, however, one would prefer to engraft a specific type of cardiomyocytes. In the setting of chronic heart failure or myocardial infarction, for instance, the cell type needed is ventricular cardiomyocytes, not a sinus-nodal type; the latter could be arrythmogenic and might cause considerable morbidity [76]. To-date, no human clinical studies have been initiated because of both ethical issues and also the possibility of teratoma formation suggested by a study injecting ES cells in skeletal muscles [77].

In recent years, evidence has accumulated suggesting that the heart has endogenous regenerative potential. Undifferentiated colonogenic cells have been separated from both human and murine hearts. These cells have been separated and phenotyped [78,79]. They are responsible for replacement of ongoing turnover and for minor repair. Moreover they may represent a therapeutic target that, if enhanced, could induce cardiac self-repair [2]. Clusters of self-adherent cells (cardiospheres) that grew from cultured adult cardiac tissue derived from both human and murine hearts have been recently described [80]. These cells have been shown to be colonogenic and capable of transdifferentiation in vitro and to induce both myocardial and vascular regeneration [81]. A side population cells capable of differentiating into cardiac and hematopoietic lineages in vitro was separated from both developing and adult heart [82]. Cardiac stem cells are reported to increase in number immediately

after myocardial infarction [83] and it has been suggested that BM may represent a reservoir for cardiac stem cells, the depletion of which might contribute to diminished cardiac repair [84]. To date there are no clinical trials of human cardiac stem cells.

Autologous skeletal myocytes or satellite cells are another potential source for cardiac repair. They are the reservoir of regenerative cells for skeletal muscle tissue and are solely committed to myogenic lineage. Experimental studies and initial clinical trials have shown engraftment of donor cells and improvement in global cardiac pump function [66,85,86].

One of the commonly used sources for stem cells is the BM. In humans, after orthotopic transplantation of female hearts into males, up to 15% of cardiac myocytes can be donor derived. The only possible source is BM-derived stem cells. Apparently, there is an intrinsic repair mechanism for minor cardiac damage within the BM but it is inadequate to repair larger damage [2]. Therapeutic benefit has been demonstrated in mice with experimentally induced myocardial infarcts that receive intracardiac injection of whole marrow (or Kit+ BM cells) during the initial post infarct period [70]. The use of green florescent protein (GFP)-positive donor BM demonstrated that the donor cells contributed to both cardiomyocytes and endothelial cell formation [87]. This regenerative therapy can be executed by either direct injection into the peri-infarct rim or by GCSF-induced mobilization of the stem cells from the BM to home to the site of injury [67,88]. Using the latter approach endothelial and smooth muscles were proliferating, but new myocytes growth predominated [89]. However differentiation of lineage negative, C-Kit+ cells into myocytes was denied by other workers [90,91]. Yet beneficial effect was reported in all studies. Other studies provided evidence that precursors of both cardiomyocytes and endothelial cells exist within the mononuclear cell fraction of bone marrow (BMMNCs). Studies by three groups are of special importance as they included controls. The first group [92] included 10 patients who received autologous BMMNCs reinfused into the infarct-related artery 7 days after myocardial infarction (MI); the control group included 10 patients who refused the intervention. The second study group [93,94] randomized 59 patients after acute MI to receive either intracoronary infusion of BMMNCs or ex vivo expanded circulating progenitor cells. The cells were delivered in the infarct-related artery 4 days after MI. The third group [95] randomized 60 patients after successful percutaneous coronary intervention for acute MI to receive either intracoronary BMMNCs or standard therapy. Taken together, the studies suggested that BM-MNCS are safe and may improve cardiac function by a substantial and clinically meaningful degree following MI. In contrast to MI setting, patients with chronic ischemic cardiomyopathy are unlikely to release signals from damaged myocardium to induce stem cell homing. Therefore endomyocardial injection of cells is needed to deliver the cells to the exact location where their effect is required [96].

The other type of stem cells used is MSCs; they are found in BM, muscle, skin and adipose tissue (vide supra). Studies showed that MSCs transdifferentiate in vivo into cardiomyocytes and endothelial cells [97-100] with significant increase in capillary formation and improved cardiac function [101,102]. In one study performed in pigs, allogeneic MSCs were used with no evidence of rejection.

In an elegant study performed by Miyahara et al., 2006 [69] in a rat model, Mesenchymal stem cell sheets were prepared in vitro from adipose tissue. Four weeks after coronary ligation, monolayer mesenchymal cells were transplanted onto the scarred myocardium. To identify the transplanted cells in myocardial sections, the authors used GFP-expressing cell grafts. They grafted monolayer MSCs onto the scar area of the anterior wall. After transplantation, the engrafted sheet gradually grew to form a thick stratum that included newly formed vessels (12.2%±0.6%), undifferentiated cells (57.8%± 2.2%) few smooth muscles (5.0%±0.3%) and few cardiomyocytes (5.3%±0.3%). No evidence of apoptosis was detected in the transplanted cells. Cardiac functions improved in all the mice and they all survived indicating that fatal arrythmogenic problems were not caused by integration of the MSC tissue. These data suggested that the major improvement may be mainly explained by growth factor-mediated paracrine effects leading to improvement of vascular supply rather than direct transdifferentiation into cardiomyocytes. It is worth men-

tioning that the MSC tissue included a large number of newly formed blood vessels. These vessels were composed of graft-derived cells, host-derived cells or both. The MSC sheet secreted a large amount of angiogenic and antiapoptotic cytokines, including VEGF and HGF. These results suggested that MSCs induce neovascularization not only through their ability to differentiate into vascular cells but also through growth factor mediated paracrine regulation. MSCs have also been used clinically [103]; 69 patients were randomized after acute MI to receive intracoronary autologous MSCs or placebo. Significant improvement in global and regional left ventricular function and significant reduction in the size of the perfusion defect was observed.

Another type of cells used in cardiac regenerative therapy is EPCs. After MI, injected EPCs homed to the infarct region within 48 hours [88]; at 14 days there was a marked increase in the number of capillaries in the infarct zone and the peri-infarct rim resulting from the induction of both vasculogenesis and angiogenesis leading to prevention of apoptosis as well as to some degree of cardiomyocytes regeneration [104]. EPCs can be obtained from BM using CD34+ cells which are enriched in EPCs and they can be expanded ex vivo and reinfused via the coronary artery [105].

