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Review/Revue

Stem cell therapy for chronic heart failure. Lessons from a 15-year experience<sup>☆</sup>

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## ABSTRACT

Although cell therapy has entered the clinical arena since 2000, its benefits are still controversial. This is partly due to a shift of the whole paradigm from the mere provision of new cells intended to replenish the pool of dead cardiomyocytes to the exploitation of the cell's paracrine effects to activate host-associated cytoprotective signalling pathways, particularly those involved in angiogenesis, prevention of apoptosis and possibly recruitment of endogenous cells capable to mature into functional cardiomyocytes. This review will discuss how these two basic mechanisms (direct donor cell-derived myocardial regeneration versus paracrine signalling) underlie the rational selection of cells in light of the target clinical indication, with a particular focus on chronic heart failure, and will emphasize the importance of optimizing cell delivery and survival to fully exploit the potential benefits of this novel approach to acute and chronic heart diseases.

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## R É S U M É

Bien que les essais cliniques de thérapie cellulaire aient commencé en 2000, les bénéfices de cette approche restent encore discutés. Cette incertitude est en partie liée au changement du paradigme qui a évolué de la seule provision de cellules « externes » destinées à remplacer physiquement celles qui avaient été détruites à l'exploitation des effets paracrines des cellules greffées sur diverses voies de signalisation cytoprotectrices, particulièrement celles impliquées dans l'angiogenèse, la limitation de l'apoptose et peut-être le recrutement de cellules endogènes capables d'acquiescer un phénotype contractile. Cette revue discutera en quoi la prise en compte de ces deux mécanismes (régénération directe à partir des cellules greffées ou effets paracrines) a de profondes implications sur le choix du type cellulaire le plus approprié en fonction de la pathologie à traiter, avec un accent mis tout particulièrement sur l'insuffisance cardiaque chronique. Elle insistera aussi sur l'importance d'optimiser le transfert et la survie des cellules pour pleinement exploiter les bénéfices thérapeutiques de cette nouvelle approche des pathologies cardiaques.

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## 1. Introduction

Cell therapy is currently generating a great deal of interest as a potential means of treating different kinds of cardiac diseases, which include acute myocardial infarction

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tion, refractory angina and chronic heart failure. The current practice of cardiac surgery provides the opportunity to treat an increasing number of patients of the last category, because of the growing incidence of heart failure [1], and the present review will therefore focus on this group. In contrast to myocardial infarction and refractory angina where the primary objective of cell therapy is to provide growth factors and cytokines which may activate host-associated cytoprotective pathways to rescue still reversibly damaged cardiomyocytes and induce neovascularization [2], the more challenging aim assigned to cells delivered in the context of chronic heart failure is to regenerate, at least partially, areas of scarred myocardium to make them functional again. This review will thus address some of the basic questions which have emerged from the basic and clinical experience accumulated over these past 15 years.

## 2. Analysis of clinical data

Whereas most patients with acute myocardial infarction or angina have been treated by catheter-based cell delivery techniques (i.e., intracoronary or endocardial injections), those with heart failure have primarily been given cells as an adjunct to coronary artery bypass grafting (CABG). Two cell types have been used in this context: skeletal myoblasts and bone marrow-derived cells. Skeletal myoblasts had initially been selected because they featured distinct advantages: (1) an autologous origin which made accessibility easy and avoided immune rejection; (2) a high degree of scalability in culture (one billion cells can be yielded from an initial small biopsy over a 2–3-week time frame); (3) a myogenic lineage restriction which provided a safeguard against tumor formation; and (4) a high resistance to ischemia, which was deemed a major advantage given the poorly vascularized environment in which they were to be implanted. Following several years of experimental work in small and large animal models which had yielded positive results (myoblasts injected into postinfarction scars differentiated into typical multinucleated myotubes and this engraftment was associated with a sustained improvement in LV function), we initiated a first human trial in 2000 and, following a pilot safety and feasibility study, proceeded to a randomized controlled double blind, placebo-controlled trial (MAGIC, an acronym for Myoblast Autologous Grafting in Ischemic Cardiomyopathy). This trial included 97 patients with severe left ventricular (LV) dysfunction who underwent transepical injections of autologous skeletal myoblasts (at two doses) or a placebo medium at the time of CABG. However, in contrast to the initial expectations, at the 6-month study point, myoblast injections failed to improve LV function beyond that seen in the placebo group, even though the high dose group experienced a significant reduction in LV volumes (which was a prespecified secondary end point) [3]. Similar data have been reported in the SEISMIC trial where endocardially-delivered myoblasts failed to improve global or regional LV function at a 4-year posttreatment time point [4]. There is only one study [5] which has reported 1-year improvements in quality of life and echocardiographically-

measured LV dimensions, but the relevance of these data is weakened by the small sample size (12 treated patients). Aside from myoblasts, bone marrow-derived cells have also been tested either as an adjunct to CABG (reviewed in [6]) or as stand-alone procedures entailing intracoronary delivery of cells in both ischemic [7] and nonischemic [8] cardiomyopathy settings. Despite some overenthusiastic claims, a fair analysis of the data cannot unequivocally conclude to the benefits of the technique and leads to one conclude that until now myocardial regeneration has remained a wishful thinking rather than a clinically proven reality. In this context, the improved outcomes reported in some methodologically sound studies with either bone marrow mononuclear cells [9,10] or CD133<sup>+</sup> [11] progenitors likely reflect the angiogenic potency of these cells rather than a putative donor cell-derived remuscularization.

