Stem cells for clinical use in cardiovascular medicine
Current limitations and future perspectives
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Summary
Cell transplantation is currently gaining growing interest as a potential new means of improving the prognosis of patients with cardiac failure. The basic assumption is that left ventricular dysfunction is largely due to the loss of a critical number of cardiomyocytes and that it can be partly reversed by implantation of new contractile cells into the postinfarction scars. Primarily for practical reasons, autologous skeletal myoblasts have been the first to undergo clinical trials and now that the feasibility of the procedure is well established, efficacy data are expected from the ongoing randomized studies. Bone marrow stem cells are also generating a great deal of interest, particularly in patients with acute myocardial infarction, and are currently undergoing extensive clinical testing although recent data have raised a cautionary note about the transdifferentiation potential of these cells. While experimental studies and early-phase clinical trials tend to support the concept that cell therapy may enhance cardiac repair, several key issues still need to be addressed including (1) the optimal type of donor cells in relation to the clinical profile of the patients, (2) the mechanism by which cell engraftment improves cardiac function, (3) the optimization of cell survival, (4) the development of less invasive cell delivery techniques and (5) the potential benefits of cell transplantation in nonischemic heart failure. Current evidence suggests, however, that adult stem cells (myogenic or marrow-derived) fail to electromechanically integrate within the recipient heart, thereby mandating the search for second generation cell types able to achieve this goal which is the prerequisite for an effective enhancement of contractile function. Preliminary data suggest that cells that feature a true cardiomyogenic phenotype such as cardiac stem cells and cardiac-precommitted embryonic stem cells may fall in this category and carry the potential for ensuring a true regeneration of dead myocardium.

Keywords
Stem cells, myocardial infarction, heart failure, skeletal myoblasts, bone marrow, embryonic stem cells

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Introduction
Cell therapy is currently emerging as a potential new treatment for heart failure with the underlying assumption that recolonization of the areas of scarred myocardium with exogenously supplied surrogates or precursors of cardiomyocytes could restore function and ultimately affect clinical outcomes.

So far, various cell types have been tested experimentally for cardiac repair but for obvious practical reasons, only those of autologous origin have yet undergone clinical testing. This encompasses both bone marrow-derived cells and skeletal myoblasts.

Current status of cell therapy clinical trials
Chronologically, skeletal myoblasts were the first to enter the clinical arena at the end of almost a decade of extensive laboratory investigations (1) which have established that injected myoblasts stably engrafted into postinfarction scars and, although remaining physically isolated from the surrounding residual cardiomyocytes, were able to improve left ventricular function. Indeed, myoblasts should be better termed precursor cells in that they do not have the plasticity of true stem cells and are already engaged in a tissue-specific myogenic differentiation pathway. Normally lying in a quiescent state under the basal membrane of skeletal muscular fibers, they are rapidly mobiliz-

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ed upon skeletal muscle fiber injury and effect regeneration of the damaged fibers through active proliferation and fusion. Aside from their autologous origin and ease of procurement, a great in vitro scalability under appropriate culture conditions and a relatively high resistance to ischemia have strongly contributed to their consideration for clinical use. It should, however, be acknowledged that the consistent documentation of improved functional outcomes following skeletal myoblast transplantation contrasts with the persisting uncertainties regarding the mechanisms of these benefits; current hypotheses include (in a non mutually exclusive fashion) limitation of left ventricular remodelling by a girdling effect, occasional contraction of chimeric cells resulting from the fusion of myoblasts with recipient cardiomyocytes at the graft-host interface (2, 3) and paracrine effects (4) exerted by cell-released growth factors and cytokines on several putative targets (angiogenesis, recruitment of resident quiescent cardiac cells or extracellular matrix composition).

Despite these unsettled mechanistic issues, the bulk of animal data has been deemed convincing enough to justify a move towards clinical applications that actually started in June, 2000, when we performed the first human transplantation of autologous myoblasts in a patient with severe ischemic heart failure. This case was the first in a series of 10 which primarily allowed to document the feasibility of myoblast culture and expansion according to Good Manufacturing Practice conditions and the safety of multiple intraoperative injections of the final cell yield across the postinfarction scar (5). This early phase I trial also unravelled a potential proarrhythmic risk of myoblast implantation. Although differences in the electrical properties between implanted myoblasts and host cardiomyocytes could set the stage for micro-reentry circuits, only the results of the ongoing placebo-controlled randomised trial (see below) will allow to assess, in an unbiased fashion, the incidence of postengraftment arrhythmias and thus to determine to what extent the risk (if any) is increased above the arrhythmic background inherent in heart failure.

