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REVIEW

Stem cell therapy for cardiovascular disease: the demise of alchemy and rise of pharmacology

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Regenerative medicine holds great promise as a way of addressing the limitations of current treatments of ischaemic disease. In preclinical models, transplantation of different types of stem cells or progenitor cells results in improved recovery from ischaemia. Furthermore, experimental studies indicate that cell therapy influences a spectrum of processes, including neovascularization and cardiomyogenesis as well as inflammation, apoptosis and interstitial fibrosis. Thus, distinct strategies might be required for specific regenerative needs. Nonetheless, clinical studies have so far investigated a relatively small number of options, focusing mainly on the use of bone marrow-derived cells. Rapid clinical translation resulted in a number of small clinical trials that do not have sufficient power to address the therapeutic potential of the new approach. Moreover, full exploitation has been hindered so far by the absence of a solid theoretical framework and inadequate development plans. This article reviews the current knowledge on cell therapy and proposes a model theory for interpretation of experimental and clinical outcomes from a pharmacological perspective. Eventually, with an increased association between cell therapy and traditional pharmacotherapy, we will soon need to adopt a unified theory for understanding how the two practices additively interact for a patient's benefit.

LINKED ARTICLES

This article is part of a themed section on Regenerative Medicine and Pharmacology: A Look to the Future. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2013.169.issue-2

Abbreviations

AIV, intra-ventricular vein; AMI, acute myocardial infarction; ASC, adult stem cell; bFGF, basic fibroblast growth factor; BM, bone marrow; BMNC, bone marrow mononuclear cell; CABG, coronary artery bypass grafting; CFR, coronary flow reserve; CPC, cardiac progenitor cell; CSC, cardiac stem cell; CVD, cardiovascular disease; EC, endothelial cell; EDV, end-diastolic volume; EPC, endothelial progenitor cell; ESC, embryonic stem cell; ESV, end-systolic volume; FS, fractional shortening; hESC, human embryonic stem cell; HGF, hepatocyte growth factor; HPC, haematopoietic progenitor cell; HSC, haematopoietic stem cell; hVSEL, human very small embryonic-like; IGF-1, insulin-like growth factor 1; iPS, induced pluripotent stem; IVUS, intra-vascular ultrasound; LAD, left anterior descending; LV, left ventricle; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac event; MAPC, multipotent adult progenitor cell; MASC, multipotent adult stem cell; MCP-1, monocyte chemotactic protein-1; MCV, middle cardiac vein; mESC, murine embryonic stem cell; MI, myocardial infarction; MIAMI, marrow-isolated adult multilineage inducible; MS, microsphere; MSC, mesenchymal stem cell; MV, microvesicle; PB, peripheral blood; PBMNC, peripheral blood mononuclear cell; PCI, percutaneous coronary intervention; PGC, primordial germ cell; RCV, retrograde coronary transvenous; SDF-1α, stromal cell-derived factor-1α; Sfrp2, secreted frizzled-related protein 2; SM, skeletal myoblast; SMC, smooth muscle cell; UCB, umbilical cord blood; VEGFR2, VEGF receptor 2; VSEL, very small embryonic-like; WMS, wall motion score

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the Western world. The WHO estimates that over 17 million people around the globe die of CVD each year, and the majority of people that survive heart attacks and strokes will require continuing costly clinical care. In the UK alone, there are approximately 124 000 cases of myocardial infarction (MI) each year, and 88 000 of these MI are fatal (Scarborough *et al.*, 2010).

The main treatment option for these patients is revascularization therapy, including percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). The aim of such procedures is to restore blood flow to the ischaemic myocardium in a timely manner in an attempt to limit the degree of myocardial damage and subsequent maladaptive remodelling. The cost associated with treatment in the UK is £3248 million per annum. Moreover, most patients who suffer an MI display impaired heart function, and many develop heart failure as myocardial performance declines, thus requiring multiple pharmacological therapy, additional revascularization procedures and hospitalization (Velagaleti *et al.*, 2008). The cost associated with hospitalization and production loss is £3295 million per annum. Hence, new cost-effective treatments for true cardiac repair are urgently needed. Even though prolonging life is the basic purpose of a medical treatment, improving quality of life has become a very important goal in our ageing society. Thus, strategies concentrating purely on palliative support of residual cardiac function are neither sufficient nor free of risk. Hence, in this context, any new form of therapy must be profoundly analysed and monitored in preclinical and clinical stages to avoid serious adverse effects.

A new treatment strategy that aims to regenerate the damaged myocardium and restore pristine myocardial function is now being sought, with stem cell therapy offering much promise. Embryonic stem cells (ESCs), adult stem cells (ASCs) and more recently induced pluripotent stem cells (iPS) all demonstrate the ability to differentiate into cardiac-lineage cells *in vitro* (Xu *et al.*, 2004; Caspi *et al.*, 2007; So *et al.*, 2010). As such, many research teams have focused on utilizing these cells as possible sources for myocardial repair in animal models of MI. Experimental studies indicate that cell therapy influences a spectrum of processes, including vascular growth and cardiomyogenesis as well as inflammation, apoptosis and interstitial fibrosis. Hence, distinct cell therapy strategies might be required for specific regenerative needs to prevent heart failure. The apparent success in a majority of these studies has paved the way for a number of human clinical trials. However, results do not seem to fully replicate the achievements of experimental settings, thus casting doubts and uncertainties. Has cell therapy promised too much? Is the problem related to the lack of a solid scientific background? Can we use an available theory (i.e. pharmacology) to re-interpret and refine the approach?

An experimental animal model relevant to the clinical situation is crucial for a better understanding of underlying pathological mechanisms and consequently for tailoring successful treatment methods. Hence, discrepancies between preclinical and clinical findings immediately raise questions about the adequacy of research models. Small rodents cannot faithfully mirror the pathophysiological mechanisms of human hearts. Small heart dimension, relatively thin ventricular wall, rapid cardiac rhythm (Teng *et al.*, 2006) and different biochemical and haemodynamic response to ischaemic injury are crucial discrepancies (Boyle *et al.*, 2011). Nonetheless, the RRR principles recommend the initial use of mammals with the lowest neurophysiological sensitivity likely to produce satisfactory results in the field of investigation. Therefore, it is ethically appropriate to proceed stepwise from screening in small animals to refined large animal models on the journey towards clinical application. Studies in large animal models are mandatory to minimize the possibility of misconception and, ultimately, inadequate treatment.

It is also important to correctly interpret the clinical relevance of experimental outcomes. When comparing therapeutic effects quantitatively, we should bear in mind that, in animal studies, cell therapy is given as the sole treatment, whereas patients receive cell therapy on top of an optimal treatment. Furthermore, even we may not be able to transfer the marked changes in cardiac function observed in animal models to patients, smaller changes may lead to mortality benefits, as seen in other forms of therapy (e.g. device therapy).

Based on these seminal concepts, here we review the use of stem cell therapy from a pharmacological perspective and attempt to reframe this novel approach as a successful theory. We also evaluate possible alternative models for aspects that do not satisfy the principles of pharmacotherapy.

Which stem cell

Science has not yet established which cell is best suited to the treatment of CVD. Therefore, the investigation of all types of cells is warranted. The classification of stem cells in the following section follows a hierarchy based on potency (i.e. potential to differentiate into different cell types) rather than on therapeutic priority.

Embryonic stem cells (ESCs)

ESCs are pluripotent cells derived from the inner-cell-mass of the blastocyst (Thomson *et al.*, 1998). Their pluripotent nature means they can differentiate into any cell type of the three germ layers that make up the body. No drug possesses such a powerful activity. Hence, ESCs could be likened to a drug factory rather than to a single drug.

Owing to their infinite capacity to replicate, ESCs would provide the exponential proliferation of cells required for cardiovascular repair. Different protocols have been set up for the rapid generation of functional vascular endothelial cells (ECs) and cardiomyocytes (Tran *et al.*, 2009; Kane *et al.*, 2010). In addition, ESCs have also been used without prior differentiation in animal models of cardiovascular disease. Murine ESCs (mESCs) have been demonstrated to survive, migrate and proliferate when injected into the infarcted myocardium of rodents (Nelson *et al.*, 2006; Qiao *et al.*, 2009; Lin *et al.*, 2010). These studies also showed the potential of ESCs to provide some, albeit small, restoration of global cardiac function up to 8 weeks post transplant. One of the concerns

to arise, however, was the extremely low degree of cardiomyocyte differentiation, which is as low as 0.5% of the total number of cells delivered (Qiao *et al.*, 2009).

The use of ESCs is currently hampered by ethical controversy, immunogenic potential (Swijnenburg *et al.*, 2005; Nussbaum *et al.*, 2007) and risk of cancer formation, including teratomas (Qiao *et al.*, 2009). These limitations are certainly not confined to murine cells. When human ESCs (hESCs) were injected into healthy or infarcted myocardium, few cardiomyocytes were detected, and teratomas had formed in approximately 50% of rats (Caspi *et al.*, 2007). It is clear therefore that ESCs would need to be pre-differentiated prior to transplantation to try and avoid tumour development. Moreover, immunosuppressive therapy would need to be applied if these cells were to be used in humans. One of the possibilities to prevent rejection is by creating ESCs that are genetically identical to the patient via therapeutic cloning or generating cell lines from different genetic backgrounds so as to use the cell line that is most similar to the patient. Whether or not ESCs might become part of clinical therapy in the future is far from certain.

Induced pluripotent stem (iPS) cells

The breakthrough studies by Takahashi *et al.* (2007) and Yu *et al.* (2007) opened up the possibility of inducing pluripotency in differentiated adult cells. iPS cells can be generated from the patient's own somatic cells and then stimulated to differentiate into any cell type of the body, thus making patient-specific treatment more feasible. However, as with ESCs, iPS cells pose a risk of teratoma formation due to their pluripotent nature.

