Myoblast transplantation for heart failure: where are we heading?

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The whole cardiology community is well aware that ischemic heart failure is a major challenge for the years to come. The incidence and related health care expenditure are steadily increasing as a result of the improved survival rates after myocardial infarction and the aging of the population[1]. Despite the dramatic improvements in drug therapy, a substantial number of patients still remain severely disabled, thereby requiring to consider more aggressive options like cardiac transplantation, implantation of "destination therapy" assist devices or biventricular resynchronization. The indications of these treatments are, however, selective so that there remains ample room for alternative interventions aimed at improving functional outcomes. Over the past decade, there has been increasing experimental evidence that cell therapy could be one of them.

The rationale for cell therapy

Cell therapy is based on the assumption that heart failure develops when a critical number of cardiomyocytes has irreversibly been lost, and that, consequently, function could be improved by repopulating these areas of dysfunctional myocardium with a new pool of contractile cells. Even if self-repair endogenous mechanisms exist in the adult human heart, they are of insufficient magnitude to compensate for the infarct-related loss of cardiomyocytes, which has led to the idea that the most realistic approach was to exogenously supply a new pool of contractile cells targeted to engraft into the post-infarct scars[2]. Indeed, the question often arises as to why implanting cells in scar tissue. The answer is that it is precisely the fundamental, although admittedly challenging, objective of this therapy to regenerate dead myocardium and make it a newly functional tissue following successful engraftment. In addition, it would not be appropriate to implant cells in viable tissue as recovery of hibernating myocardium would not require additional cells but only restoration of an adequate blood flow (by angioplasty or bypass surgery) to rescue reversibly damaged cardiomyocytes.

Experimental data

Historically, the initial "proof-of-concept" experiments have entailed the use of fetal cardiomyocytes with the underlying assumption that these cells would be the most suitable for replacement of lost cardiomyocytes. Indeed, these studies have actually demonstrated in rodent models of myocardial infarction, that fetal cardiomyocytes successfully engrafted in scar tissue, established connexions with host cardiomyocytes through gap junctions, improved left ventricular function[3] and maintained their cardioprotective effects up to 6 months after transplantation[4]. However, from a clinical perspective, the multiple issues associated with the transplantation of fetal cardiac cells (ethics, availability, sensitivity to ischemia, immunogenicity) have rapidly led to question the clinical rele-

vance of this approach and to refocus on a cell type best suited for human applications.

In this context, skeletal myoblasts (which normally lie in a quiescent state under the basal membrane of skeletal muscular fibers and direct their post-injury repair through active proliferation and fusion) feature attractive characteristics: 1) an autologous origin which overcomes all problems related to availability, ethics and immunogenicity is a key factor for large-scale clinical applicability, 2) a high proliferative potential under appropriate culture conditions which allows a substantial upscale (from a few millions in the initial biopsy to one billion in the final product) over a 2-3-week time frame, 3) a commitment to a well-differentiated myogenic lineage which virtually eliminates the risk of tumorgenicity, and 4) a high resistance to ischemia, which is a major advantage given the poor vascularity of the post-infarct scars in which they are to be implanted. Almost a decade of pre-clinical work has established that the injected myoblasts differentiate into typical multinucleated myotubes, that this engraftment is associated with an improvement in left ventricular function both in small and large animal models of myocardial infarction[5] and that these functional benefits are sustained over time[6], possibly because of the appearance of a composite population of fibers co-expressing, in addition to the skeletal muscle-specific fast myosin, the slow-type myosin isoform which should increase the graft resistance to fatigue and thus allow it to better withstand a cardiac-type workload.