Our initial trial was soon followed by five other phase I studies of autologous skeletal myoblast transplantation, three of which were surgical, i.e., entailed myoblast implantation associated with coronary artery bypass grafting (6–8) while the two others were designed as catheter-based stand-alone procedures (9, 10). Altogether, these studies, in addition to confirm safety and feasibility, have also provided pathologic evidence for myotube engraftment in the human infarcted heart (11). Conversely, they do not allow to draw meaningful conclusions regarding efficacy because they were neither designed nor powered to address this end point. Indeed, the interpretation of functional outcomes is confounded by several factors such as the inhomogeneities in cell culture processes, the variability in the methods used for assessing viability of the grafted scars, and the revascularization or lack of revascularization of these segments. For this reason, we have implemented the MAGIC (Myoblast Autologous Grafting In Ischemic Cardiomyopathy) trial which features a multicentric, randomised, placebo-controlled, dose-ranging and double-blind protocol design. The primary and secondary end points of the study are the recovery of left ventricular function and the 6-month clinical outcomes, respectively, of patients meeting the following three inclusion criteria: (1) a severe left ventricular dysfunction reflected by an echocardiographically measured ejection fraction < 0.35, (2) a postinfarction discrete akinetic and nonviable scar, as assessed by dobutamine echocardiography, and (3) an indication for coronary artery bypass surgery in remote ischemic areas, i.e., areas different from those in which the cells (or placebo) are injected. The study is currently under way and the interim efficacy analysis of the first 75 patients should be performed before the end of 2005. One particular feature of the MAGIC protocol is that all patients are implanted with an internal cardioverter-defibrillator (ICD) both for safety reasons and also because most of the MAGIC patients match the MADIT II criteria. As previously mentioned, this protocol offers the additional advantage of providing a recording of arrhythmic events and, thus, to compare their incidence between treated patients and those receiving placebo injections. So far, the unblinded safety analysis of the first patients whose ICD readouts are available (and analysed by an independent event adjudication committee) looks rather reassuring and suggests that even if myoblast implantation was to facilitate ventricular arrhythmias, the latter are manageable by an appropriate drug regimen combining beta-blockers and amiodarone given in the perioperative period. If the MAGIC and related trials ultimately demonstrate some efficacy, diffusion of the technique will undoubtedly require less invasive methods of delivery than open-chest surgery or even thoracoscopy. While the size of myoblasts precludes their direct intracoronary injection, percutaneous injections utilizing endoventricular (9) or trans-coronary sinus catheters (10) have already been tested. In our experience, the latter technique looks more reliable, accurate and user-friendly (12).

Bone marrow-derived cells have been the second cell product that has been used clinically in patients with ischemic heart disease. The pioneering studies conducted in Germany stemmed from the seminal experiments of Orlic and coworkers (13) showing, in a mouse model of myocardial infarction, that injection of a phenotypically well-defined population of bone marrow cells (lin–c-kit+) 5 hours after coronary artery ligation resulted in a repopulation of the infarct areas by the engrafted cells, their conversion into cardiomyocytes and endothelial cells and, ultimately, an improvement of function, at least in the short term since the assessment was done only 9 days after transplantation. In addition to the conceptually attractive idea that, in contrast to skeletal myoblasts, bone marrow cells could have a plasticity potential allowing them to adopt a cardiac phenotype in response to environmental cues, the ease of bone marrow harvest and the relatively loose regulatory constraints inherent in the extemporaneous use of the cells (with absent or very limited in vitro expansion) have greatly contributed to the enthusiasm of many clinicians and driven the implementation of a flurry of trials.