IPS cells form embryoid bodies with spontaneously contracting clusters (So *et al.*, 2010) and display a genetic profile similar to ESC-derived cardiomyocytes (Gupta *et al.*, 2010; van Laake *et al.*, 2010). These contracting clusters express cardiac mesoderm and cardiomyocyte markers and demonstrate electrophysiological properties (So *et al.*, 2010). Nonetheless, iPS cell-derived cardiac cells are still significantly different in comparison to mature cardiomyocytes (Xi *et al.*, 2010).

IPS cells have been assessed as a potential cell source for the treatment of acute myocardial infarction (AMI). Transplantation of spontaneously contracting embryoid bodies into infarcted mouse hearts reportedly produces an improvement in the contractility indices and a normalization of systolic wall motion (Nelson *et al.*, 2009). In another study using NOD-SCID mice, transplanted iPS cell-derived cardiomyocytes were able to survive after grafting, showed no marker of pluripotency and did not form tumours, suggesting that, like ESCs, the more differentiated the cells the less tumour risk they pose (van Laake *et al.*, 2010).

One current limitation is the small number of cardiomyocytes being produced with currently available differentiation protocols (Burridge *et al.*, 2011; Fujiwara *et al.*, 2011; Zwi-Dantsis *et al.*, 2011). A recent advance in this technology is that the initial requirement of viral vectors for genetic reprogramming of somatic cells is now overcome with the use of transcription factors or direct transdifferentiation (Wernig *et al.*, 2008). However, the efficiency of these new technologies needs to be refined.

A number of studies are broadening our understanding of the genetic modifications that occur during the reprogramming process and differences between iPS cells derived from different somatic cell lines have been revealed by analysing their epigenetic profiles (Polo *et al.*, 2010) It has been reported that iPS cells retain methylation patterns similar to their somatic cell of origin and retain a greater efficiency in differentiating along the donor cell lineage, highlighting the fact that iPS cells retain an epigenetic memory that can influence their subsequent differentiation potential (K Kim *et al.*, 2010; Polo *et al.*, 2010). This phenomenon appears to be attenuated with increased passaging (Polo *et al.*, 2010) or through tertiary reprogramming (K Kim *et al.*, 2010). However, there are still questions over the true pluripotent nature of iPS cells compared with that of ESCs or nuclear transfer techniques.

Of more concern is the potential genomic instability of reprogrammed cells (Gore *et al.*, 2011; Hussein *et al.*, 2011; Lister *et al.*, 2011; Pasi *et al.*, 2011). Genetic analysis appears to show that the reprogramming process leads to genomic aberrations with mutations occurring at both genetic and epigenetic levels, particularly in large genes and genes associated with cancer development; this is thought to be as a result of replicative stress (Gore *et al.*, 2011; Pasi *et al.*, 2011). It is clear therefore that thorough testing of genome integrity should be performed as part of a quality control process before iPS cells are used as a therapeutic agent.

Resident adult stem cells (ASCs)

ASCs are multipotent cells that reside in adult organs and have the ability to self-renew and differentiate into any cell type of a particular organ or system in which they are found. They have been identified in a number of tissues including the bone marrow (BM), heart, skeletal muscle and adipose tissue. Moreover, progenitor cells with limited plasticity populate adult tissues and may be used for specialized cell therapy.

Marrow reconstitution is a well-established procedure in cancer and myeloproliferative disease. This explains why BM cells were rapidly used in cardiovascular regenerative medicine. BM cell therapy may be capable of promoting myocardial repair after MI by contributing to neovascularization and cardiomyogenesis, thereby limiting myocardial remodelling, preserving overall function and preventing the decline to heart failure. One issue arising with BM cells is the low efficacy of cardiac homing and differentiation. With advances in genetic engineering and basic understanding of the signalling pathways involved, these cells can be modified *ex vivo* prior to transplantation to enhance their differentiation potential and functional capacities (Haider *et al.*, 2008; Jiang *et al.*, 2008; Lian *et al.*, 2011). For example, BM cells modified to overexpress insulin-like growth factor 1 (IGF-1) are provided with elements that make them better able to differentiate into cardiovascular lineage cells. Hence these cells improve cardiac remodelling and function to a greater extent than unmodified cells (Haider *et al.*, 2008; Lian *et al.*, 2011).

Apart from haematopoietic stem cells (HSCs), the marrow contains non-haematopoietic cell populations: endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), very small embryonic-like (VSEL) stem cells, multipotent adult progenitor cells (MAPC), marrow-isolated adult multilineage

inducible (MIAMI) cells and multipotent adult stem cells (MASC). It is not clear whether the whole BM or specific sub-fractions are required for regenerative purposes.

Haematopoietic and endothelial lineages are thought to derive from a common ancestor cell, the haemangioblast. In human postnatal haematopoietic tissues, a small subset of $CD34^+$ cells expressing the VEGF receptor 2 (VEGFR2, also known as KDR) is reportedly enriched pluripotent HSCs and capable of vascular regeneration in ischaemic models (Botta *et al.*, 2004). Orlic and colleagues were the first to transplant murine HSCs into the infarcted rodent heart (Orlic *et al.*, 2001). They reported the presence of proliferating HSC-derived cardiomyocytes, ECs and smooth muscle cells (SMCs) occupying around 68% of the infarcted area at 9 days post transplantation, thus suggesting the ability of HSCs to transdifferentiate into cardiac and vascular-lineage cells (Orlic *et al.*, 2001). Since then, a number of other studies have been published supporting the concept of HSC transdifferentiation (Jackson *et al.*, 2001). However, many have disputed these results, suggesting that cell fusion rather than transdifferentiation could occur (Murry *et al.*, 2004; Deten *et al.*, 2005; Norol *et al.*, 2007).

EPCs – cells with endothelial surface marker expression and functional characteristics of ECs – can not only be grown from HSC expressing the markers CD34 or CD133 but also from monocytic cells (Asahara *et al.*, 1999; Madeddu *et al.*, 2004; Urbich and Dimmeler, 2004). These specialized populations are seemingly able to support neovascularization of the ischaemic heart (Suuronen *et al.*, 2007; Dubois *et al.*, 2010), resulting in indirect benefits for cardiomyocyte survival and function (Kocher *et al.*, 2001; Schuh *et al.*, 2008). A continuous effort has been made to determine the precise characteristics of EPC based on antigenic profiles (H Kim *et al.*, 2010) and clonal analysis (Yoder *et al.*, 2007).

MSCs are relatively easy to isolate and proliferate from the BM; they are considered to be immunoprivileged and have been shown to be capable of differentiating into ECs (Oswald *et al.*, 2004) and cardiomyocytes both *in vitro* and *in vivo* (Toma *et al.*, 2002). Intramyocardial injection of *ex vivo* expanded MSCs to the infarcted heart, immediately or shortly following MI, improves cardiac recovery (Imanishi *et al.*, 2008; Chacko *et al.*, 2009; Li *et al.*, 2010). Besides being capable of transdifferentiation, MSCs act as potent activators of angiogenesis through the release of angiogenic factors. This led to increased vessel density in the peri-infarct area and a restoration of myocyte mechanical function and overall cardiac function.

Other sources of MSCs include peripheral (PB) and umbilical cord blood (UCB) and adipose tissue. Adipose tissue-derived MSCs have been delivered via intramyocardial injection immediately after MI induction in mouse and pig models (Alt *et al.*, 2010; Yu *et al.*, 2010). In animal studies these cells have been shown to increase the vessel density, reduced scar size and induce a functional improvement, as evidenced by increased left ventricular ejection fraction (LVEF) and fractional shortening (FS). However, results from recent studies have indicated that MSCs can induce calcification, cancer and lose their immunomodulatory activity as they differentiate in the recipient tissue (Djouad *et al.*, 2003; Huang *et al.*, 2010; Jeong *et al.*, 2011; Katare *et al.*, 2011).

Human VSEL (hVSEL) cells are a rare subpopulation of stem cells identified in BM as well as in PB and UCB (Kucia *et al.*, 2007). They are characterized by $CD133^+CXCR4^+Lin^-CD45^-$ surface markers, very small size, high nucleus to cytoplasm ratio, open type chromatin, abundance of mitochondria and diploid number of chromosomes. *In vitro*, they are able to differentiate into all three germ layers (Ratajczak *et al.*, 2011). In contrast to pluripotent stem cells, VSEL cells do not complete blastocyst development and noticeably do not form teratomas, probably due to specific methylation of imprinted genes (Ratajczak *et al.*, 2011). Developmentally, VSEL cells are the most primitive stem cells in BM, acting as a common precursor of HSCs and MSCs. They are similar to primordial germ cells (PGCs) (Ratajczak *et al.*, 2011), very closely related to pluripotent epiblast stem cells (Surani *et al.*, 2007). Apart from overlapping markers, these subpopulations (VSEL cells, PGCs and HSCs) are characterized by high sensitivity to a stromal cell-derived factor-1α (SDF-1α) gradient and associated migratory response. Therefore, besides being developmentally related, these cells could be responsive to similar chemoattractants from injured tissues, which guide their mobilization and migration to ischaemic tissues (Paczkowska *et al.*, 2009; Wojakowski *et al.*, 2009; Ratajczak *et al.*, 2011).

A few non-haematopoietic stem cell subpopulations in the BM, including MSCs, MAPCs, MIAMI cells and MASCs, display several features of pluripotent stem cells (Zuba-Surma *et al.*, 2009). It is possible that these cell types and VSEL cells belong to the same species of stem cell, yet they remain differently categorized simply because of different experimental procedures of isolation and characterization (Ratajczak *et al.*, 2008). Moreover, there is a strong interaction between VSEL cells and BM fibroblasts, which implicates internal cell processing/hiding, also termed emperipolesis. Thus, BM-derived stromal cells might be contaminated with VSEL cells, which would account for the high 'plasticity' of fibroblastic cells (e.g. MSCs or MAPCs) (Zuba-Surma *et al.*, 2009). Additionally, to support close developmental relation, VSEL cells are able to differentiate into MSCs *in vivo* and *in vitro* (Dawn *et al.*, 2008; Zuba-Surma *et al.*, 2011). The use of VSEL cells does not raise the ethical concerns associated with ESCs. From a clinical point of view, however, obtaining high numbers of VSEL cells in culture is difficult and this may limit their usefulness.