Umbilical cord stem cells were used in few experimental animal studies [106-108] but no clinical studies have been reported.

Stem cell therapy for neurological disorders:

The mammalian central nervous system (CNS) is an enormously complex organ. In the adult CNS, this complexity presents profound complications for useful regeneration in disease or damage; diverse insults are repaired with non-functional astrocytic scarring [109]. At present not a single neurodegenerative disorder can be reversed, none halted, and the evidence that any can even be slowed down is very slight. The modest and frequently inadequate or abortive replacement of CNS cells lost by ageing or disease falls far short of the regenerative capacity observed in most other organs. This has stimulated the search for more imaginative, regenerative treatments, generally based on cell implantation [110]. Scientists and clinicians, however, recognize the need to move cautiously towards cell implantation goals, as damaging results due to premature clinical testing would be devastating to patients as well as to the emerging stem cell neural repair field [111]. Three main challenges have to be considered. First the timing of implantation bearing in mind the reversibility of acute deficits and the irreversible axon loss of chronic lesions [112]. Second, the site of implantation could be problematic in widely disseminated diseases. Third the optimum cell type to be used in different situations [110]. Neuronal tissue is a vast collection of highly specialized cell types, each with unique roles. Directed differentiation and isolation of specific neuronal subtypes will need to be achieved [1]. Even if directed differentiation is able to yield specific neuronal subtypes, another concern in their use remains. Unlike cells of the hemopoietic system, there is no evidence that when delivered via the circulation, neurons can home to their appropriate location in the nervous system; thus they must be delivered to the correct site surgically [1]. However Akiyama et al. [113,114] have shown that intravenous infusion of BM cells can lead to myelination in the spinal cord. Also an opposing statement was delivered by Sanberg, [115] in the Lifecell conference at Chennai, Jan-2006 "It is now evident that the circulatory system can be used as a route to deliver specific cells (stem cells) to the damaged brain to facilitate repair and recovery". Furthermore, in order to properly function, neurons require synaptic input from neighboring neurons; regenerated neurons will need to integrate properly with existing, fully developed neurons to re-establish functional neural network. In addition to not restoring function, failure to integrate properly could result in epileptiform activity [1].

Many cell types have been used for the treatment of neurological diseases including ESC, embryonic neural precursor cells, adult stem cells and BM stem cells. In addition to these, other cell types have been used in repair of spinal cord injury mainly, Schwann cells and olfactory nervous system cells.

ESCs were first isolated in 1981. Their substantial proliferative potential carries the advantage that numerous grafts can be prepared from a single sample. The significant risk of teratoma formation [116] was denied by Zhang et al. [117] who reported wide migration and appropriate differentiation of these cells in the neonatal

brain without teratoma formation. However, this is the general outcome when cells are implanted in the developing brain and cannot be extrapolated to cell therapy in the adults [118]. Away from the ethical problems, hESC have been grown in culture [119] and differentiated to neuronal cells [120].

Embryonic neuronal precursor cells develop from ESC; they are a mixed population of stem and progenitor cells with a more limited proliferation and differentiation capacity confined to different types of neural cells. Adult neural stem cells (NSC) are also thought to be capable of dividing asymmetrically to produce a more committed progenitor and an identical daughter cell [109]. Neural stem cells isolated from human fetuses have been differentiated, in vitro, into oligodendrocytes [the myelinated cells lost in multiple sclerosis (MS)] [121,122] and dopaminergic neurons (lost in Parkinson's disease) [123]. Improved functional benefit has been observed in animal models of Parkinson's disease following, in vitro, expansion and differentiation of neural progenitors [124]. Early clinical trials using unselected human embryonic mesencephalic tissue demonstrated practical problems; several fetuses are required to secure enough cells for each transplant [125].

Adult neural stem cells have been found in the brains of both rodents and humans [126,127]. Precursors of oligodendrites have been differentiated from adult rat brain and have demonstrated the capacity to produce myelin in vitro [122]. Adult neuronal progenitor cells (NPC) have also been differentiated into neurons when they engraft in certain areas of the CNS [128].

Bone marrow stem cells provide a relatively accessible source of different types of stem cells. BM-derived cells can be easily expanded in vitro and manipulated to express markers of neuroectodermal lineage [129-132]. Although the expression of neural markers does not imply functionality, rodent studies have demonstrated that focal implantation or intravenous infusion of BM cells can lead to myelination in the spinal cord [13,114]. Similarly, BM-derived cells injected into focal areas of cerebral ischemia [132] or infused peripherally [133] led to functional improvements. Also, appropriate neural differentiation and amelioration of neurological deficit was reported in an animal model of Parkinson's disease following transplantation of BM stromal cells [134].

Like with other systems, the transplanted cells may not exert their influence merely by transdifferentiation into functional cells replacing lost, damaged or dysfunctional cells but may act on the host environment to increase plasticity or resistance to disease. The possibility of grafting cells capable of secreting inhibitory neurotransmitters into foci of epileptic activity has been explored [135].

Self-evidently, if stem cell transplantation is to be a useful therapeutic modality, the transplanted cells must produce a therapeutic benefit without significant harm. A balance must be struck between a primitive stem cell capable of multilineage differentiation and proliferation but which has a risk of malignancy and a cell with reduced differentiation and lineage potential but which is still capable of providing sufficient numbers of the appropriate cell phenotype and/or functional benefit. The recent appreciation that adult stem cells have much of the differential potential previously associated only with embryonic stem cells has encouraged those who have ethical objections to the use of human embryonic or fetal material, and such cells may have the additional advantage of being easier to control in vivo. Adult stem cells, particularly those originating outside the CNS, could be a source of autologous transplant material that is relatively easy to obtain and may also have increased resistance to CNS pathology [109].

Stem cells in specific neurological diseases:

The specificity of cell types damaged by the pathological process has a significant impact on how amenable the disease is likely to be to cell replacement therapy. The more diffuse the damage, the more invasive the replacement strategy may need to be [109]. To follow are some examples of neurological diseases amenable to treatment with stem cells.

Parkinson's disease (PD): The symptoms of idiopathic PD result from the focal degeneration of dopaminergic neurons in the substantia nigra. A focal approach to replace a small number of spatially discrete neurons might be clinically beneficial. Clinical trials originally suggested that intrastriatal transplantation of fetal dopaminergic neurons could be beneficial

[136,137] but results of more robust randomized trial were generally disappointing [138,139]. However, transplantation of adult BM stromal cells into an animal model of Parkinson's disease showed some appropriate differentiation and amelioration of the deficit [134].