So far, most of these trials (14) have focused on patients presenting with an acute myocardial infarction and undergoing an emergency percutaneous revascularization procedure (angioplasty and stenting). The standard protocols have then entailed bone marrow (or peripheral blood) harvest and immediate intra-coronary re-injection of the unfractionated cell yield during a brief period of occlusion of the target vessel intended to facilitate diffusion of the cells into the myocardial tissue. Overall, the procedure has turned out to be remarkably safe, although a caution-
ary note has been raised about a potential risk of atheromatous plaque overgrowth which, however, has not been really documented. Until a recent past, all studies have also consistently reported improved outcomes with regard to global and regional left ventricular function, tissue perfusion and viability. Importantly, however, all these studies have been observational, comparing their results with those of historical controls or case-matched cohorts, and the only randomised trial (the BOOST study) did not include a placebo-controlled group. These methodological limitations and the resulting care with which their results should be interpreted are highlighted by the recent report (communication by Dr Stefan Janssens at the late-breaking clinical trials of the 2005 American College of Cardiology meeting) of the first randomised, placebo-controlled, double-blind study of intracoronary bone marrow cell injections in 66 patients with acute myocardial infarction who underwent a percutaneous coronary intervention within 4 hours of symptom onset. In contrast to the precedent trials, this study failed to document a significant increase in left ventricular ejection fraction in the 32 bone marrow-treated patients compared with the 34 placebo-injected controls. However, magnetic resonance imaging demonstrated a reduction in infarct size which did not translate into an improvement in global (or even) regional function, a finding attributed to the high percentage of patients with microvascular obstruction. Clearly, these data mandate additional carefully designed and strongly powered studies to better delineate the place of bone marrow cell intracoronary injections in the setting of the acutely revascularized myocardial infarction. Fewer studies have addressed the effects of this mode of therapy in patients with refractory myocardial ischemia (15) or ischemic heart failure (16) and while their results have confirmed the feasibility and the safety of the procedure (whether it is surgical or catheter-based), they have not (yet?) provided compelling efficacy data. In addition, the concept of transferrationalization has been increasingly challenged (17, 18) by the observation of fusion events and there is now growing evidence that the primary effect of bone marrow cells is to secrete a wide variety of growth factors (19), making them probably more suitable for paracrinally enhancing angiogenesis than for generating intrinsically contractile cells.

Limitations and perspectives

At least three major issues need to be addressed.

The fate of engrafted cells

A major limitation of cell therapy is the high death rate of the injected cells within a few hours and days following transplantation. This attrition is likely to result from the interplay of several factors, particularly physical strain-related damage incurred by cells during manual poorly pressure-controlled injections, hypoxia and necrosis related to the poor vascularity of the target scars, apoptosis possibly enhanced by the inadequate patterning of the transplanted cells relative to the extracellular matrix and inflammation. There is evidence that the surviving fraction subsequently proliferates (20), an event possibly enhanced by electrical cardiac fluxes and cardiomyocyte-released factors (21) and soluble factors released by myoblast-induced macrophage chemotaxis (22), but not to the point that it can catch up the initial attrition rate. Additionally, a fraction of the cells injected inoperatively is likely to rapidly escape into the systemic circulation through the venous system of the heart and correspondingly reduces the amount of cells remaining trapped within the recipient myocardium. The same holds true for intracoronarily infused unfractonated mononuclear cells whose retention in myocardial tissue is extremely low (in the range of 1–3%) (23) compared with the high “graft” enrapment rate in other organs like the lungs or the spleen (of note, this percentage was found to be substantially higher after intracoronary infusion of a selectively enriched CD34+ population). These considerations are clinically relevant in that, at least in the case of skeletal myoblasts, a tight correlation has been established between the number of injected cells and the functional outcome (24). Clearly, additional studies have to be performed to better clarify the dose-effect relationships for the various sources of cells.

These observations clearly emphasize the importance of developing strategies targeted at enhancing cell survival to optimise the expected benefits of cell therapy. The design of these strategies should be the result of a balanced trade-off between conceptually sound scientific hypotheses, consistent animal data and clinical practicality. Among those, two look particularly appealing. The first is based on an optimisation of the graft blood supply, which can be achieved in different ways (concurrent revascularization by coronary bypass or an interventional procedure, co-injection of angiogenic growth factors (25) or transplantation of cells engineered so as to overexpress some of these factors (26)). A second possible strategy consists of embedding cells into bioresorbable tridimensional matrices (27) that can help in physically limiting cell leakage while protecting them against harmful environmental stresses like invasion of inflammatory cells. Both strategies are currently undergoing laboratory testing. In addition, enhancement of cell survival can probably benefit from improvements in cell delivery devices and, in the setting of percutaneous procedures, it should be emphasized that more bench work is still required for clarifying the possible interactions between cells and catheter materials. Finally, an objective and quantitative assessment of the efficacy of these various strategies requires the development of reliable imaging techniques allowing a non-invasive tracking of the fate of the transplanted cells (28).