Regarding safety, the main concern has been the occurrence of ventricular arrhythmias following myoblast implantation. Because we had observed such episodes during our early experience, all patients in the MAGIC trial were fitted with an internal cardioverter-defibrillator. Of note, at the 6-month time point, the proportion of patients experiencing arrhythmias did not differ significantly between the myoblast-grafted patients and those of the control placebo-injected group [3], suggesting that, even though inexcitable cells (like myoblasts or bone marrow cells) can be pro-arrhythmic by acting as current sinks [12], other mechanisms than the cell phenotype were likely in play, such as the technique of cell delivery, donor-recipient cell mismatches in size, alignment and action potential patterns, and transmural heterogeneity of ventricular repolarization [12].

## 3. Potential mechanisms of action of transplanted cells

There are indeed two major mechanisms which can be considered and are not mutually exclusive.

The first mechanism, which is the most convincingly demonstrated so far, relies on paracrine effects whereby cytokines and growth factors released by the grafted cells, (skeletal myoblasts [13] or bone marrow-derived cells [14]) favourably influence the myocardial microenvironment by triggering host-associated signalling pathways [15] leading to increased angiogenesis, decreased apoptosis, extracellular matrix remodelling and, possibly, induction of endogenous cardiomyocyte generation. The latter mechanism has recently generated a great interest on the basis of studies showing that epicardial cells, under the influence of appropriate signals, primarily transforming growth factor- $\beta$ , could reactivate their embryonic developmental program and undergo an epithelial-to-mesenchymal transition allowing them to turn into cells which could subsequently differentiate into different heart lineages, including cardiomyocytes [16]. Of note, however, the clinical relevance of this sequence needs to be confirmed because epicardial cells have been reported to be absent in human pathological hearts [17], possibly because repeat episodes of ischemia have led to their exhaustion. Regardless of the involved pathways, the most compelling evidence for the robustness of the paracrine

paradigm has come from studies showing that the benefits of cells could be duplicated by intravascular injection of conditioned media from mesenchymal stem cells (MSC) [18], thereby suggesting that the major action of the cells was not to physically substitute for host dead cardiomyocytes and directly contribute to the heart's contractile function but rather to behave as biofactories. A most extensive documentation of this paracrine hypothesis could have profound implications in terms of pharmacological development in that it might lead to shift from injection of cells to that of their derived products, provided they can be accurately characterized and efficiently delivered [2].

The second mechanism implies that the transplanted cells physically substitute for those of the native heart which have been irreversibly lost. The prerequisite here is that the donor cells feature from the onset or acquire in situ the phenotypic features of true cardiomyocytes, including the fundamental property of electromechanical coupling with host cardiac cells, thereby allowing synchronous graft-host contractions and a subsequent improvement in contractility. Unfortunately, and despite the initial hopes of a milieu-induced "transdifferentiation" of adult somatic cells, it is now recognized that both skeletal myoblasts and bone marrow "stem" cells lack the degree of plasticity allowing them to cross their lineage boundaries and convert into cardiomyocytes [19,20]. This implies that the objective of myocardial regeneration requires to use either adult cells in which a cardiopoietic program has been forcefully induced or pluripotent cells which can be committed towards a cardiomyocytic phenotype (see below).

#### 4. Matching the cell type to the clinical targets

Unlike myocardial infarction and refractory angina in which rescue of reversibly injured cardiomyocytes in the border zone and increased angiogenesis, respectively, might be theoretically achieved by the cells' paracrine effects, restoring function of the chronically failed myocardium rather mandates the provision of cells endowed with an innate cardiomyogenic differentiation potential.