The robustness and consistency of these data sharply contrast with those yielded by bone marrow-derived cells in the context of *chronic* post-infarction scars. In this context, most experimental studies have failed to document a sustained engraftment of hematopoietic progenitors[7] as well as a conversion of cells into cardiomyocytes[8,9]. Indeed, there is increasing agreement that bone marrow cells are unlikely to be effective in inducing myogenesis and that their primary effect is to increase angiogenesis through the release of cytokines and growth factors[10]. This might explain the good results reported with their administration at the *acute* stage of myocardial infarction[11] where the appropriate signaling pathways may still be harbored in the ischemic border zone whereas they are no longer present in scar tissue. Thus, we do not think that there is a "competition" between skeletal myoblasts and bone marrow cells but, rather, that each lineage is more electively indicated in a given myocardial environment and, consequently, fits different patient populations.

The consistent documentation of the improvement in functional outcomes associated with skeletal myoblast transplantation contrasts with the persisting uncertainties regarding the mechanisms of these benefits. Limitation of left ventricular remodeling by a girdling effect is a first possibility that has been demonstrated experimentally[12] although we speculate that while this mechanism might be operative when cells are injected at a relatively early stage after the infarction, before ventricular dilation has occurred, it is less likely to be effective for reversing an already completed remodeling process. A second hypothesis is that the cells contribute to improve function through their contractile properties. This hypothesis is challenged by the observation that engrafted myoblasts are not physically connected to host cardiomyocytes through connexin 43-supported gap junctions and do not beat in synchrony with them[13] although our electrophysiological findings have shown that the engrafted myotubes retain their excitable and contractile properties[13]. These data are consistent with a recent report that transplanted myoblasts can fuse with neighboring cardiac cells, thereby resulting in chimeric cells primarily located at the graft-host interface[14]. These cells can occasionally contract synchronously with the host cardiomyocytes but they are scarce and, as such, it remains uncertain whether they can significantly contribute to increase systolic function. Finally, the myoblast-induced enhancement of contractile function could be mediated by cell-released paracrine factors that could mobilize resident quiescent cardiac cells[15], thereby endogenously increasing the number of contractile elements and/or affect extracellular matrix remodeling[16].

Clinical applications

Despite the unsettled mechanistic issues associated with myoblast transplantation, the bulk of animal data has been deemed convincing enough to justify a move toward clinical applications that actually started in June, 2000[17]. So far, six phase I safety and feasibility pilot studies of autologous skeletal myoblast transplantation have been performed, four of which were surgical, i.e., myoblast implantation at the time of coronary artery bypass grafting[18-21] while the two others were designed as catheter-based stand-alone procedures[22,23]. Basically, these trials have shown that myoblast expansion from a small biopsy could be performed efficiently under Good Manufacturing Practice conditions and that multiple cell injections could then be implemented without specific procedural complications. An additional important piece of information, based on pathology studies, has been that myotube engraftment was sustained over time[24,25]. These early trials have also raised the concern that intramyocardial skeletal muscle grafts might represent arrhythmogenic substrates but, as discussed later, this issue may require to be revisited.

While the initial clinical experience with myoblast transplantation has yielded valuable feasibility and safety data, it does not allow to draw meaningful conclusions regarding efficacy because these studies were neither designed nor powered to address this issue. Indeed, the interpretation of functional outcomes is clouded by several confounding factors such as the dif-

ferences in cell culture processes, the variable type of examinations used for assessing viability, and the revascularization or lack of revascularization of the grafted scar. For this reason, we have implemented the MAGIC (Myoblast Autologous Grafting in Ischemic Cardiomyopathy) trial which has been specifically designed to assess the effects of myoblast transplantation on functional recovery of the injected areas and clinical outcomes in patients meeting the following three inclusion criteria: 1) a severe left ventricular dysfunction reflected by an echocardiographically measured ejection fraction ≤ 35%), 2) a post-infarction discrete akinetic and non-viable 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.

This study whose sponsorship involves both a public institution (Assistance Publique-Hôpitaux de Paris) and an industry sponsor (Genzyme) representing MG Biotherapeutics, a joint venture of Medtronic Inc. and Genzyme Corporation, is multicenter, randomized, placebo-controlled, dose-ranging and double-blind. It currently involves 29 centers in Europe, of which 5 are located in Italy (Milan, Genoa, Bologna, Udine and Treviso). Additional centers should soon be included in Europe and Canada.