Multiple sclerosis (MS): Neurological function is impaired in MS because of damage to myelin and the myelin-producing cells (oligodendrocytes), resulting in the disruption of the electric signaling. Spontaneous remyelination is known to occur in MS but it is inadequate and unsustained [140]. MS is, by definition, multifocal and injection of cells into each and every lesion is not practical. However, only small percentage of lesions is largely contributing to the disability. Targeted implantation at the site of symptomatic spinal cord or brain stem plaques might be initially beneficial but a more global treatment would be needed in the long term. This will necessitate a more complex strategy for cell delivery unless the cells themselves retain tropism for areas of pathology and can migrate to sites of damage following intraventricular or intrathecal delivery. Intravenous delivery of stem cells would also be effective due to the breakdown of the blood-brain barrier [109]. Oligodendrocytes are the cells responsible for most spontaneous remyelination. They would be the candidate of choice but the number of these cells is limited and their migration through normal brain is considerably impeded [141]. An alternative cell type is fetal neuronal stem cells but adult neuronal stem cells may be used preferentially. These can be directed to start differentiating along the oligodendrocyte lineage prior to transplantation [109], a commitment that appears necessary for efficient myelination [142]. Another cell source is autologous BM; cells of BM origin can be induced to express oligodendrocyte antigens in vitro [143]. Myelination has been shown to occur in the rodent spinal cord following both focal implantation and IV infusion of BM-derived cells [113,114].

Huntington's disease (HD): HD is characterized by spatial disruption and loss of complex connections of the medium spiny neurons. Attempts to reverse deficit using implanted fetal striatal neurons were reported as early as 1983 [144]. Several studies have now reported that fetal striatal neurones engraft, survive [144-147], establish afferent [148] and efferent connections [149], restore striatal sensitivity to dopamine [146] and reverse behavioral deficits in animal models of HD [150,151]. Encouragingly, primate studies suggest that the resultant benefit might extend beyond improvement in locomotor deficit and that there may also be amelioration of cognitive dysfunction [152].

Alzheimer's disease (AD): The physiologic cause of AD is loss of neurons and neuronal dysfunction in the frontal and parietal association neocortex. Many neurotransmitter systems are involved in the pathology of this disease, but the most affected are the cholinergic, noradrenergic and serotonergic systems. Drug therapies and other intervention strategies to prevent or delay the progression of AD have been limited, at best [153]. Neuroreplacement therapy will undoubtedly become more feasible. It is well established that the olfactory sensory pathway is pathologically affected in AD. Severe loss (as much as 75%) of the anterior olfactory nuclei neurons in early-onset AD has been reported. Because of their vulnerability to toxic substances in the environment, olfactory sensory neurons readily degenerate and are replenished continuously from a population of stem cells at the base of the olfactory epithelium. Stem cells originating from the subventricular zone are known to migrate into the olfactory system [154]. Furthermore, these NSCs migrate into the hippocampus [155] and other parts of brain [156], which may be important for proper maintenance of cognitive function. Thus, deficits in normal neurogenesis and differentiation of NSCs may be implicated in a cascade of impairment in olfactory function and cognitive function, as observed in AD. Mutations in amyloid-b precursor protein (APP) and the presenilins (PS1 and PS2) are evidenced in familial early-onset AD. It has been reported that the adult human brain retains multipotent progenitors [157], suggesting that regeneration of CNS cell types may occur throughout life. Since NSCs carrying defected PS may be found in the familial AD brain, and because these NSCs may not properly respond to differentiation factors released from damaged neurons (due to deficits in the Notch signaling pathway), it was therefore suggested that AD patients may have impaired olfactory and cognitive functions as a combined consequence of progressive neuronal loss coupled with a defective neuroreplacement system [153]. Thus, for familial AD, a therapeutic strategy

by which HNPs carrying defected presenilin are replaced by HNPs carrying wild type presenilin was proposed by Sugaya and Brenen, [153]. As a result of this therapy, healthy HNPs respond to endogenous differentiation factors, and migrate and differentiate in the affected brain to locales where they are needed.

Amyotrophic lateral sclerosis (ALS): ALS or classical motor neurone disease is a progressive condition that results in widespread muscle denervation due to the loss of both central and peripheral motor neurones. Both hematogenous and intrathecal delivery of stem cells have been considered, and the latter used in clinical trials with peripheral blood stem cells [158]. This is particularly ambitious given that axons will be expected to extend distances measured in tens of centimeters.

Stroke: Stroke is a common cause of neurological disability and death. The recovery that occurs following ischemic damage to the brain may be attributed both to neuronal plasticity, as well as neurogenesis following ischemia [159,160]. This has encouraged attempts to supplement endogenous repair using stem cells. Beneficial effects could potentially be mediated through the exogenous supply of cells capable of neurogenesis and/or neovascularization or via modulation of the environment to enhance plasticity or the survival and differentiation of host cells. Intracerebral transplantation of cells derived from neural cell lines have been reported to show some benefit in rat models of stroke [161,162] and in humans [163,164]. Animal studies have suggested that BM cells are recruited to ischemic brain [165]. BM-derived cells have also been demonstrated to contribute to functional improvements in animal models of stroke when injected focally [132] or delivered intravenously [133]. The exact mechanism underlying this remains unclear, but the transplanted bone marrow cells have been shown to be associated with new vessel formation [122], increased levels of brain-derived neurotrophic factor and nerve growth factor [128], as well as the expression of neuronal markers [132,166].

Cell Therapy for Spinal Cord Injury (SCI):

Different approaches with different cell types have been used in a trial to repair SCI.

Peripheral nerve grafts with various combinations of therapy were reported to promote recovery with regeneration of supraspinal axons into, through and beyond grafts in adult rats [167,168]. This approach has also shown some success in treatment of chronic incomplete human SCI [169] but it did not prove successful in people with complete SCI [170].

Schwann cells from peripheral nerves have been transplanted into rat models of SCI. After contusion and implantation of Schwann cells, cavitation is reduced and sensory and spinal axons extend into grafts, and many are remyelinated [171]. Recovery of hind limb function was reported in some [171] but not all studies [172]. Human Schwann cells have also been transplanted into the transected spinal cord of rats with attenuated immune systems; functional improvement was also reported [173]. So far, there have been no peer-reviewed reports of clinical trials involving the transplantation of Schwann cells after SCI [174].