Growing attention is being paid to cardiac resident stem/progenitor cells (CSCs/CPCs) as an endogenous source of cells for direct cardiomyogenesis. Beltrami's study was the first to show that isolated $c\text{-}kit^+/Lin^-$ CSCs behave as self-renewing, clonogenic and multipotent cells that give rise to cardiomyocytes, ECs and SMCs (Beltrami *et al.*, 2003). It is thought that these cells are activated after heart injury and migrate to damaged regions to generate new cardiomyocytes (Gonzalez *et al.*, 2008). The use of CSCs could be achieved through either cell transplantation or endogenous activation. CSCs are reportedly capable of differentiating into cardiomyocytes *in situ*; therefore, there would be no need to pre-differentiate these cells prior to transplantation (Smits *et al.*, 2009). Murine and human CSCs have been cultured and expanded *in vitro* and delivered to the infarcted rodent heart by intravenous, intracoronary and intramyocardial injection (Beltrami *et al.*, 2003; Oh *et al.*, 2003; Dawn *et al.*, 2005; Wang

et al., 2006; Smits *et al.*, 2009). These studies show that CSCs are capable of migrating to the ischaemic heart where they promote increased vessel density, cardiomyocyte differentiation and reduced myocyte apoptosis. It appears that at least part of the benefit is through paracrine mechanisms (i.e. release of protective factors by transplanted CSCs). One clinical caveat with CPCs, as for other cells that require expansion, is that they cannot be used immediately after an MI, when they would probably be most effective for replacing the acute cardiomyocyte loss (Tang *et al.*, 2010).

Endogenous CSCs express receptors for a number of growth factors. Activation of these receptors by exogenously administered ligands represents a strategy to increase CSC proliferation and migration *in vivo* (Urbanek *et al.*, 2005; Rota *et al.*, 2008; Ellison *et al.*, 2011) and thereby improve myocardial performance (Urbanek *et al.*, 2005). Epicardial CPCs can be activated by thymosin β4 (Tβ4) restoring pluripotency (Smart *et al.*, 2007). Chromosome painting of labelled donor epicardial CPCs revealed transdifferentiation to myocytes and vascular cells in the absence of cell fusion. Derived cardiomyocytes were shown to structurally and functionally integrate with resident muscle, suggesting that stimulation of this adult progenitor pool may represent a useful means for cell therapy in ischaemic heart disease (Smart *et al.*, 2011).

Like the heart, the vasculature also possesses its own repertoire of stem cells, which is strategically located in specialized niches across the vessel wall. Our recent work shows that clonogenic pericyte-like progenitor cells are located in the adventitial niche and can be extracted and expanded from vein leftovers of CABG patients to obtain millions of cells for therapeutic use. Injection of human pericytes in immunocompetent mice improves the recovery from MI through the stabilization of vascularization, containment of infarct extension and inhibition of fibrosis, by a mechanism involving the microRNA-132 and its target genes, Ras-GTPase activating protein and methyl-CpG-binding protein 2 (Campagnolo *et al.*, 2010; Katare *et al.*, 2011). Interestingly, human pericytes conserve a peri-vascular memory; that is, they accumulate around neovessels after intramyocardial injection. Therefore, they represent a unique example of therapeutic cells that can be selectively delivered to a specific structural component of the heart for targeted repair. Furthermore, other perivascular cells from the BM and epicardium are seemingly endowed with relevant reparative potential (Crisan *et al.*, 2008; Chong *et al.*, 2011), although only a few of them have been tested in MI models (Galli *et al.*, 2005).

Skeletal myoblasts (SMs) develop from skeletal muscle satellite cells and already have contractile functions. Transplantation of SMs into models of MI showed that they have the ability to replace cardiomyocyte loss and restore a degree of cardiac function (Taylor *et al.*, 1998; Reinecke and Murry, 2000; Horackova *et al.*, 2004). To improve the efficacy of SM transplantation, many teams have focused on overexpressing growth factors such as VEGF (Ye *et al.*, 2007; 2008; Aharinejad *et al.*, 2008; Rong *et al.*, 2008). It appears that overexpression of these factors make SMs more efficient at promoting angiogenesis and preventing cardiomyocyte apoptosis, leading to improved blood flow to infarcted areas and increased cell survival in AMI models (Ye *et al.*, 2007; 2008; Rong *et al.*, 2008).

Transplanted SMs are short-lived, however, with a significant number of transplanted cells no longer being detectable 72 h post-grafting (Suzuki *et al.*, 2004). Those that survive retain their skeletal muscle cell characteristics (Taylor *et al.*, 1998) and are unable to integrate into myocardial tissue (Reinecke and Murry, 2000). This lack of integration increases the risk of ventricular arrhythmias (Fernandes *et al.*, 2006), and early clinical trials have reported a number of patients developing either sustained or non-sustained ventricular tachycardia, predominantly within the initial post-operative period (Menasche *et al.*, 2003; Pagani *et al.*, 2003; Siminiak *et al.*, 2004; Dib *et al.*, 2009).

The lesson of clinical trials

Several small-size clinical trials, a large randomized controlled trial (Repair-AMI) and meta-analyses indicate that cell therapy reduces infarct size and improves LV function and perfusion in patients with AMI or ischaemic cardiomyopathy (Abdel-Latif *et al.*, 2007; Burt *et al.*, 2008; Kang *et al.*, 2008; Martin-Rendon *et al.*, 2008; Dill *et al.*, 2009) (Table 1). The modest clinical improvement reported so far suggests the need for further investigation. The EU-FP7 has recently funded a consortium of 17 clinical centres to perform a definitive outcome study on BM cells in 3000 MI patients (BAMI trial). Additional trials now explore the potential of resident cells, like expanded autologous CPCs, in patients with myocardial ischaemia (Bolli *et al.*, 2011). Although *ad interim* results are promising, it is premature to draw definitive conclusions on efficacy and safety before trial conclusion.

The early randomized, controlled TOPCARE-AMI trial using intra-coronary infusion of either BM mononuclear cells (BMNCs) or blood-derived progenitors 4 days post AMI led to significant improvements in global LVEF and wall motion score (WMS) at the infarct border zone at 4 months follow-up (Assmus *et al.*, 2002). There was also a significant improvement in myocardial viability within the infarct area and a normalization of coronary flow reserve (CFR) in patients without restenosis. These initial reports were based on the first 20 patients enrolled, although when 59 patients were followed up to 1 year, there was also a significant improvement in LVEF and infarct size compared with the controls (Assmus *et al.*, 2002). The results of this small trial suggest that BMNCs have the potential to significantly improve vessel and myocardial function after AMI.

The only trial with a substantial follow-up time has been the BOOST randomized, controlled trial. This study confirmed that BMNCs can significantly improve LVEF and WMS up to 6 months (Wollert *et al.*, 2004). When followed up to 18 months and 5 years, however, the initial increase in LVEF could no longer be detected (Meyer *et al.*, 2009), suggesting that any functional benefit resulting from BMNC transplantation is short-lived.

The larger, randomized, double-blind, placebo-controlled REPAIR-AMI trial found that the absolute change in LVEF was significantly greater in the BMNC group versus the control ($P = 0.01$). The same pattern was found with regards to myocardial contractility, but no significant changes in end-systolic volume (ESV) or end-diastolic volume (EDV) were observed in

Table 1

Summary table of key trials utilizing direct transplantation of BMNCs in AMI

Study reference	Study design	No. of patients	Cell dose	Procedure time	Follow-up	Outcomes
(Assmus *et al.*, 2002; Schachinger *et al.*, 2004) (TOPCARE-AMI Trial)	Randomized controlled	n = 29 BMNC; n = 30 CPC; n = 11 Control	7.35 ± 7.31 × 10^6 $CD34^+/CD45^+$ cells	~4 days post AMI	4 months and 1 year	Cell therapy significantly improved LVEF and WMS at border zone. No significant difference between cell therapy groups. CFR normalized and viability in infarct-zone significantly increased with cell therapy. At 1 year, LVEF and infarct size significantly improved with cell therapy.
(Wollert *et al.*, 2004; Meyer *et al.*, 2009) (BOOST Trial)	Randomized controlled	n = 30 BMNC; n = 30 Control	24.6 × 10^8 nucleated cells, 9.5 × 10^6 $CD34^+$cells, 3.6 × 10^6 haemopoietic colony-forming cells	~5 days post-AMI	6 months and 5 years	LVEF and WMS at border zone significantly improved in BMNC group versus controls at 6 months. EDV, ESV, LV mass index and myocardial injury did not significantly differ from control. No significant difference in MACEs or restenosis at 5 years. No significant improvement in LVEF at 5 years.
(Schachinger *et al.*, 2006a,b; Erbs *et al.*, 2007) (REPAIR-AMI Trial)	Randomized double-blind Placebo-controlled multi-centred	n = 101 BMNC; n = 101 Control	236 ± 174 × 10^6	3–7 days post-PCI	4 months and 1 year	Significant improvement in LVEF and regional contractility in control and BMNC groups. Absolute change in LVEF significantly greater in BMNC versus control. Inverse relationship between baseline and absolute change in LVEF in treatment group. Trend for increased contractile function in patients treated >4 days post PCI. CFR normalized and vascular resistance decreased in treatment group. No MACEs at 1 year.
(Tendera *et al.*, 2009) (REGENT Trial)	Randomized controlled multi-centred	n = 80 $CD34^+/CXCR4^+$ cells; n = 80 BMNC; n = 40 control	1.90 × 10^6 $CD34^+/CXCR4^+$ cells; 1.78 × 10^8 BMNC	7 days post-PCI	6 months	Procedure is safe. No significant difference in absolute change in LVEF or EDV and ESV between groups. Inverse correlation between baseline LVEF and its change after cell therapy in patients treated with $CD34^+/CXCR4^+$ cells but not with BMNCs. Significant improvement in patients receiving any cell therapy when baseline LVEF was <median. No significant difference in MACEs or restenosis between groups
(Bolli *et al.*, 2011) SCIPIO Trial; Phase 1	Randomized Open-label single-centred	n = 16 $c\text{-}kit^+/Lin^-$CSC; n = 7 control	1 × 10^6 $c\text{-}kit^+/Lin^-$CSC	Mean of 113 days post CABG (by IC infusion)	4 months and 1 year	No CSC-related adverse effects were observed. In 14 CSC-treated patients who were analysed, LVEF increased from 30.3% before application of cells to 38.5% at 4 months after infusion. At 1 year in eight patients, LVEF increased by 12.3 ejection fraction units versus baseline. In 7 patients cardiac MRI study showed, that infarct size decreased from 32.6 g by 24% at 4 months and 30% at 1 year.

either the control or treated groups at 4 months (Schachinger *et al.*, 2006a). A small sub-study associated with the REPAIR-AMI trial also showed a normalization of CFR and reduction of microvascular resistance in the BMNC-treated group, in agreement with the TOPCARE-AMI trial, which is in keeping with the promotion of vascular repair (Erbs *et al.*, 2007).