In the setting of cardiac-committed cell transplantation, the first candidates, which have already entered the clinical arena are the cardiac stem cells, whose putative existence challenges the long-standing dogma that the heart is a terminally differentiated organ. In the three ongoing clinical trials, these cells are harvested by an endomyocardial biopsy or during a CABG procedure, expanded in vitro, and then reinjected into the coronary arteries (CADUCEUS and SCIPIO trials) or directly in the myocardium (ALCADIA trial which combines cell delivery with implantation of a  $\beta$ -Fibroblast Growth Factor controlled-release gelatin hydrogel sheet). However, this approach raises several issues. First, the phenotype of these cells is still controversial, as demonstrated by the multiplicity of markers that have been proposed to identify them [21] (in the CADUCEUS trial, cardiac stem cells are grown as aggregates of mixed cell populations known as cardiospheres while in SCIPIO they are selected on the basis of a positive staining for *c-kit*). Second, their in vitro

upscale remains challenging. The third and possibly the most important concern pertains to their persistence in adult, ischemically-diseased hearts patients [22] and the loss of cardiac stem niches over time is indeed illustrated by the finding, in myocardial tissue specimens harvested during pediatric heart surgery, that the number of these cells rapidly decreases beyond the first two years of life [23]. The recently reported observation that the capacity of the neonatal mouse heart to fully regenerate is lost as early as seven days after birth points in the same direction [24] and it is indeed noteworthy that the group which has developed the technique of cardiospheres and has implemented it in clinical practice now recognizes that most of its benefits are due to paracrine effects rather than direct myocardial regeneration from the sphere-derived putative cardiac stem cells [25].

Another option is to use pluripotent stem cells and to commit them towards a cardiac lineage in vitro prior to their delivery. Human embryonic stem cells (ESC) currently remain at the frontline of this approach since they can generate cardiac progenitor cells which, once engrafted, finish by differentiating into cardiomyocytes [26] under the influence of local cues with an attendant improvement in function [27]. Besides from ethical issues, the major safety issue associated with ESC is the development of a teratoma, which requires one to optimize sorting techniques allowing to yield purified populations of progenitors devoid of residual contaminating pluripotent cells. Another challenge is that these allogeneic cells are intrinsically immunogenic and will be rejected without immunomodulation [28]. Two recently approved US trials, one using ESC-derived oligodendocytes in patients with spinal cord injury and the other using retinal progenitors in patients with macular degeneration demonstrate that the field is rapidly moving forward due to improvements in cell scale-up, lineage-specific commitment and purification procedures. However, it is likely that the clinical development of this therapy will largely depend on the ability (or not) to develop immunomodulatory strategies robust enough to blunt rejection but without the side-effects of conventional pharmacologic immunosuppression. In this setting, induction of tolerance is particularly appealing and the fact that a state of self-tolerance could be established in type I diabetic patients treated by a specific antibody to the point that insulin needs were reduced [29] makes plausible that the concept could be successfully extended to allogeneic cell transplantation. The successful promotion of ESC engraftment by a short-course of co-stimulatory molecule blockers is another strategy worth considering [30].

Alternatively, this immune issue can be solved by the use of induced pluripotent stem (iPS) cells, i.e., somatic cells taken from various sources in the patient himself (skin, hair, blood) and reprogrammed to an embryonic-like pluripotent state from which they are redifferentiated towards the selected lineage [31]. However, a potential therapeutic use of iPS cells for regenerative purposes is still plagued by the low efficiency of reprogramming, the potential toxicity of the reprogramming agents (even though the initial cocktail of integrative viral vectors tends to be progressively replaced by safer small molecules), the

potential occurrence of genomic and epigenomic abnormalities [32] and the impairment of directed differentiation [33]. Put together, these concerns explain why iPS cells are currently considered as useful tools for modelling diseases and screening drugs on patient-derived disease-specific cells while their therapeutic applications still remain further down the road.

Studies of ESC and iPS cells have unravelled some signalling pathways which play a key role in heart development. Identification of the molecules involved in these pathways has then led a group of investigators to develop a cardiopoietic cocktail and to use it for forcing naive MSC (which fail to transdifferentiate into cardiomyocytes) to re-enter into a cardiomyogenic developmental program [34]. Indeed, MSC feature distinct advantages such as easy harvestability (from the bone marrow or adipose tissue) and straightforward *in vitro* expandability although culture expansion may result in genetic instability [35]. They have also been credited for an immune privilege, thereby raising the hope that they could be banked and used as an allogeneic, readily available “off-the-shelf” product matching the regulatory constraints with regard to identity, potency, purity, reproducibility, microbiological qualification, consistency and robustness of release criteria (which contrasts with the intrinsic functional variability of autologous cells). This immune privilege, however, has been challenged by recent studies showing allogeneic MSC were actually rejected [36]. In fact, the above-mentioned trial assessing the effects of cardiac-committed MSC uses the patients' own cells. So far, it has reported positive short-term outcomes which need to be confirmed and, in any case, do not necessarily imply that the benefits are due to an effective regeneration by the transformed MSC and not to the well documented paracrine effects of these cells.