Olfactory nervous system cells from the embryonic and adult olfactory bulb or mucosa have been transplanted after SCI. Functional recovery and/or CNS axon regeneration has been reported when cells are transplanted immediately or up to 2 months after SCI in adult rats [175-177], although whether olfactory cells directly myelinate axons after SCI remains controversial [178]. Transplantation of cells from the olfactory nervous system does not, however, promote CNS axon regeneration and functional recovery under all circumstances [171,179-181]. Transplants from fetal olfactory bulbs or adult mucosa were reported to be performed in more than 400 humans in China, Portugal and Colombia [170,182,183]. Improvement in motor and sensory functions was reported [170,182] but it is difficult to gauge safety and efficacy of this intervention [174].

Embryonic CNS tissue was also tried as a source of cells in SCI. After contusion and transplantation of fetal spinal cord into the lesion site, small but significant improvement of function was observed in rats [184,185] and cats [186]. The difficulty of obtaining fetal tissue for transplantation is a limiting factor for that approach in humans.

Embryonic stem/progenitor cells transplantation for SCI faces three major challenges namely controlling the survival, integration and

differentiation of transplanted cells [174]. Different types of stem cells were tried to repair injured adult rodent spinal cord including stem cells [187,188] or progenitor cells [189-191] derived from rodent embryonic or human umbilical cord; modest improvement in functional recovery was reported by some [187,192,193]. Neural progenitors derived from human fetuses have been transplanted into immunosuppressed mice [194] and non-human primates [195] after contusion. In both cases, the transplanted cells survived and differentiated into cells with characteristics of oligodendrocytes and neurons, and were associated with locomotor improvements [194,195]. The best approach is to use progenitor cells that have been pre-differentiated to a desired lineage before transplantation. Transplantation of hESC-derived oligodendrocyte-restricted progenitor cells into the adult rat spinal cord 7 days after injury enhanced remyelination and promoted improvement of motor function. The cells survived, migrated over short distances and differentiated into oligodendrocytes. By contrast, when cells were transplanted 10 months after injury, there was no enhanced remyelination or locomotor recovery [196,197].

Adult stem/progenitor cells are now being considered for CNS transplantation. Transplantation of HSCs promotes functional recovery after compression-induced SCI in mice [198,199] and transplantation of BMSCs significantly improves hind limb function after SCI in mice and rats [199-201]. A small scale human trial was conducted in which autologous BMSCs were intravenously delivered to nine patients with SCI [202]. The improvements observed appeared to fall within an expected range of spontaneous recovery, and one participant advanced from ASIA category B to D. However it can be concluded only that a measure of procedural safety was demonstrated. Adult neural progenitor cells (NPCs), isolated from the dentate gyrus, the subventricular zone or spinal cord, have been shown to self-renew, and to be multipotent in vitro and after transplantation into the CNS [203,204]. After transplantation of adult NPCs into the intact and injured murine spinal cord, differentiation into only astrocytes or oligodendrocytes is observed [205-206]. NPCs transplanted 2 weeks post-injury survived, migrated, integrated in the injured spinal cord tissue, generated mature oligodendrocytes that remyelinated the injured axons, and promoted some functional recovery. However, NPCs transplanted 8 weeks post-injury did not survive, and failed to exert similar effects [207]. Therefore, there is a need to find and neutralize the inhibitory obstacles present in chronic SCI that interfere with NPC survival after transplantation [174].

Damage to the spinal cord often results in progressive tissue loss and subsequently in cavity formation. These cavities may be of substantial diameter leaving only a small rim of white matter [208].

However to bridge a large gap in the injured tissue may be difficult if not impossible without tissue engineering. A scaffold grafted into the site of injury may provide necessary mechanical support for the transplanted cells, guide axonal growth and promote better integration with host tissue. Different compounds were used [193,209] but the potential problem may be based on the type of cells used to populate the scaffold as well as on the development of a glial scar around the injury. An alternative approach utilizing enzyme chondroitinase ABC has been reported [210]. It is well-known that at the site of the spinal cord injury a glial scar forms containing extracellular matrix molecules including chondroitin sulphate proteoglycans which are inhibitory to axonal growth. In a recent study the investigators have used specific enzyme chondroitinase ABC to degrade chondroitin sulphate [211].

Stem cell therapy for liver diseases:

Orthotopic liver transplantation has proven to be effective in the treatment of a variety of life-threatening liver diseases; however, significant morbidity and mortality remains. In addition, the growing disparity between the number of donated organs and the disproportionately large number of patients awaiting transplantation has provided an impetus for developing alternative therapies for the treatment of liver failure [212]. Novel strategies designed to increase the number of organs transplanted, such as the use of adult living donors, are not without significant risk to both the donor and recipient [213].

The hepatic parenchyma is made up of hepatocytes and cholangiocytes. Unlike other organs, liver cell mass is restored primarily through division of the majority of mature hepatocytes

and not via a dedicated stem cell population. At times of overwhelming cell loss with long standing iterative injury (e.g. chronic viral hepatitis), or when hepatocytes replication is impeded, regeneration seems to occur via a second cell compartment [214,215]. In rodents, these are oval cells but in human they are more aptly called hepatic progenitor cells [216]; attempts to identify the originating stem cell are hampered by lack of markers [217].

Many cell sources have been tried for hepatic regeneration including fetal and adult hepatocytes, embryonic cells and BM-derived cells.