Worth noting is the REGENT trial, another large randomized controlled trial, that found no significant difference in the absolute change in LVEF between any of the treatment groups or controls at 6 months (Tendera *et al.*, 2009), which is in contrast to the previous trials. However, in accord with the results from previous trials, an interesting finding from both the REPAIR-AMI (Schachinger *et al.*, 2006a,b) and REGENT (Tendera *et al.*, 2009) trials is that the improvement in LV function with cell therapy was greater in patients with worse baseline LVEF scores.

No major adverse cardiac events (MACE) have been reported after an intracoronary infusion in any of the trials discussed, suggesting that intracoronary infusion is a safe way of administering treatments. In fact, the REPAIR-AMI trial reported that the number of deaths, recurrent MI and revascularization was significantly lower in the treatment group versus control (Schachinger *et al.*, 2006a,b). Additionally, the combination of deaths, recurrent MI and hospitalization for chronic heart failure occurred less frequently in the treatment group.

Recent meta-analysis studies of randomized, controlled trials utilizing intracoronary infusion of BMNCs in AMI suggests that overall the increase in LVEF of those treated with BMNCs is significantly higher compared with controls (Lipinski *et al.*, 2007; Zhang *et al.*, 2009). Moreover, these meta-analyses support the conclusion that intracoronary infusion of BMNCs is safe and feasible.

The lesson from the first generation of clinical trials represents a solid building block for designing second-generation studies.

Can pharmacology provide a framework for cell therapy refinement?

One major struggle in physical science is to find a unifying theory for describing phenomena within a wide dimensional range. Wherever these ranges overlap, different theories agree and become part of the same model; yet, owing to their diversity, a vast proportion of physical phenomena cannot be described by a single unified theory. This results in the adoption of a network of theories that are good to explain the dualisms and paradoxes of physical reality. A similar approach may be useful to reconcile the differences between pharmacotherapy and cell therapeutics (Table 1). Traditional drugs and cells differ in size, mass and solubility and; for some properties, it is even impossible to classify them by the use of a common template. Researchers still endeavour to find the ideal recipe (i.e. the best cell) and its superior correlation to clinical benefit, a procedure not distant from alchemy. A multidisciplinary effort bridging these gaps might eventually lead to new knowledge guiding specialists and even general practitioners to use cell or cell-derived bioproducts, alone or in association with conventional drugs, to treat cardiovascular patients in the near future.

In the next section, we will try to address the issue of whether the classical pharmacological model can be used or adapted to better classify and exploit the potential of cell therapy. We will also discuss the applicability of the pharmacological model for the delivery of new cell products to the healthcare system.

Drug production

The preparation of a natural human medication – stem cells – involves harvesting of ingredients (from BM/specific tissue), their characterization and cultivation in a favourable environment under good manufacturing practise (GMP) conditions. Moreover, the pre-formulation of a drug requires the additives to be compatible with the active cell component to obtain a stable and tolerable final product.

During cell preparation, viability must be strictly measured with pre-injection level around 95%. This is hardly achievable with operative procedures that require cryopreservation, storage and thawing of the cell product. Therefore, a great deal of attention is focusing on improving preservation of viability during cell processing and banking (Healy *et al.*, 2011). Noteworthy, as for clinically used drugs, bioproducts need to be microbiologically tested to exclude endogenous (viral, bacterial) and exogenous contaminations (Strauer and Steinhoff, 2011).

One major distinction in stem cell production is the use of autologous and allogeneic cell therapy. Autologous stem cells might be a first step towards personalized medicine, where the donor is also the recipient. In line with Pharma investments in pharmacogenetics and pharmacometabolomics to generate new compounds for personalized or stratified medicine, tissue-derived molecular information may pave the way to analogous improvement in customized cell therapy. Owing to the limited amount and depressed functionality of patient's cells, these bioproducts may require expansion, modification and final checking for viability, purity and therapeutic activity. Noteworthy, the reduction in the cells' functionality, mentioned previously, is associated with an accumulation of DNA damage and alterations in gene expression throughout the lifespan, which may require genetic and epigenetic screening, before and after expansion, prior to autologous transplantation (Ratajczak *et al.*, 2010). On the other hand, widely available, off-the-shelf bioproducts made of allogenic stem cell lines may be stocked in bio-banks and used for thousands of patients as needed. A few trials with allogeneic stem cells have already been approved. In one such trial, a cryopreserved neural stem cell line, ReN001, derived from 12-week-old fetal tissue was used to treat patients with sequelae from ischaemic stroke (Mack, 2011).

General characteristics: physical properties and formulations

Stem cells have much higher mass and dimensions compared with common drugs (Table 1). Substantial differences also

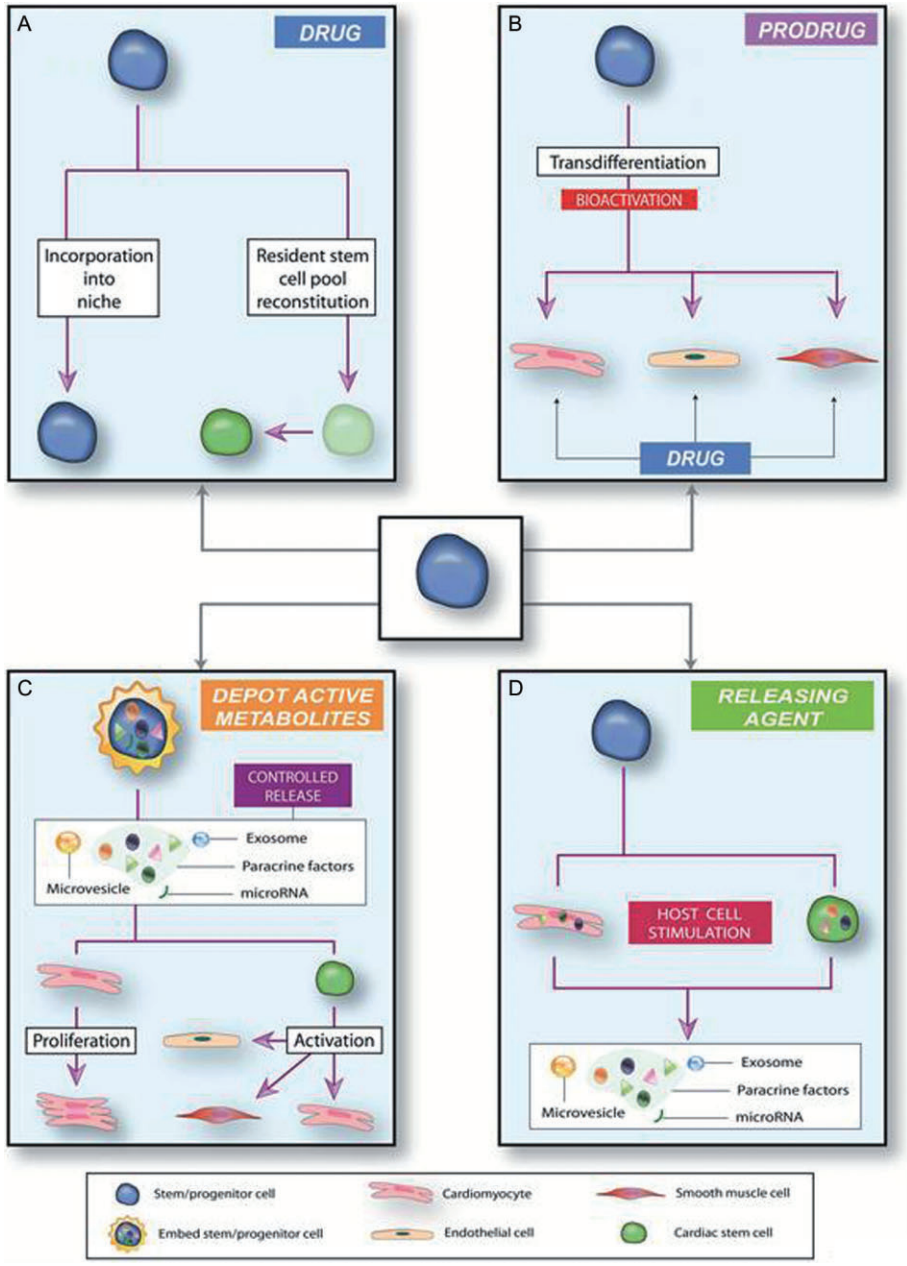

Figure 1

Mechanisms of stem/progenitor cell action. Stem/progenitor cell, acting as a *drug*, incorporates into the niche or reconstitutes resident pool of cardiac stem cells (A). Stem/progenitor cell, acting as a *pro-drug*, is bioactivated by transdifferentiation (B). Stem/progenitor cell, acting as *metabolite-releasing agent*, release paracrine factors in controlled way – depot form (C). Stem/progenitor cell, acting as an *indirect releasing agent*, stimulate host cells to release paracrine factors, microRNAs, microvesicles and exosomes (D).

exist in dosage and biodistribution. Furthermore, stem cells are metabolically active; that is, they extract energy from the external environment, absorb drugs and interact with them either intra- and/or extracellularly. The kinetic energy of a stem cell in the niche is very low. However, upon injection into the circulation or ischaemic tissues, stem cells acquire velocity, and hence kinetic energy, being attracted by chemical cues. Noteworthy, changes in expression of surface receptors and cytoskeleton rearrangement could confer stem cells with additional kinetic energy to use during migration. The model theory of and laws governing cell motility simply do not apply to phamacotherapeutics.