Features of the MAGIC study

Some key methodologic features of the protocol need to be briefly outlined. First, the trial is randomized: once a potential candidate has been screened in one of the participating centers and has signed the informed consent form, his (her) echo tapes (rest + dobutamine) are shipped to the echocardiographic core laboratories headed by Professors Albert A. Hagège (Department of Cardiology, Hôpital Européen Georges Pompidou in Paris) for European patients and Scott Solomon (Brigham's and Women Hospital, Boston, MA, USA) for north-American patients. If the inclusion is validated on the basis of an ejection fraction between 15 and 35% and an akinesia of at least three non-septal contiguous segments without response to low-dose dobutamine, the patient is randomized into one of the following three groups: control (placebo solution), cells at low dose (400×10^6) and cells at high dose (800×10^6) in combination, irrespective of the group, with bypass surgery. The placebo group is deemed important to rule out any effect of multiple needle punctures if, at the end of the study, a benefit is demonstrated in the treated cohorts. For those patients allocated to this placebo group, the biopsy is kept frozen and can subsequently be grown for a later catheter-based cell delivery. Given the rapid industry-driven development of these percutaneous less invasive approaches, such an anticipation is not unreasonable.

All patients included in the MAGIC trial are implanted with an internal cardioverter-defibrillator (ICD). This decision has been taken for the following reasons: 1) the initial phase I trials have outlined a possible proarrhythmic risk associated with myoblast implantation, making ethically mandatory to offer these patients a safety net; 2) by virtue of the inclusion criteria, most of the MAGIC patients match the MADIT II criteria[26] and it is therefore not unreasonable to maximize protective measures by implanting a device which is expected to confer a survival benefit in this heart failure population; 3) finally, the readouts of the defibrillators provide the only means of objectively assessing the incidence of ventricular arrhythmic events in the treated patients compared with those receiving placebo injections. There is no specified timing of ICD implantation (before or after the operation) provided that the device is in place at the time of the final patient hospital discharge.

Once the patient has been randomized, the local investigators define a date for the biopsy in coordination with a central operational center. There are two cell production sites, one in Paris (Hôpital Saint Louis, which supplies the French and German centers) and the other in Cambridge, MA, USA (Genzyme laboratories, which supply the other European centers and, in the near future, Canada). Extensive pre-clinical studies have allowed defining a transportation medium which ensures adequate cell viability up to 72 hours and the details of the complex logistical issues associated with these long distance shipments have also been worked out successfully. The myoblast cultivation procedures have been tightly harmonized so as to guarantee the similarity of the final cell therapy product between the two production sites. After a 3-week expansion period and once the product has successfully passed the stringent quality controls required for final release (viability, purity, sterility), it is shipped back to the transplantation center for intramyocardial implantation. The procedure is straightforward and entails approximately 30 injections of 200 μl each with a total volume of 6 ml across the scar segments identified by echocardiography as non-viable, including the borders. This requires a 15 min extra-time of aortic cross-clamping which is not considered as an additional risk factor given the efficacy of the current myocardial protection methods (although off-pump surgery is permitted as well).

As previously mentioned, the cell-grafted area is not bypassed and because the reason for this feature of the protocol is often questioned, it is important to explain its rationale. Indeed, three major considerations account for this decision: 1) virtually all pre-clinical studies have used permanent coronary artery occlusion models and this has not precluded the injected myoblasts to successfully engraft. The likely reason is that, even in animal models, the scar area is never fully avascular and that some residual blood flow persists that can afford cell survival; this is even more true in pa-