Hepatocyte transplantation has several advantages over whole liver transplantation. Intact liver has to be transplanted within a short time; isolated liver cells may be cryopreserved for later use [218]. However it is still unclear whether cryopreserved cells can engraft and function as well as fresh cells [219]. Another advantage of hepatocyte transplantation is that a single donor could potentially provide hepatocytes for several patients. However, despite unequivocal evidence of function in some patients, the efficacy of hepatocyte transplantation has been difficult to prove [219]. Although transplanted hepatocytes become integrated into host parenchyma, function, and survive, they proliferate poorly in the host liver. Animal models have shown that extensive repopulation by transplanted hepatocytes requires exposure of the transplanted hepatocytes to proliferation stimuli and selective loss of the host parenchyma [220-222]. The liver and spleen are the most reliable sites for hepatocyte engraftment and function. The peritoneal cavity may also be a site for transplantation of encapsulated or matrix-attached hepatocytes [216]. The expanded extracellular matrix associated with liver cirrhosis increases the endothelial barrier to engraftment in the liver. However, transplanted hepatocytes can migrate into cirrhotic nodules and integrate into liver plates following intraportal infusion in rodents. Furthermore, transplanted hepatocytes express enzymes associated with normal liver function, such as glucose-6-phosphatase and glycogen, and are capable of significant expansion following transplantation, as long as there is no ongoing injury to the liver [223]. Data indicates that transplanted hepatocytes that are resistant to the underlying disease could potentially repopulate a severely diseased cirrhotic liver. Several issues, however, may limit hepatocyte transplantation into the cirrhotic liver. Portal-systemic shunts will result in translocation of hepatocytes to the pulmonary circulation. While hepatocytes do not engraft in this location and are rapidly cleared, translocation of a large number of transplanted cells may produce pulmonary emboli with resultant cardiopulmonary compromise. More importantly, the presence of portal hypertension increases the risk of portal vein thrombosis; potentially further compromising host liver function. Finally, it is unclear whether the transplanted cells can function within cirrhotic nodules when there is ongoing injury or whether enough cells can engraft in the decompensated cirrhotic liver to significantly affect overall liver function. Transplantation experiments in urokinase-type plasminogen activator (uPA) transgenic mice and fumarylacetoacetate hydrolase (FAH)-deficient mice have suggested that the proliferative potential of adult hepatocytes is infinite [224,225]. However, treatment for a number of acute liver failure or end-stage liver disease is limited. Several experiments in animal models of liver failure have shown that hepatocyte transplantation resulted in a significant prolongation of survival time [226-228]. Clinical trials of hepatocyte transplantation to treat acute or chronic liver failure and inherited liver disorders have been performed [229-231]. Although the clinical efficacy of hepatocyte transplantation varies with the case of the liver diseases, hepatocyte transplantation is considered a potential treatment for metabolic liver diseases and a bridge for patients awaiting a donor liver for liver transplantation. Living or cadaveric livers as well as livers not used for liver transplantation are possible sources of hepatocytes. However, their availability is limited due to the shortage of donors [232]. Accordingly if hepatocytes can be generated, in vitro, from various types of stem cell; this might constitute a more available source for hepatocyte transplantation.

Embryonic cells would, logically, be the best candidate. Differentiation of ES in vitro seems to recapitulate early embryonic development [220]. When mouse embryonic cells are cultured in Petri dishes, embryoid bodies (EBs) form within few days [233]. Cultured EBs start to express the hepatocyte-related genes within a couple of weeks [234-238]. Albumin-expressing cells are observed in cultured EBs as clusters

in a multilayered structure [234-238]. Combinations of HGF with other growth factors, including oncostatin M and nerve growth factor (NGF) are used to induce hepatocyte-related gene expression in cultured EBs [239,240]. Generally, the effect of growth factors and the extracellular matrices on hepatocyte differentiation in EBs is limited, suggesting the difficulty of inducing ESCs to differentiate into hepatocytes in cultured EBs. A group reported spontaneous differentiation of functional hepatocytes in cultured EBs in the absence of exogenous growth factors [234,236]. It seems likely that hepatic differentiation proceeds by cytokines secreted by other cells in the developing EBs, and presumably via cell-cell interactions. Animal experiments have proved that ESC, per se or after in vitro manipulation, can differentiate into hepatocytes in vivo. Teratomas resulting from transplantation of mouse ESCs were shown to contain cells with mature hepatocyte phenotype [241,242]. When hepatocytes were isolated from the teratoma and transplanted into injured mouse liver, they integrated without forming teratoma [242]. Transplantation of EBs obtained from in vitro culture of mouse ESCs resulted in the formation of hepatocytes expressing albumin, however, teratoma formation was frequently observed [243]. Thus, elimination of tumorigenic cells from EBs is an important task for ES cell-based cell replacement therapy to become feasible. This can be achieved by Percoll discontinuous gradient centrifugation [244], with antibodies or with a suicidal gene [245]. The Hepatocyte-rich fraction was found to improve the prothrombin time and total bilirubin markers [244], to suppress fibrosis [246] and hence improve liver injury [220]. Human and Monkey ESCs were also shown to be capable of differentiation into hepatocytes in vitro under the effect of growth factors [247-250]. Inspite of all the progress, hepatocyte differentiation by ESCs is inefficient, and the mechanism of liver development needs to be understood to direct the hepatocyte differentiation from ESCs [220].

Bone marrow-derived cells constitute an appealing source of stem cells for regenerative therapy of liver diseases as it is with other organs. Using Y chromosome tracking, a sparse number of hepatocytes seemed to be originating from the BM in male recipients of female orthotopic liver transplants, and in females who had received BMT from male donors [251,252]. In perhaps the most exciting demonstration of BMSC plasticity, transplantation of Lin-Kit$^+$Sca$^+$Thy1^{lo} (KTLS) BM cells to irradiated hosts was used to treat an inborn error of metabolism. This was performed on an animal model of hereditary type I tryosinaemia, the fumarylacetoacetate hydrolase knockout mouse [FAH (-/-)]. With time, it became apparent that these initial observations were difficult to reproduce, and later elegant studies in the same FAH (-/-) mouse model conclusively showed that monocyte-hepatocyte fusion was the explanation for the restored normal phenotype to the FAH-deficient liver, in which hepatocytes formed by fusion expanded rapidly owing to a considerable survival advantage [253,254]. Unfortunately, in the absence of a strong selective pressure, it seems that stable long-term engraftment of BM-derived parenchymal cells is unusual. In the hepatitis B surface antigen transgenic mouse, the BM contributed to hepatocyte repopulation through cell fusion, but only at a very modest rate. In this model, constitutive HBsAg expression induces chronic low-grade hepatocyte turnover with nodule formation, and inhibition of hepatocyte replication with retrorsine provokes an oval cell response. Here, the contribution from BM-derived cells to hepatocyte repopulation waned to just 1.6% by 6 months, presumably owing to lack of a sustained selection advantage [255]. Low level repopulation was also documented in other animal models including C Cl4-induced liver damage [256]. The current balance of evidence therefore suggests that, under circumstances of severe or repeated injury, BM cells can contribute to only a minor amount of liver parenchymal regeneration, primarily through cell fusion. In therapeutic terms, cell fusion may be a powerful tool to correct metabolic disorders of hepatic origin. This has been exploited in a number of isolated clinical scenarios. For example, sequential healthy donor hepatocyte transplantation was able to moderate the clinical phenotype of argininosuccinate lyase deficiency, an inborn error of metabolism, in an affected child for periods of one year. Histological engraftment through cell fusion of over 10% was detectable together with an improvement in clinical and metabolic indices [257]. The evidence as to which type of BMSC is responsible for liver repopulation is conflicting. In early studies looking at BM contribution to hepatocytes in the FAH mouse, it seemed that HSCs were the stem cell fraction