Bioproducts including stem cells can be assimilated to the classical formulations of drug, pro-drug, controlled-release drug and releasing agent (Figure 1). Cells are used as a *direct drug* to reconstitute the resident pool of stem cells or other components of the niche. This classical approach has long since been employed for BM reconstitution after chemotherapy and now extended to reconstitute the cardiac stem cell pool. Cells can also be used as *pro-bioproducts* or *pro-drugs*

that require a bioactivation process to become therapeutically active. The bioactivation process consists of stem cell differentiation into cardiomyocytes as well as the production of a surplus of accessory cells to support nutrition, perfusion and structural solidity (i.e. vascular cells, interstitial cells and fibroblasts). ESCs, iPS cells, CPCs, pericytes and VSEL cells are typical examples of pro-bioproducts for cardiac and vascular reconstitution.

However, despite strenuous work, there is no convincing evidence that adult BM cells transdifferentiate into functional cardiomyocytes (O'Malley and Scott, 2004), and their action seems to be mainly due to release of growth factors and angiogenic or immunomodulatory cytokines. For these cells, the most appropriate term is *controlled release agent*, which corresponds to formulations, like tablets and capsules, used in pharmacotherapy to release the active ingredient after absorption at the target site. This apparently minor function may turn BM cells and mobilized haematopoietic cells, including monocytes, from a poor affiliate of pluripotent stem cells into a powerhouse. Likewise, other stem cells and progenitor cells may exploit paracrine mechanisms for promotion of tissue healing. Functioning metabolites working in a paracrine fashion seem to be responsible for a spectrum of actions, including neo-angiogenesis (Mazhari and Hare, 2007), extracellular matrix modulation (Jain *et al.*, 2001), anti-apoptosis and probably stimulation of resident progenitor cell proliferation (Deindl *et al.*, 2006; Loffredo *et al.*, 2011), BM/heart axis adjustment (Lapidot and Petit, 2002) and even apoptotic-mediated local reaction ('dying stem cell hypothesis') (Thum *et al.*, 2005). These active metabolites include VEGF, hepatocyte growth factor (HGF), IGF-1, basic fibroblast growth factor (bFGF), MCP-1, SDF-1α, secreted frizzled-related protein 2 (Sfrp2), PKB (Akt) (Uemura *et al.*, 2006) as well as microRNAs (Katare *et al.*, 2011; Sahoo *et al.*, 2011), exosomes and microvesicles (MVs). As postulated by Ratajczak, MVs may act through three different mechanisms: (i) stimulation of host cells directly by surface expressed ligands; (ii) delivery of protein, mRNA, miRNA, bioactive lipids and mitochondria, as MVs contain some cytoplasm from original cell; and (iii) transfer of surface receptors between cells. Furthermore, it has been proposed that it is possible to construct superior MVs by engineering MSC overexpressing favourable factors (Ratajczak, 2011).

Importantly, there are examples of cells like MSCs that encompass properties of both *pro-bioproducts* and *controlled release bioproducts* owing to their combined capacities of cell reconstitution and release of therapeutic ingredients (Pittenger *et al.*, 1999; Kinnaird *et al.*, 2004a,b). The evolution of these concepts has already generated new systems in which MSCs are embedded in biocompatible beads, allowing for controlled paracrine release, longer engraftment and protection from immunological reaction of the host (Tang *et al.*, 2011). Moreover, cells can be either genetically modified (Noiseux *et al.*, 2006) or hypoxia-preconditioned to improve their activity as *releasing agents of bioproducts* (Rehman *et al.*, 2004).

Finally, transplanted cells can act as *indirect releasing agents*. This last term is used in pharmacology for drugs that stimulate the release of neural transmitters or other active substances from human cells. Likewise, using species-specific primers, it is possible to demonstrate that human cells stimulate the release of angiocrine factors by resident and recruited cells of the recipient (Jeong *et al.*, 2009; Katare *et al.*, 2011).

Pharmacodynamics and pharmacokinetics

The model theory of interference is well established in physics. Waves travelling through the same medium at the same time may interfere by superposition. The net effect of this process might be both amplification and reduction of resultant wave amplitude. Interacting waves must be coherent with each other (correlated in phase, amplitude and frequency).

Interference also represents a founder concept in pharmacology. The interaction between drugs and biological systems encompasses the effect of drugs on the organism (pharmacodynamics) and the modification of drugs by the organism (pharmacokinetics). With regard to cell therapy and MI, optimal synchronization is required to obtain a satisfactory outcome in the form of significantly improved heart function (Figure 2). Instead of amplitude, wave frequency and phase, we have to monitor the following variables: cell type, route of administration, time interval between MI and cell administration, injection velocity and pressure, needle diameter, single or multiple injections, place of administration (border-zone/necrotic myocardium), injection into beating or arrested heart, application during reperfusion or occlusion, preconditioning effect, injectate volume and cell concentration.

Routes of administration

As in pharmacotherapy, different administration systems have been used for cell therapy either having the advantage of easy delivery via the systemic circulation or direct administration into the myocardium to facilitate cell retention and integration (Wu *et al.*, 2011b) (Figure 3).

Peripheral i.v. administration is the easiest and safest but the not the most efficient cell delivery method with deliverance effectiveness of <1% (Barbash *et al.*, 2003; Chin *et al.*, 2003; Hofmann *et al.*, 2005; Kupatt *et al.*, 2005; Freyman *et al.*, 2006; Kang *et al.*, 2006). The contact with the infarct-related region is very limited due to the cells dissolution in plasma volume and small (~3%) coronary blood flow contribution of cardiac output (Strauer, 1979). Hence, cells administered i.v. require numerous passages through the infarcted myocardium to achieve an effective homing concentration. It appears that the majority of cells are retained within the liver and spleen during the first passage (Hofmann *et al.*, 2005). As a result, no functional benefit can be seen at 3 or 6 months post treatment (Hare *et al.*, 2009).

Intracoronary administration is generally performed during routine PCI and shows superior homing efficiency compared to the i.v. route because stem cells are delivered directly in the vicinity of the infarcted region (Hofmann *et al.*, 2005; Hou *et al.*, 2005; Blocklet *et al.*, 2006; Kang *et al.*, 2006; Caveliers *et al.*, 2007; Doyle *et al.*, 2007; Penicka *et al.*, 2007; Qian *et al.*, 2007; Schachinger *et al.*, 2008). To avoid immediate washout and enhance the effectiveness of delivery, cells are administered via an over-the-wire balloon cath-

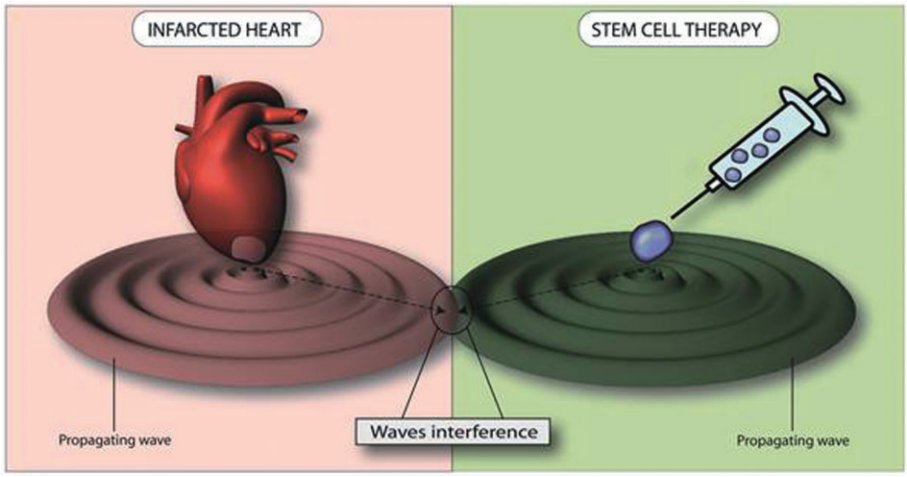

Figure 2

Synchronization of stem cell therapy and myocardial infarction is required to obtain significant improvement of heart function. The following variables must be optimized: cell type, route of administration, time interval between myocardial infarction and administration of cells, injection velocity and pressure, needle diameter, single or multiple injections, site of administration (border-zone/necrotic myocardium), injection into beating or arrested heart, application during reperfusion or occlusion, preconditioning effect, injectate volume and cell concentration.

eter, which facilitates cell trans-endothelial migration by prolonging contact time. Moreover, local ischaemia caused by balloon dilatation seems to be important for cell homing (Gyongyosi *et al.*, 2010).

Stem cell permeation into the myocardium depends in the first instance on myocardial vascularity and coronary blood flow. Moreover, the type of cell does matter, as myocardial retention of enriched BM cells is superior by several orders of magnitude to non-enriched ones (Kraitchman *et al.*, 2005). Comparing BM cell retention in acute and chronic phases of MI, Penicka *et al*. have shown that 20 h after intracoronary administration, cells were present in the target area only in patients with an acute event (Penicka *et al.*, 2007). Thus, it seems that the intensity of local biochemical processes influences the engraftment efficiency.