tients whose infarcts typically feature a patchy pattern with islands of subnormal myocardium interspersed with islands of necrosis; 2) in most cases, the coronary artery subserving the infarct area is completely occluded or at least so badly diseased that it is not reasonably amenable to any form of revascularization; 3) finally, the present trial should primarily be viewed as confirmatory; as such, it aims at establishing the proof of principle, which intends to show that implantation of myoblasts in scar areas restores some functionality in the formerly akinetic territories; an associated revascularization would then be a major confounding factor, making virtually impossible to distinguish between the effects of cell grafting versus those of coronary bypass if the kinetics of the target zone are ultimately found to improve (for that same reason, patients should not undergo a concomitant mitral valve procedure either). However, in the future, and *after* efficacy, if any, has been established, the cell-implanted area will likely be revascularized concomitantly (whenever feasible) as it makes sense to optimize the graft blood supply and, thus, to minimize the ischemic component of cell death which is quantitatively important[27] and likely hampers the benefits of the procedure.

As alluded to in the preceding paragraph, the primary endpoint of the trial is the improvement in contractility of the myoblast-grafted myocardial segments 6 months after the operation, as assessed in the core laboratory by a blinded echocardiographist. Secondary endpoints include changes in global left ventricular function (assessed by echocardiographic and scintigraphic measurements of ejection fraction) and major adverse cardiac events at 1 year after transplantation. A flow chart of the protocol is depicted in figure 1. The trial is carefully scrutinized by an independent Data Monitoring Committee that analyzes safety data at regular intervals. It is important to report that the first blinded assessment of the arrhythmic events is rather reassuring as only 5 out of 44 patients whose defibrillators have been interrogated experienced some form of ventricular tachycardia and only 2 patients out of 44 required ICD therapy. This incidence is certainly lower than expected from the phase I experience; our assumption is that strict maintenance of the beta-blocker treatment combined with administration of amiodarone (starting at the time of the muscular biopsy and continued uninterruptedly thereafter until 2 months after the operation) has been pivotal in reducing the incidence or at least the severity of the arrhythmic events potentially triggered by myoblast implantation.

Overall, the study has been powered so as to include a total number of 300 patients. However, safety and efficacy *interim* analyses will be performed at regular intervals and their results will determine the ultimate outcome of the trial.

Conclusion

In conclusion, cell therapy is now founded on robust and consistent experimental grounds but we know that data collected in animal models are not easily extrapolated to our patients. It is therefore likely that the ongoing clinical trials will provide additional insights with regard to the safety and efficacy of this novel approach which is, so far, the only one to really target regeneration of non-functional myocardial tissue. Among these trials, MAGIC holds a unique place because of its size, design, compliance with international monitoring standards and rigorousness of assessment. The earlier we have exploitable data, the earlier we know to what extent myoblast transplantation can really impact on the management of heart failure and this should have major implications for routine patient care and future research plans. It is therefore important that each cardiologist who has to take care of these difficult patients feels him(her)self committed to contribute to this study by *thinking* of identifying potentially eligible candidates and referring them for further evaluation to the closest participating center. This is the key for an active

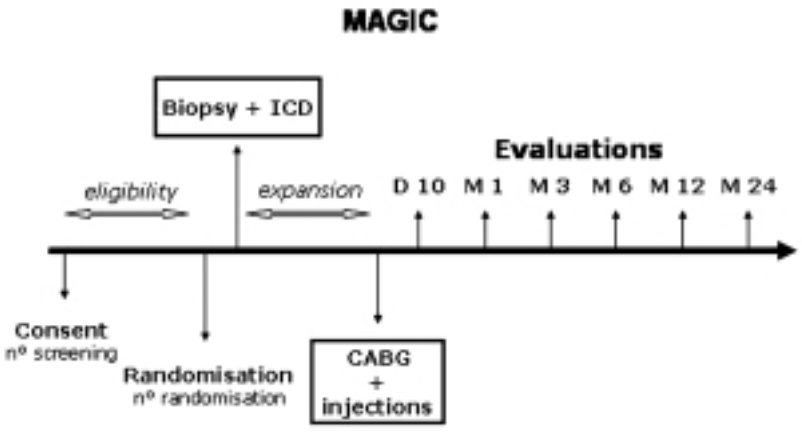

Figure 1. *Flow chart of the MAGIC protocol. CABG = coronary artery bypass grafting; D = day; ICD = implantable cardioverter-defibrillator; M = month.*