involved [258]. The HSCs seemed to be the key cell in BMT experiments of CCl4 liver injury in irradiated C57/B6 mice [259]. In vitro, HSCs can be induced to differentiate into hepatocyte-like cells, given the appropriate medium containing HGF. More importantly, when cocultured with injured hepatocytes across a barrier through which soluble mediators can pass, HSCs could be induced to differentiate into hepatocytes [260]. Conversely, when human BMSC fractions were directly xenografted into rat liver damaged with allyl alcohol, only the MSC fraction seemed to give rise to hepatocyte-like progeny, positive for mRNA albumin expression [261]. Also in vitro transdifferentiation of MSCs into hepatocytes can be demonstrated when co-cultured with fetal liver cells [262]. Whether it is the HSC or the MSC compartment that contributes to BM-derived hepatocytes, or whether it can be both, remains unresolved [217]. Monocytes, though not stem cells, were also claimed to contribute. When treated with macrophage-colony stimulating factor and interleukin-3 and subsequently conditioned with hepatocyte medium, cells with the morphology, marker gene expression and metabolic function of hepatocytes were found. On transplantation into NOD/SCID mice, these cells showed liver integration and albumin expression. One study has shown that rodent and human multipotent adult MAPCs can be induced to adopt a hepatocyte phenotype in vitro and can display limited hepatocyte function (e.g., secrete urea, cytochrome P450 activity) [263]. MAPCs can also apparently differentiate into hepatocytes when infused in vivo into nonirradiated mice, although function was not determined [264]. However, it is worth noting that other laboratories have found it notoriously difficult to propagate MAPCs from BM [265].

In contrast to hepatocytes, where derivation from the BM is limited, there is a significant contribution from BMSCs to the non–parenchymal cells within the liver. The sinusoidal endothelium seems to have BM origins. Circulating EPCs, which are of BM origin, participate in the formation of new blood vessels at ischemic sites throughout the body including the liver. These EPCs may have extra beneficial effects on hepatocyte regeneration, and fibrosis resolution [217]. There is also evidence that fibrogenic cells in the liver originate from BM. Hepatic damage during chronic liver disease is usually accompanied by progressive fibrosis. As a consequence of liver inflammation, hepatic stellate cells (HpSCs) become activated, proliferate and synthesize collagen. They display a myofibroblast phenotype histologically distinguished by expression of a smooth muscle actin, and are thought to be central to the pathogenesis of liver fibrosis; there is, therefore, much interest in being able to clinically modify their activity. It has been suggested that HpSCs have their embryological origins in the septum transversum mesenchyme. There is in fact a growing body of evidence to indicate that the myofibroblast population, at least in part, derives from BMSCs. In gender crossover BMT experiments using CCl4 and thioacetamide models of liver injury, up to 70% of HpSCs and myofibroblasts associated with septal scars were BM derived [266]. In contrast, a recent study, using CCl4 induced fibrosis model, transplantation of MSC separated from BM and propagated in culture for 4 weeks was associated with decreased fibrosis and improved liver function [267]. Certainly in the liver, it is likely that there is more than one population of collagen-producing cell disparate in derivation. A similar situation was encountered in human. In across gender transplantations, 6-22% of hepatic scar associated myofibroblasts were derived from BM. Recurrence of hepatitis C, accompanied by rapid and aggressive liver fibrosis, is a major cause of graft dysfunction and failure. The implication here is that a significant proportion of the fibrotic response is attributable to the recipient's cells rather than a property of the donor organ. Human BM-derived myofibroblasts have also been found in other tissues including the intestine, the lung [268,269], the skin and kidney, the location being dependent on the site of injury [270-272]. The BM cell that gives rise to the myofibroblast is controversial but the suggestion is that the main protagonist is the MSC. In effect, more than one BMSC compartment may be contributing to the scar-forming cells within the damaged liver. Likewise, different studies have collectively shown that both HSCs and MSCs may repopulate the liver or ameliorate liver disease by promoting regeneration or attenuating fibrosis. At present, the specific role of each BMSC is incompletely defined and the validity of future work is crucially dependent on exactly how donor BMSCs are isolated and characterized.

The mechanisms of homing of BM cells to the liver are extensively investigated. HSCs express the cellular receptor CXCR4, to which the natural ligand is stromal derived factor-1 (SDF-1). When the SDF-1 concentration within the BM is reduced, HSCs are recruited into the circulation and migrate along a concentration gradient **[273,274]**. It has been shown that injurious stimuli such as irradiation and inflammation upregulate hepatic SDF-1 production **[275]**. Inoculation of human SDF-1 increases homing of HSCs to the liver, and blockade of CXCR4 abrogates it. The CXCR4 receptor has also been shown on oval cells, which in vitro seem to migrate along a SDF-1 gradient. HGF, upregulated during hepatic regeneration, can augment CXCR4 expression on HSCs and potentiate SDF-1-induced migration. Stem cell factor, the production of which localizes to the same area in the liver, acts synergistically with SDF-1 to induce HSC migration in vitro. HSCs express c-kit, the receptor for stem cell factor. Other factors such as matrix metalloproteinase-9 (MMP-9), which augments HSC release from the BM, and IL-8, which is upregulated in liver disease and stimulates granulocyte production of MMP-9, are also likely to be important. The literature on what determines MSC homing is more conflicting. It seems at best that only a small proportion of MSCs can express functionally active CXCR4 **[276]**. Using green fluorescent protein (GFP) as a cell marker, MSC migration to pancreatic islets in response to SDF-1 has been demonstrated, but no in vivo experiments have investigated MSC homing to the liver **[277]**. Clearly, the clarification of the factors controlling BMSC migration has important implications for future treatment in liver disease. In particular, if the precise precursor of the BM-derived myofibroblast is identified and its migration pathway elucidated, then the development of liver-specific anti-fibrotic therapies may become possible **[217]**. Proper homing of exogenously applied stem cells is likely to depend on whether they can integrate into their respective niches. This may depend on whether the existing stem cells within the niche have been disrupted or depleted. In the BM, myeloablation through irradiation will have this effect. In the liver, toxic damage (e.g., with CCl4) can alter the local niche. The fate of transplanted BM cells may thus be determined by whether they are introduced locally into the liver or whether their inoculation is peripheral, via the BM. The prior manipulation of the stem cell niche in the recipient is likely to be an important factor in the outcome.