Clinical and functional improvement using the intracoronary route is far from desirable with mixed results being reported (Stamm *et al.*, 2003; Wollert *et al.*, 2004; Schachinger *et al.*, 2006a,b; Meyer *et al.*, 2009; Grajek *et al.*, 2010) (Table 1). In addition, intracoronary injection is generally safe, but not completely free of potential complications. For instance, intimal dissection, micro-infarctions due to embolization after cell injection (especially MSCs due to relatively large cell diameter) (Vulliet *et al.*, 2004; Furlani *et al.*, 2009), difficulties in accessing chronic occlusions (Bourassa *et al.*, 1995), distribution of the cells in non-targeted organs (Caveliers *et al.*, 2007; Penicka *et al.*, 2007), decrease in coronary blood flow (Freyman *et al.*, 2006) and in-stent restenosis (Bartunek *et al.*, 2005) have been reported.

Three delivery strategies are available for intramyocardial injection: transendocardial, retrograde coronary transvenous injection (both percutaneous methods) and transepicardial application (surgical method). The most important advantage of intramyocardial administration is the direct and immediate placement into the myocardial compartment. Stem cell permeation is vasculature-independent and 'drug solubility' in the myocardium depends predominantly on the cells interaction with the microenvironment.

Transendocardial administration has shown high delivery efficiency (up to 54% at 40 min after application) (Mitchell *et al.*, 2010). Modern intraventricular mapping systems (NOGA and ESI) allow for the identification of viable tissue and facilitate precise injection of cells into the infarct border zone. The NOGA system is a contact procedure, whereas ESI is a non-contact technique. The former has been used in already completed (Smits *et al.*, 2003; Charwat *et al.*, 2010) as well as in ongoing clinical trials (NCT00555828, NCT00314366; http://www.clinicaltrials.gov). An example is the study by Krause *et al.* who utilized percutaneous intramyocardial injection on 20 patients with AMI. No adverse procedural effects were reported and an initial improvement in LVEF within the first 6 months was seen (Krause *et al.*, 2009). The ESI is still in a preclinical stage, but has shown successful cell transplantation and significant LVEF improvement in a porcine model of MI (Wei *et al.*, 2010). The procedure is generally safe but not free of potential drawbacks. Perforation and electrical abnormalities during contact mapping and cell injection have been reported (Gyongyosi *et al.*, 2009). Moreover, the procedure is expensive and requires training.

Retrograde coronary transvenous injection (RCV) is also attractive (Thompson *et al.*, 2003; Siminiak *et al.*, 2005). Intravascular ultrasound (IVUS) guidance is employed to facilitate precise localization of the region of interest as well as reduce the possibility of venous wall damage and pericardial haemorrhage by an incorrectly positioned needle (Brasselet *et al.*, 2005; Siminiak *et al.*, 2005). Despite the lower cost and relatively short-time performance compared with the transendocardial approach, RCV allows intervention only along veins. The anterior intraventricular vein (AIV) runs parallel to the left anterior descending artery (LAD), whereas the middle cardiac vein (MCV) runs in the posterior inter-

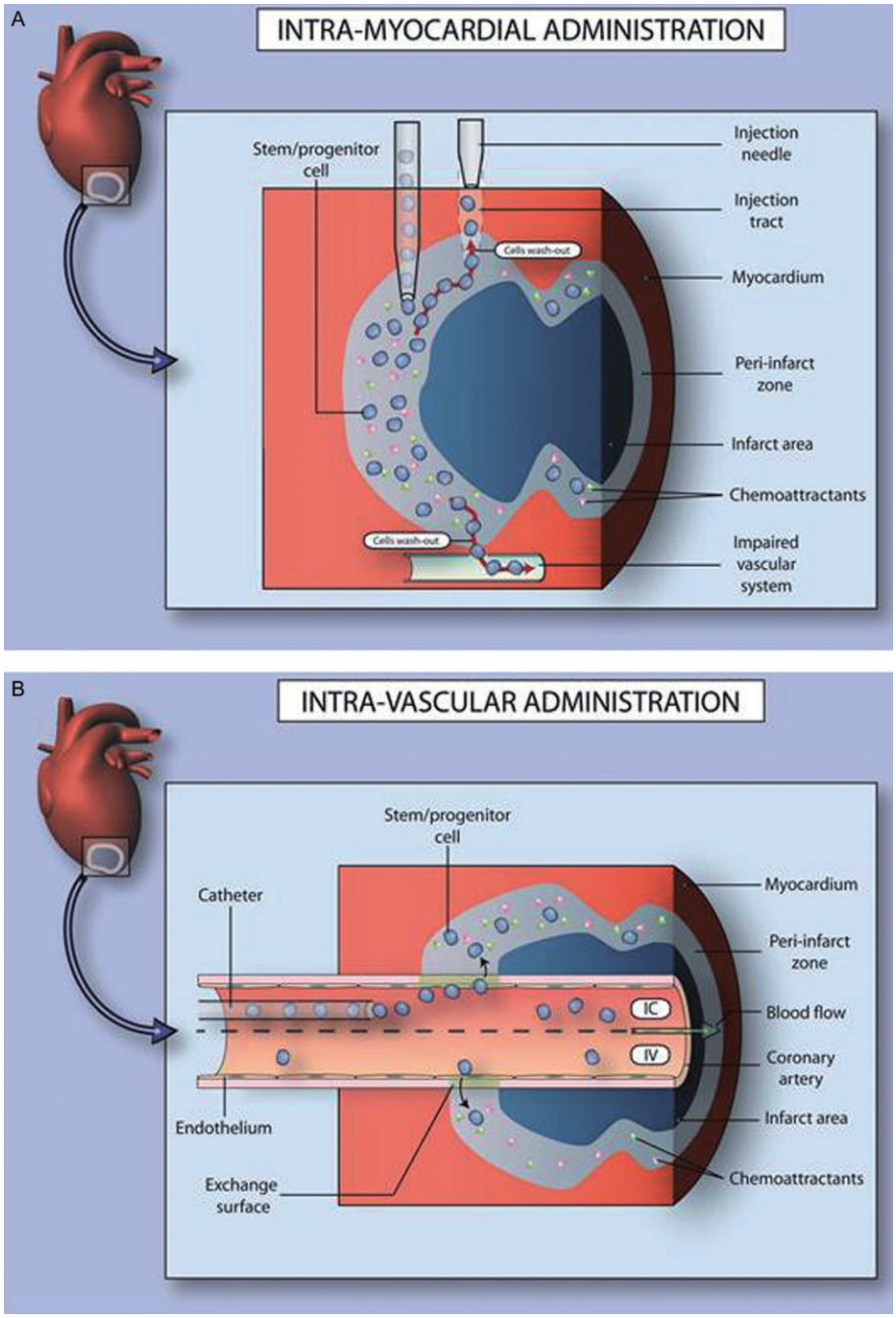

Figure 3

Retention of stem/progenitor cells after intramyocardial (A) and intravascular – intracoronary and i.v – (B) administration. The wash-out of cells through the injection tract and/or impaired vascular system is proposed to be responsible for early mechanical loss. Stem/progenitor cell permeation depends on the chemoattractants concentration gradient, the number of cells having contact with infarct-related artery wall (exchange surface), cell concentration and coronary blood flow.

ventricular sulcus, draining blood from the posterolateral wall of the LV (Herity *et al.*, 2000). Of note, the coronary vein anatomy is characterized by significant variability.

Transepicardial delivery provides a high rate of deliverance to the heart. Mitchell and co-workers achieved 57% retention of EPCs 40 min post implementation (Mitchell *et al.*, 2010). Transepicardial application is performed during CABG procedure, which allows direct access to the ischaemic border-zone (Patel *et al.*, 2005; Stamm *et al.*, 2007). In addition, transepicardial injection can be performed through microthoracotomy (Klein *et al.*, 2007; Pompilio *et al.*, 2008).

Similar to RCV, intracoronary vein infusion can be used as an alternative choice in patients with coronary artery occlusion and poor collaterals (Wu *et al.*, 2011a). It is worth noting

that the adjustment of balloon inflation pressure during the procedure improves the delivery efficiency (Hou *et al.*, 2005). Peripheral vein access, required to reach coronary veins through the coronary sinus, is clinically more favourable compared with the arterial artery approach due to less frequent bleeding and ischaemia during balloon occlusion. In fact, AVI balloon inflation has no influence on LAD blood flow up to 30 min (Herity *et al.*, 2000). Interestingly, the number of veno-venous connections seems to limit the efficiency of the procedure (Herity *et al.*, 2000). In general, intracoronary vein administration results in cell retention levels as low as 2% to 3% and prevalent accumulation in the lungs (Hou *et al.*, 2005). This procedure is analogous to the intracoronary vein approach, but balloon inflation occurs directly in the coronary sinus. It was shown that in the short term and long term this method is safe, feasible, relatively cheap and effective. Ninety days and 1 year after cell delivery in patients with stable angina, both perfusion and collateral vessel supply were reportedly improved in >60% of individuals (Vicario *et al.*, 2004; 2005).

So far no human clinical study has been performed using an intrapericardial route of administration. However, injection into the pericardial sac seems an ideal route, providing direct contact with the heart, minimal dispersion and slow turnover. Moreover, the results from preclinical studies are encouraging (Ladage *et al.*, 2011). According to Hermans *et al.* (2002), small molecules injected intrapericardially reach the myocardium, but larger particles do not. In healthy mice, stem cells migrate into myocardium after intrapericardial injection, but they preserve their immature morphology. Nevertheless, in the presence of vasculopathy, cell differentiation occurred (Steele *et al.*, 2005). Furthermore, in pigs, a marked myocardial homing of cells was observed following MI (Branco *et al.*, 2009). Potential risks of this method are acknowledged to be low as the approach is routinely used for drug delivery in patients with pericarditis (Maisch *et al.*, 2004). Noteworthy, pericardial effusion accompanying pericarditis reduces the risk of wall puncture and tamponade during drug application. The risk might be higher in MI patients owing to the lower amount of 'protecting' fluid in the pericardial sac. New percutaneous access (with PerDUCER) and upgraded devices (AttachLifter) simplify the procedure (Maisch *et al.*, 2000; 2001).