enrollment rate leading to meaningful conclusions in a foreseeable future. It is also noteworthy that irrespective of the actual results, the MAGIC trial has yet the merits to have set, in close collaboration with the different regulatory authorities, the guidelines for cell therapy trials. This should turn out to be time- and energy-saving when time comes to consider the next generation of cells more directly targeted at truly "rebuilding" mended hearts.

References

1. Jessup M, Brozena S. Heart failure. N Engl J Med 2003; 348: 2007-18.
2. Melo LG, Pachori AS, Kong D, et al. Molecular and cell-based therapies for protection, rescue, and repair of ischemic myocardium: reasons for cautious optimism. Circulation 2004; 109: 2386-93.
3. Li RK, Jia ZQ, Weisel RD, et al. Cardiomyocyte transplantation improves heart function. Ann Thorac Surg 1996; 62: 654-61.
4. Müller-Ehmsen J, Peterson KL, Kedes L, et al. Rebuilding a damaged heart: long-term survival of transplanted neonatal rat cardiomyocytes after myocardial infarction and effect on cardiac function. Circulation 2002; 105: 1720-6.
5. Dowell J, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. Cardiovasc Res 2003; 58: 336-50.
6. Al Attar N, Carrion C, Ghostine S, et al. Long-term (1 year) functional and histological results of autologous skeletal muscle cells transplantation in rat. Cardiovasc Res 2003; 58: 142-8.
7. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004; 428: 664-8.
8. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004; 428: 668-73.
9. Thomson RB, Emani SM, Davis BH, et al. Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells. Circulation 2003; 108 (Suppl 1): II264-II271.
10. Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004; 94: 678-85.
11. Mathur A, Martin JF. Stem cells and repair of the heart. Lancet 2004; 364: 183-92.
12. Ghostine S, Carrion C, Souza LC, et al. Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. Circulation 2002; 106 (Suppl I): I131-I136.
13. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci USA 2003; 100: 7808-11.
14. Reinecke H, Minami E, Poppa V, Murry CE. Evidence for fusion between cardiac and skeletal muscle cells. Circ Res 2004; 94: E56-E60.
15. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114: 763-76.
16. Murtuza B, Suzuki K, Bou-Gharios G, et al. Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. Proc Natl Acad Sci USA 2004; 101: 4216-21.
17. Menasche P, Hagege AA, Scorsin M, et al. Myoblast transplantation for heart failure. Lancet 2001; 357: 279-80.
18. Menasche P, Hagege AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003; 41: 1078-83.
19. Herreros J, Prosper F, Perez A, et al. Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. Eur Heart J 2003; 24: 2012-20.
20. Siminiak T, Kalawski R, Fiszer D, et al. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. Am Heart J 2004; 148: 531-7.
21. Dib N, McCarthy P, Dinsmore J, et al. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: interim analysis from the United States experience. (abstr) Circulation 2002; 106 (Suppl II): II-463.
22. Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. J Am Coll Cardiol 2003; 42: 2063-9.
23. Siminiak T, Fiszer D, Jerykcwska O, et al. Percutaneous transvenous transplantation of autologous myoblasts in the treatment of postinfarction heart failure - the POZNAN trial. (abstr) Eur Heart J 2004; 25 (Suppl): 264.
24. Hagege AA, Carrion C, Menasche P, et al. Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy. Lancet 2003; 361: 491-2.
25. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. J Am Coll Cardiol 2003; 41: 879-88.
26. Moss AJ, Zareba W, Hall J, et al, for the Multicenter Automatic Defibrillator Implantation Trial II Investigators. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. N Engl J Med 2002; 346: 877-83.
27. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. J Mol Cell Cardiol 2001; 33: 907-21.