The therapeutic potential of BM-derived cells is documented in animal models; whether or not engraftment and organ reconstitution continues in the long term has not been answered. One pathway by which recovery can occur in chronic liver disease is through a reduction in hepatic fibrosis. When MSCs in vitro were induced to adopt a hepatocyte phenotype and then transplanted intravenously into non-irradiated CCl4-damaged recipients, a histological decrease in hepatic fibrosis and a rise in serum albumin were noted **[278]**. Likewise in a similar animal model and experimental paradigm, the transplantation of a BM mononuclear MSC subpopulation led to a reduction in liver fibrosis when infused early enough after the onset of injury **[279]**. It may be, at least in part, that the anti-fibrotic property of BM cells is conferred by the infusion of macrophages (which express MMPs central to the degradation of collagen bands) (280). It has been clearly shown that BM-derived macrophages are crucial to the resolution of CCl4-induced liver fibrosis during the recovery phase after injury **[281]**. Another possible explanation for the reduction in fibrosis is that migrating BM cells increase hepatocyte proliferation and suppress fibrogenesis by supplying growth factors and cytokines critical to the recovery process. Amelioration of liver fibrosis was also achieved with EPC treatment, in the presence of increased HGF and vascular endothelial growth factor, and a reduction in the pro-fibrotic mediator transforming growth factor-β **[282]**. The application of BM cell treatment in liver is not as advanced as it is in cardiac diseases. In patients with chronic liver disease, there does not seem to be an increase in circulating BM-derived stem cells (defined as CD34+) at times of acute decompensation **[283]**. Nor does there seem to be a consistent improvement in liver function when G-CSF is given to patients with cirrhosis to increase the CD34+ cell count in peripheral blood, though isolated improvements in some biochemical indices are noted **[284]**. There are only a handful of clinical trials, all of which are small-scale, uncontrolled feasibility studies. The first study looked at patients with liver cancer undergoing portal vein embolization to induce contralateral lobe hypertrophy and there-

by increase the size of the future remnant liver volume before an extensive partial hepatectomy [285]. Accelerated hepatic regeneration was demonstrated in three of these patients after the infusion of autologous CD133+ BM cells. By CT criteria, the left lateral segments hypertrophied by two and a half times more than in non-BM cell-treated controls. Another preliminary uncontrolled study in five patients with cirrhosis showed a transient improvement in clinical parameters such as serum bilirubin and albumin over 60 days after portal vein or hepatic artery infusion of $1x10^6$ to $2x10^8$ autologous CD34+ BMSCs. Again feasibility and safety were demonstrated [286]. The only other published clinical trial involved nine patients with cirrhosis who received portal vein infusion of 5.2 x 10^9 autologous unsorted BM cells [287]. Follow-up was longer, at 24 weeks, and patients showed some improvement in Child-Pugh score and albumin. Liver biopsies, when taken, showed increases in proliferating cell nuclear antigen staining, an indirect marker of hepatocyte turnover; however, there was no control arm. A recent case report describes the use of autologous BMSCs as rescue treatment for hepatic failure in a 67-year-old man ineligible for liver transplantation [288]. The portal venous infusion of 5 x 10^6 CD34+ cells, obtained from peripheral blood after G-CSF induction, led to an apparent rapid improvement in hepatic synthetic function in this patient, although BMSCs were not identifiable as they were not labeled with markers before transplantation. In none of the clinical trials so far has colonization or even engraftment of transplanted cells been demonstrated in recipient livers. It is conceivable that the variable change in parameters of hepatic function may be occurring through the supply of growth factors promoting liver regeneration and fibrosis resolution. This in itself may be a sufficiently satisfactory end point.

Thus, currently, there is very little evidence that BMSCs can make hepatocytes at a level that could be clinically useful, nor has stable or long term engraftment been demonstrated. It is more probable that a realistic goal of BMSC treatment is to stimulate the regeneration of endogenous parenchymal cells or enhance fibrous matrix degradation. It appears that BMSC treatment can create a milieu conductive to liver regeneration through the transient supply of growth factors, but it is likely that repeated treatment would be required in clinical practice; this has not yet been studied. It is important to take into account the potential that stem cells may have for malignant transformation. It has become increasingly evident that the cellular origin of HCC is the oval cell or hepatic progenitor cell. This raises a theoretical concern that BMSC treatment may accelerate carcinogenesis in patients with liver disease. There is already a well-documented incidence of HCC in patients with cirrhosis, the precise cohort for which stem cell treatment may be most needed. There is of course the theoretical potential to exploit the BM-hepatic fibrogenic axis to influence and deliver antifibrotic treatments through the BM. This is an area in which future investigation may prove rewarding.

Stem cell therapy for skeletal muscle repair:

There are more than 20 types of muscular dystrophy and numerous other muscle disorders, but treatment options are almost nonexistent [289].

Many cell types have been used in animal models of Duchenne's muscular dystrophy (DMD) including BM-derived cells, synovial membrane-derived MSC and mesoangioblasts.

Injection of marrow cells into damaged muscles leads to marrow derived cells with myocytes-specific gene expression [290]; functionality of the marrow derived myocytes is as yet unclear. An elegant study using transplantation of GFP+ marrow cells documents the engraftment kinetics of BM derived myocytes after transplantation of whole marrow contributing to approximately 3.5% of the muscle fibers in response to exercise [291]. A case report is that of a boy who was diagnosed with relatively mild DMD at the age of 12. The boy had received Allogeneic BM transplantation at the age of one year. It was suggested that healthy muscle fibers forming from the donor BM might have decreased the severity of the disease. Rare donor derived nuclei expressing normal dystrophin (0.5-0.9) were detected in the skeletal muscle fibers [292].