Tissue bioengineering is another promising method for cell delivery. Biodegradable epicardial patches (Rosen *et al.*, 2007), scaffolds (Davis *et al.*, 2005) or intramyocardial injectable gels may be particularly effective (Kofidis *et al.*, 2005; Zhang *et al.*, 2006). Biomaterials (natural or synthetic) provide an optimal microenvironment (Forte *et al.*, 2008) and prolonged delivery time (Leor *et al.*, 2000), thus facilitating cell attachment and migration. Moreover, novel biointerface technology allows biomaterial programming to influence and regulate the behaviour of the cell phase (Place *et al.*, 2009).

Duration of action, homing and retention

The duration of action of a drug is known as its half-life and depends on the drug distribution and rate of clearance. Owing to its half-life, a given drug might need to be administered from one to several times a day. Interpreting the half-life of cell therapy in relation to its biodistribution and clearance represents a puzzling task, as the duration of therapeutic effects of a single application largely exceeds the persistence of cells in injected tissues. In this respect, the productiveness of cell therapy on the recipient can be compared with the irreversible changes induced by some enzyme inhibitors, which produce covalently modified 'dead-end complexes'. In living organisms, such modifications can occur at the level of the DNA and histones, resulting in epigenetic imprinting responsible for persistent functional alterations. It is likely that such an epigenetic memory of cell therapy is triggered by agents released by the cells rather than by the cells themselves.

Retention represents the Achilles's heel of cell therapy. Early cell loss is observed within the first minutes of implantation (Teng *et al.*, 2006), possibly be due to cell retention in the syringe's dead space (Muller-Ehmsen *et al.*, 2002), leakage from the puncture site into the ventricular cavity or pericardium (Grossman *et al.*, 2002; Muller-Ehmsen *et al.*, 2002; Teng *et al.*, 2006), dispersion through impaired local vascular system (Grossman *et al.*, 2002; Muller-Ehmsen *et al.*, 2002; Teng *et al.*, 2006) or a combination of the above. In order to better understand the nature of the process, researchers have used microspheres (MSs) instead of stem cells (Grossman *et al.*, 2002; Teng *et al.*, 2006; Hudson *et al.*, 2007; Anderl *et al.*, 2009). The main advantage of this delivery system is the exclusion of microenvironment influences on cell survival rate. On the other hand, lack of membrane adhesion molecules and cell plasticity underestimates clearance halftime when using MSs (Teng *et al.*, 2006). Of note, the diameter of the MSs administered was devised to mimic cell size (Grossman *et al.*, 2002; Hudson *et al.*, 2007). However, increasing the MS diameter to 40 μm does not affect the retention level (Anderl *et al.*, 2009).

Clearance of intramyocardial implanted stem cells reportedly follows a three-phase kinetics model (Teng *et al.*, 2006): (i) rapid and extensive loss through injection tract or impaired vascular system; (ii) gradual decrease due to microenvironmental factors (apoptosis, ischaemia, reactive oxygen species); and (iii) slight increase due to cell proliferation. Regarding phase 1, a linear correlation between the number of cells injected and their clearance is generally observed. Unfortunately, simply increasing the number of implanted cells may not give the result expected due to a limit in the capacity of the myocardium to accommodate an excess of cells and the cytotoxic effects of the necrotic core, which is more likely to develop with a large injectate volume (Muller-Ehmsen *et al.*, 2002). Remarkably, the influence of pressure caused by myocardial contraction may also play a crucial role in mechanical cell loss. Generally, the overall effect of contraction pressure seems to be negatively correlated with the level of cells retained (Grossman *et al.*, 2002; Teng *et al.*, 2006). Moreover, Mitchell *et al.* (2010) have shown that mechanical forces acting on cells after subendocardial and subepicardial injection vary due to the systolic and diastolic transmural pressure gradient, accounting for superior retention following subendocardial injection (Grossman *et al.*, 2002; Mitchell *et al.*, 2010). Accordingly, *ex vivo* animal studies using MSs show that cells injected into arrested hearts are more effective; the retention rate in non-beating hearts was almost seven times higher than that in contracting hearts (Teng *et al.*, 2006). Moreover, an ascending gradient was observed from subepicardial (beating) to subendocardial

(beating) and a stilled heart (Grossman *et al.*, 2002). In contrast, an *in vivo* study using cardiopulmonary bypass model corresponding to CABG with cardioplegia showed no difference between beating and arrested hearts (Hudson *et al.*, 2007). It is also proposed that complete surgical heart isolation may improve cell engraftment (Bridges *et al.*, 2005).

It seems that improved cell engraftment is associated with multiple, lower volume injections (Grossman *et al.*, 2002). It is also worth noting that injection pressure influences delivery efficiency (Hou *et al.*, 2005). The forces acting on cells during their administration may have two opposite effects: cell destruction as well as stretch preconditioning (Muller-Ehmsen *et al.*, 2002). Moreover, the issue of cell application during reperfusion and occlusion is also interesting. According to Grogaard *et al.* (2007), reperfusion is advantageous to cell homing after intracoronary administration.

The myocardium viability seems to inversely correlate with cell homing (Schachinger *et al.*, 2008). The emerging question is whether inflammatory processes in the infarct zone promote or inhibit cell engraftment. On the one hand, inflammation acts as an activating factor for cell homing and proliferation via the production of chemoattractants (chemokines, cytokines, bioactive lipids; Cui and Madeddu, 2011; Ratajczak *et al.*, 2012; Wojakowski *et al.*, 2012) as well as complement activation (Ratajczak *et al.*, 2012). On the other hand, a hostile microenvironment may impair cell engraftment, survival and differentiation (Swijnenburg *et al.*, 2010; Sheikh *et al.*, 2012). Early kinetics of BM cells (up to day 4 post MI) is different in ischaemic and non-injured myocardium. In the first group, homing is impaired but reaches the same value as that in non-injured myocardium after 4 days, suggesting that ischaemia induces cell proliferation (Sheikh *et al.*, 2012). It was thought that anti-apoptotic, pro-angiogenic, anti-free radical and heat shock treatments can significantly improve cell survival (Teng *et al.*, 2006).

Dispersion and clearance

Heterogeneity of the medium in which the wave propagates may cause its dispersion. Similarly, stem cell wave travelling through the body encounters different environments that may contribute to a specific biodistribution of injected cells. The character of this process depends largely on the route of administration and cell type. The highest intensity of cell dispersion is observed after intravascular injection, the lowest and clinically more preferable, after direct intramyocardial application. The stem cell pulse propagating along the circulatory system loses its 'energy' mainly in the liver, spleen and lungs.

Many studies have confirmed a significant contribution of the liver to stem cell absorption (Gao *et al.*, 2001; Brenner *et al.*, 2004; Hofmann *et al.*, 2005; Blocklet *et al.*, 2006; Freyman *et al.*, 2006; Kang *et al.*, 2006; Doyle *et al.*, 2007; Kurpisz *et al.*, 2007; Qian *et al.*, 2007; Schachinger *et al.*, 2008). Hofmann *et al.* (2005) have shown that i.v. and intracoronary administration of unselected BM cells in MI patients is associated with >85% retention in the liver and spleen ~1 h after injection. However, in the same study intracoronary injection of enriched $CD34^+$ cells improved myocardial and reduced liver and spleen retention (>55%). $CD34^+$ cell kinetics were also observed by Blocklet *et al.* (2006) who noted 48 ± 35% and 71 ± 10% cell retention 1 h and 19 h post injection, respectively. A time-dependent increase in cell number might be dependent on the type of cell and mode of mobilization. In fact, different kinetics were observed with G-CSF-mobilized HSCs (Kang *et al.*, 2006), circulating pro-angiogenic progenitor cells (Schachinger *et al.*, 2008) and peripheral blood $CD133^+$ cells (Caveliers *et al.*, 2007).

The spleen is another non-target organ that absorbs stem cells (Gao *et al.*, 2001; Brenner *et al.*, 2004; Hofmann *et al.*, 2005; Blocklet *et al.*, 2006; Doyle *et al.*, 2007; Kurpisz *et al.*, 2007; Qian *et al.*, 2007; Schachinger *et al.*, 2008). Early (1 h post administration), single-time-point observations showed that 17.3 ± 1.1% and 17 ± 6% of intracoronary injected BMNCs (Qian *et al.*, 2007) and intraventricularly administered $CD34^+$ haematopoietic progenitor cells (HPCs) were retained in the spleen, respectively (Brenner *et al.*, 2004). Moreover, dynamic measurements of cell accumulation in the spleen demonstrated a stable level 3- to 4-days following their intracoronary administration with a slight increase after 24 h (Schachinger *et al.*, 2008). Likewise, Blocklet *et al.* (2006) showed consistent levels of cells during a 19 h period of observation (29 ± 19% and 29 ± 10% 1 h and 19 h post-injection, respectively). Similar kinetic values were reported by Caveliers *et al.*, but the retention level was lower (3.1–3.7% and 3.5–3.8% 1–2 h and 12 h post-$CD133^+$ cell administration, respectively). In contrast, i.v. administration of MSC was found to be associated with an increased cellular uptake by the spleen from 2.3 ± 2.7% (immediately after injection) to 5.6 ± 1.9% (1 day after injection) (Kraitchman *et al.*, 2005). It is worth noting that the study by Kang *et al.* (2006) discloses steady kinetics 2–4 h post administration (12.5%) with a significant decline after 20 h (2.4%). Interestingly, direct intraventricular injection of HPCs was associated with very high (86.6 ± 27.0%) accumulation 24 h post application (Nowak *et al.*, 2007). EPCs administered by the same route localize mainly in lymphoid follicles around marginal zones (Aicher *et al.*, 2003).