The optimal cell type in relation with the patient population clinical status

As previously mentioned, current data tend to support the use of bone marrow cells at the acute stage of myocardial infarction, when the freshly ischemic tissue still harbours the appropriate differentiation signalling pathways whereas there is less evidence for the benefits of these cells when implanted later in fibrotic postinfarction scars. It remains, however, to determine whether it is appropriate to use the mononuclear cell yield, as currently done, or whether a given subpopulation would be more functionally effective. In this regard, endothelial or hematopoietic progenitors are rather credited for an angiogenic potential but their low percentage in the peripheral blood (or the bone marrow) raises the issue of scalability and it remains to be established whether this problem can be safely and efficaciously addressed by cytokine mobilization. So far, the results reported...
with intraoperative in-scar injections of CD133+ have not provided compelling efficacy data, possibly because of the relative scarcity of available cells (29). On the other end, mesenchymal cells (which, by itself, are also an heterogeneous population) could be better suited for myogenesis (30) and currently generate a great deal of interest because of their purported immunoprivilege, possibly related to a prostaglandin E2-mediated induction of tolerance (31) and which might allow to use them as an allogeneic on-shelf product. Indeed, a clinical trial entailing intravenous injection of allogeneic mesenchymal cells in patients with recent myocardial infarction has just been launched in the United States. Of note, however, the recently identified population of human bone marrow mesenchymal cells credited for a differentiation into cardiomyocytes (30) only featured these phenotypic changes after co-cultures with neonatal rat cardiomyocytes, which may limit its clinical applicability. A selection process could also be relevant to skeletal myoblasts which represent an heterogeneous population that could be sorted to isolate a fraction recently shown to exhibit cardiomyocyte characteristics (32).

The mechanisms of action

Although this may remain, like for drugs, a provisional issue, our ignorance about the mechanisms by which transplanted cells improve heart function is a limitation because a better mechanistic insight would have not only cognitive but also practical implications. For example, if a given cell type acts primarily through limitation of remodelling, it sounds logical to deliver it as early as possible following the ischemic insult. Conversely, if the benefit predominantly involves an augmented systolic function through the provision of new contracting elements, the intervention should remain effective at any time during the course of the disease.

Regardless of the exact mechanism, the increased recognition that adult stem cells have a much more limited plasticity than initially thought leads to the concept that neither skeletal myoblasts nor bone marrow cells are likely to electromechanically integrate within the recipient myocardium, which is the prerequisite for the formation of a functional syncytium and a direct synchronous contribution to pump function. This limitation has actually been clear from the onset in the case of skeletal myoblasts which do not express gap junction proteins and seem to remain electrically insulated within the scar tissue (33, 34). In the case of bone marrow cells, the issue is still controversial but a recent study shows that although bone marrow cells pretreated by 5-azacytidine can be in synchrony with co-cultured neonatal rat cardiomyocytes, the metabolic cell-to-cell coupling, assayed by a gap junction-permeable fluorescent dye, is only weak (35). It then appears that the cells best suited for achieving this objective and replacing dead cardiomyocytes are likely to be cardiac cells themselves (36). In regard, two potential sources can be considered. The first is the putative population of resident cardiac stem cells (37, 38) which, in theory, could be therapeutically exploited through either pharmacologic mobilization or an harvest-expansion-reinjection procedure. Assuming that cardiac stem cells are still present in the human adult chronically ischemic heart, this road, however, is probably going to be rocky because of the numerous obstacles that still need to be surmounted, particularly the possibility of reliably localizing these cardioblasts, harvesting them with a minimum of invasiveness and growing them in vitro under GMP conditions without loss of their differentiation potential. The second potential source of cardiac cells is the embryonic stem cell which can be appropriately precommitted towards a cardiomogenic phenotype (39) and has been shown to electrically connect with host cardiomyocytes (40) and to improve postinfarction left ventricular function (39, 41). Problems related to procurement, expansion and immunogenicity currently hamper the clinical use of these cells and the next years will tell whether they can be appropriately addressed by the active research which is ongoing in this area.

Cell transplantation is currently in its infancy but the amount of resources and efforts devoted to this field lead to reasonably anticipate that a meaningful assessment of the risk-benefit ratio yielded by this novel therapy should be available in a near future. These conclusions will be derived from the combination of animal data and clinical trials provided they are carefully conducted in accordance to the strict methodologic guidelines in use for drug studies. It should then become possible to determine whether and to what extent cell therapy, and which cells, may impact on left ventricular function and clinical outcomes of patients suffering from ischemic cardiomyopathy. Furthermore, although the bulk of studies reported so far have dealt with ischemic models, recent laboratory data suggest that the benefits of these cells might extend to nonischemic globally dilated cardiomyopathies (42). Should these results be confirmed, they could open new important therapeutic perspectives for these patients who cannot currently be offered any option other than cardiac transplantation.

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