Adult human mesenchymal stem cells isolated from synovial membrane (hSM-MSCs) were shown to have myogenic potential in vitro [293]. In a later study, by the same group, their myogenic differentiation was characterized in a nude mouse model of skeletal muscle regen-

eration and their therapeutic potential was tested in the mdx mouse model of DMD. Differentiation was sensitive to environmental cues, since hSM-MSCs injected into the blood stream engrafted in several tissues, but acquired the muscle phenotype only within skeletal muscles.

In a recent study Sampaolesi et al. [294] used a novel type of stem cells, termed mesoangioblast which can be harvested fro small blood vessels [295]. These cells have a number of advantages; they are relatively easy to isolate, and their number can be expanded greatly in tissue culture without losing the ability to form muscles. A dog model of DMD was used. The mesoangioblast cells transplanted were either healthy cells or genetically corrected autologous cells. The cells were infused in a major hind limb artery and the dogs received 5 injections at monthly interval. One dog received the infusion into the aorta. All dogs showed marked improvement that was especially evident in the last one that was able to walk 5 months after the last injection. In general dogs receiving donor cells improved more than those receiving corrected autologous mesoangioblasts. This method is promising for potential clinical application in DMD as well as a variety of other muscle diseases.

Stem cell therapy for renal regeneration:

Most researchers agree that the kidney should likely possess stem cells but evidence for functional renal stem cells within adult mammals remains elusive and their regenerative ability is incomplete [296]. A non-hematopoietic population of CD133 cells has been isolated from human kidney, cloned in vitro and found able to contribute to tubular regeneration in severe combined immune deficiency (SCID) mice [297]. In addition, cells with attributes of mesenchymal stem cells (including differentiation into fat and bone) have been cultured from glomeruli and whole kidneys of mice [298] although their ability to generate epithelial cell types was not explored. The contribution of extra-renal stem cells is suggested by the presence in tubules of appropriately differentiated epithelial cells that are of extra-renal origin, e.g. the epithelial nucleus bears an unexpected Y-chromosome in either a male recipient of a female renal allograft, or in a female recipient of a male bone marrow graft [299].

There is evidence that circulating endothelial precursors originating from the BM can contribute to repairing the damage in kidney graft [300]. There is also evidence of extra-renal origin of mesangial cells [301], myofibroblasts [302,303] and podocytes [299,304-305]. BM transplantation weeks or months before induction of renal injury was shown to result in differentiation of some of the transplanted cells into renal tubular cells capable of division though their long term survival is not known [306]. Other studies have shown engraftment of BMSC into nonepithelial mesangial cells and interstitial cells within the kidney [307,309]. In an elegant study, Held et al. [310] were able to generate a renal phenotype in the FAH (–/–) mouse mentioned earlier; up to 50% of the tubular cells expressed the FAH+ donor phenotype. Sugimoto et al. [311] recently reported that grafting whole wild type BM into young mice lacking the expression of the a3 chain of procollagen IV leads to an astonishing partial restoration of expression of the missing collagen chain (with incorporation of $\alpha3$, $\alpha4$, $\alpha5$ triple helices in renal basement membranes), expression of $\alpha3$ chain mRNA by podocytes, accompanied by improved architecture of the glomerular basement membrane (GBM) and, importantly, improved renal function [311]. Their results are especially remarkable in that renal injury was already established at the time of rescue with unaffected BM. By comparison, results of Poulsom et al. [312], using an Alport model indicated that renal function and survival was better in recipients of normal BM than Alport BM. The authors concluded that any benefits of BM in Alport mice are derived from the HSC compartment as they have demonstrated that MSC are ineffective. The data collectively show that BM-derived stem cells could be potentially helpful in treatment of renal diseases.

Stem cell therapy for type 1 diabetes:

Type 1 diabetes is a T cell-mediated, organ specific autoimmune disorder, in which the body's own immune system attacks β cells and damages them sufficiently resulting in reduced insulin production. Recently it was shown that liver stem cells/hepatocytes can transdifferentiate into insulin-producing cells [313]. Such liver-derived insulin-producing cells may overcome immunosuppression. Moreover cells transfected with the human insulin gene produce insulin [314] including human BM MSC [315].

A wide range of cell sources are being used to grow β cells for the treatment of diabetes including embryonic stem cells, BM stem cells, pancreatic ductal cells, mature β cells and hepatic cells.

Both derivatives of embryonic and adult stem cells are shown to secrete insulin in vitro. Human embryonic cells were reported to be induced to form islet-like clusters similar to immature pancreatic cells **[316]** with the possibility of reversal of hyperglycemia through the transplantation of embryonic stem cells derived insulin-producing cells **[317]**. Intraperitoneal transplantation of encapsulated ESC may protect them from immune attack **[318]**, however the effect of encapsulation on differentiation of stem cells needs to be addressed **[319]**.

Various types of adult stem cells were shown to transdifferentiate in vitro and/or in vivo into insulin producing cells; these include brain-derived neuronal progenitor cells **[320]**, rat neural stem cells **[321]**, umbilical cord blood cells **[322]**, BM stem cells **[323]**, and multipotent pancreatic progenitor cells **[324]**. The use of adult stem cells to produce insulin-secreting β cells for improving the disease condition in type 1 diabetes offers a new window of opportunity for effective treatment and cure. The major hurdle, however, is autoimmunity which could be overcome by engineering stem cells to escape recognition by the immune system **[319]**. Stem cell therapy for diabetes, however, is still in the early animal phases and its premature use in human may put patients at risk.

Currently the american diabetes association recommends:

- Genetic engineering of nonpancreatic cells into glucose-sensitive insulin-producing cells.
- Transforming stem cells or pancreatic endocrine cell lines into glucose-sensitive insulin-producing cells.
- Xenograft of nonhuman islet cells **[320]**.

In Conclusion:

The field of regenerative therapy is extremely promising. The progress in experimental applications is tremendous. Clinical progress, however, still faces many limitations. Apart from ethical considerations, results in experimental animals are still controversial in many situations; these results may not be reproducible in human. A lot of questions about which cells, how much and where to be injected have not yet been satisfactorily answered. The mechanisms of stem cell transdifferentiation or those by which they can ameliorate disease are far from fully understood. However efficacy and safety have been proved in many situations and clinical trials are going on. Currently academic laboratory work, experimental animal studies and clinical trials are going hand in hand.

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