A significant initial 'inhalation' of stem cells into the lungs was observed predominantly after their i.v. injection. Following 'exhalation', a gradual redistribution of stem cells to the spleen, liver, kidney and BM occurs (Gao *et al.*, 2001; Chin *et al.*, 2003; Brenner *et al.*, 2004; Kraitchman *et al.*, 2005; Kang *et al.*, 2006; Love *et al.*, 2007). Early lung accumulation of injected MSCs shows asymmetrical distribution with 3:1 left to right lung ratio. Moreover, the pattern of localization is associated with local perfusion conditions as left-side positioning promotes left lung cell aggregation (Kraitchman *et al.*, 2005). Stem cell clearance from the lung is observed within 48 h post i.v. injection (Gao *et al.*, 2001; Kraitchman *et al.*, 2005). According to Kraitchman *et al.* (2005), <10% of the cells originally taken up by the lung remain there after 24 h with simultaneous redistribution of cells predominantly to the liver (from 14.5 ± 15% to 48.2 ± 2% of initial left lung uptake). Transient pulmonary cell trapping was also reported by Barbash *et al.* (2003), where 53% of injected cells were located in the lungs and by Kang *et al.* (2006) (fast, 2 h post injection lung clearance and transfer to the spleen). Nevertheless, another study showed that 14 days post injection, >20% of the MSCs injected, either i.v. or intracoronary, were accumulated in the lungs (Freyman *et al.*, 2006). Similarly, Chin *et al.* (2003) observed that a high number of cells were aggregated in the lungs 2 weeks after their infusion.

Intracoronary adminstration of circulating progenitor cells was associated with a surprisingly high (>60%) accumulation in the lungs with little uptake by the liver and spleen 60 min after infusion (Doyle *et al.*, 2007). Hou *et al.* (2005) reported that pulmonary retention of PBMNCs is 26 ± 3% (intramyocardial), 47 ± 1% (intracoronary), and 43 ± 3% (retrograde coronary venous). Additionally, direct injection of HPCs into the right ventricle results in 42.4 ± 21% distribution in the lungs 24 h post the procedure (Nowak *et al.*, 2007).

A transient high uptake of stem cells in the lungs probably results from a larger cell diameter compared with the diameter of the lung capillaries. Supportive evidence indicates that the administration of vasodilator sodium nitroprusside results in decreased stem cell accumulation in the lungs (Gao *et al.*, 2001). Notwithstanding, Li *et al.* showed that i.v. administered MSCs can be found mainly in pulmonary interstitium (D'Amario *et al.*, 2011). Interestingly, it seems that the cell type is an important factor in determining their pattern of biodistribution. Initial (post 1 h), transient pulmonary uptake of HPCs administered into the left ventricle (LV) was not observed for EPCs (Aicher *et al.*, 2003). In addition, localization of both HPCs and EPCs was comparable after 24 h (Brenner *et al.*, 2004). It should be noted that *in vivo* tracking systems using specific markers may lead to erroneous interpretation of biodistribution owing to radiotracer efflux from cells (Kuyama *et al.*, 1997; Aicher *et al.*, 2003; Brenner *et al.*, 2004) and renal and hepatobiliary excretion of the radiomarker (Aicher *et al.*, 2003; Brenner *et al.*, 2004).

Dosage and timing

According to the REPAIR-AMI trial, the optimal time for intervention is not earlier then 5 days after the cardiovascular event (Schachinger *et al.*, 2006a). For this reason, the vast majority of clinical trials to date have centred on administering autologous BM cells in patients with an AMI as compared with chronic MI. Indeed, in a meta-analysis conducted by Jiang *et al.* (2010), BM cell therapy was only seen to have a benefit in acute ($P < 0.00001$) and not chronic MI patients ($P = 0.26$). Focusing specifically on AMI trials, a statistically significant difference in LVEF improvement was found to be associated with those patients treated with BM cells within 7 days post AMI (Martin-Rendon *et al.*, 2008), suggesting that BM cell therapy may have a beneficial effect on early myocardial remodelling. Importantly, the effect of an early cell implantation has not been directly tested to date in humans. The ongoing clinical trial (NCT00939042; http://www.clinicaltrials.gov) involving the early time point (<6 h after the onset of symptoms) should give a better insight into the time-related aspect of cell application.

Current animal studies support the strategy of cell application as soon as possible, during the acute phase. In a canine model, a 1 week interval significantly reduced the clearance half-time from 74.0 ± 15.3h (cell injected immediately after infarction) to 41.3 ± 0.8h (Mitchell *et al.*, 2010). Moreover, according to Schachinger *et al.* (2008), the preferable conditions for cell engraftment are a low viability of myocardium and diminished flow reserve. However, there are contradictory data with regard to the influence of infarct size on cell homing (Qian *et al.*, 2007; Schachinger *et al.*, 2008).

Amplitude and frequency are crucial variables for wave interference. Likewise, cell number in the injectate, volume of vehicle as well as injection frequency may play an important role in the final outcome. Little attention has been paid to determine the ideal cell dose in patients, but it appears as that the effectiveness of the cells could be dose – dependent. Two studies have suggested a possible trend for an increasing cell dose being associated with a greater LVEF improvement (Meluzin *et al.*, 2006; Quyyumi *et al.*, 2011); however, the small group size makes it impossible to draw any firm conclusion. A recent meta-analysis of 52 cell therapy studies in pigs, dogs and sheep with MI showed a threshold optimal dosage of 10^8 cells (van der Spoel *et al.*, 2011). A meta-analysis of randomized, controlled clinical trials utilizing autologous BM cells in MI patients showed an overall increase in LVEF in those treated compared to controls ($P = 0.0007$), with a threshold effective dosage of 10^8 cells (Martin-Rendon *et al.*, 2008). In agreement with this, Jiang *et al.* (2010) reported that LVEF correlates positively with cell dosage in the range of 10^7 to 10^9 $CD34^+$ cells.

Pathways of exploitation

Several major indicators, including the number of clinical trials in progress, announcements by the UK government for a multimillion-pound infrastructure investment, rapid industry growth and results of the Eurobarometer poll showing substantial public awareness and support (http://ec.europa.eu/public_opinion/archives/ebs/ebs_341_en.pdf), all suggest that stem cell therapy will become an important part of the global healthcare system. This opinion also emerges from the research impact analysis recently released by the Department of Business Innovation and Skills (BIS) (http://www.bis.gov.uk/assets/biscore/innovation/docs/t/11-1056-taking-stock-of-regenerative-medicine.pdf).

Opportunities for profit are also increasing. Cell therapy alone had global sales of $410 million in 2008 and this is predicted to grow to $5.1 billion by 2014. Revenues might grow even faster with integration of regenerative medicine products into current therapeutic programmes. For example, treatment of vascular disease with aspirin, ADP receptor inhibitors and angioplasty may be soon combined with cell therapies, like Aldagen's ADL-301 (USA), Pluristem's PLX-PAD (Israel) or ReNeuron's CTX (UK), to improve tissue perfusion in patients with critical limb ischaemia or restore function in patients with stroke. Moreover, the Big Pharma interest has been revitalized with the first regenerative medicine blockbusters becoming reimbursable by US Medicare & Medicaid Services.

All the positive indicators are however balanced by uncertainty about the best model for development of a new cell therapy product. In fact, the traditional model of new molecule drug development does not necessarily fulfil the needs of the emerging cell therapy sector. In particular, very few preclinical studies have progressed to the development of first-in-man clinical trials and the eventual translation into healthcare products. Longstanding problems, like limited venture capital finance, complicated patent systems, divergence in short-term objectives of academic and industrial research, together with the current global downturn exacerbates these difficulties.

It is therefore vital that basic scientists working in the cell therapy field are aware of the necessity of designing a plan during the early stage of the discovery process. Hence, they should familiarize themselves with topics not pertaining to basic science, like the assessment of the market size, manufacturability, scalability and clinical performance of the candidate cell product. On the other hand, increasing mechanistic understanding is essential to the design of clinically relevant cell products with incremental therapeutic activity. In line with this, the UK Ministry of Health states that: 'Science cannot predict at this stage which types of cell will prove to be of most benefit and so continued research on all types of cell remains necessary to improve our knowledge' (http://www.bis.gov.uk/assets/biscore/innovation/docs/t/11-1056-taking-stock-of-regenerative-medicine.pdf). Finally, cost-effectiveness is crucial for decision making in the healthcare system, as outlined by the UK's National Institute for health and Clinical Excellence (NICE).

The strategy for exploitation varies according to the nature of the cell product. While allogenic cell therapies have a potential for retention of intellectual property and industrial participation in exploitation, autologous cell therapies offer less scope for intellectual property coverage (since a patient's own cells cannot be patented) and are generally delivered as a service embedded in existing healthcare systems.

Moving research on stem cells to treatment of patients is complex. The first step is to consult with the Medicine and Healthcare products Regulatory Agency (MHRA) and the European Medicine Agency (EMEA) to decide if a cell product is an advanced therapy medicinal product (ATMP), which in general applies to cells and tissues that have been manipulated. For an ATMP to obtain market authorization, full demonstration of quality, safety and efficacy need to be provided through an application to EMEA. If the product is not an ATMP, other regulations might apply as well. In particular, ethical permission for the use of human cells requires a licence by the HTA to ensure that procedures comply with the required quality and safety standards.

Conclusions

The number of beneficiaries of cardiovascular cell therapy is potentially enormous, yet only a few thousand patients have taken advantage of the new approach. The time for full exploitation of cell therapy depends on different factors, including financial and ethical aspects and support from society and stakeholders. However, the major impetus for stem cells to become healthcare products still derives from original and well-conducted research. Exploiting the wisdom of pharmacology might accelerate this translational process for the benefit of millions of people.

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Conflict of interest

Herein, we declare that there is no competing interest on behalf of all authors.

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