Finally, one should mention the experimental report of a direct conversion of fibroblasts into cardiomyocytes bypassing the reprogramming step which occurs with iPS cells [37]. Theoretically, this might open the way to remodel a fibrotic area into a contractile one provided the appropriate converting factors be identified and their *in vivo* action accurately controlled. The complexity of this challenge probably makes the potential therapeutic applications of this concept still far ahead. Nevertheless, one can reasonably anticipate that a better knowledge of the mechanisms of cardiac differentiation derived from these basic works may end up in the development of pharmacologic agents able to harness self-repair endogenous mechanisms. One example is the use of thymosine  $\beta$ -4, an agent which can drive epicardial cells towards a vascular (and possibly cardiomyocytic) phenotype [16] and planned for being investigated in patients with acute myocardial infarction.

## 5. The issue of cell engraftment

So far, a major factor which has impeded the efficacy of cell transplantation is the poor rate of sustained engraftment. This is first due to the low efficiency of current delivery techniques and, secondly, to the high rate of early death of that small number of cells which have been

initially retained in the target myocardium [38]. This high attrition rate results from the interplay of several factors [39], including ischemia intrinsic to the hypovascularity of the target transplanted areas, apoptosis due to the loss of survival signals associated with cell-to-cell and cell-to-matrix attachments, inflammation, and rejection if allogeneic cells are used. If reliance is only on the cells' paracrine effects, long-term survival may not be mandatory since cytokine levels triggered by the grafted cells have been shown to peak as early as four days after cell injections [40]; in this case, cells are mere natural biocarriers, a role which can be even increased by engineering them with internalized biodegradable particles which release therapeutic agents [41]. Conversely, sustained survival of the graft becomes mandatory when the objective is regeneration of the failing myocardium.

In all cases, optimization of the initial delivery is important, as shown by the relationship between the engraftment rate and the improvement in LV function [42]. There is now compelling evidence that direct intramyocardial injections (either transeptically during surgical operations or transendocardially in catheter-based procedures) are more efficient, i.e., allow one to deliver greater numbers of cells than the vascular (intravenous or intracoronary) approaches [43]. However, the injection-based concept has several drawbacks: it cannot avoid a substantial leakage of cells through puncture holes and wash-out by the venous and lymphatic systems; it results in a random distribution of cells, is poorly reproducible and creates multiple intramyocardial clusters which can be arrhythmogenic through slowing of the propagation wave [44]; finally, it sets the stage for cell death because of the proteolytic dissociation which precedes the suspension of cells into the delivery vehicle. Some of these drawbacks can be partly overcome by the use of more controlled delivery devices [45] or incorporation of cells into biomaterials such as hydrogels which polymerize *in situ* and enhance retention [46]. However, in the context of cardiac surgery where the heart can be directly approached, the best strategy is likely to replace injections by the epicardial coverage of the diseased area by a cell-seeded patch. An attractive option here is to culture cells onto temperature-sensitive dishes so that, upon cooling, they are collected as a cohesive scaffold-free sheet [47]. Several of these sheets can then be stacked and the whole construct, devoid of foreign material, is then overlaid onto the infarct area where it adheres easily. We have confirmed that, compared with suspended cells, cell sheets are associated with a greater upregulation of some key factors involved in cell adhesion and survival (H. Hamdi et al. *Cardiovasc Res*, accepted for publication). Indeed, this technology has now been widely used in different applications (particularly in patients with ocular and oesophageal diseases) including ischemic cardiomyopathy, both experimentally [48] and clinically [49]. The issue is that scaffold-free cell sheets are frail and may tear or fold easily. It is thus more user-friendly to use biocompatible scaffolds seeded with cells which are mechanically more robust and can be easily manipulated at the time of their application onto the heart surface. This approach is validated by the observation that collagen-based scaffolds

provide similar benefits as cell sheets and in both cases, these benefits are superior to those of conventional injections [50]. This rapidly evolving field of tissue engineering has recently been reviewed [51] and the major requirements of the cell-supporting scaffolds (biodegradability without causing an overt inflammatory reaction; surface properties enhancing cell attachment and proliferation; lack of toxicity; mechanical strength and good handling characteristics) are now well recognized. A growing trend is actually to co-seed the cells featuring contractile properties with support cells intended to provide them with the necessary trophic support (for example, cardiac-committed and endothelial cells) with the expectation of a cellular cross-talk that might synergize the effects of the two cell populations [52–54]. An additional advantage of this technology is that scaffolds can serve as platforms for controlled-release growth factor delivery while their surface topography can be patterned at the nanoscale level to optimize the lineage commitment of cells that they support. However, it should be acknowledged that the mechanisms by which cell-seeded patches improve function still remain elusive. While a paracrine effect of the cells is likely, the recruitment of new cardiomyocytes through reactivation of the underlying epicardial cells or the coupling of transplanted cells migrating away from the patch [55] with host cardiomyocytes, remains to be consistently demonstrated.

Survival of the initially retained cells is the second objective whenever donor cell-derived replenishment of the dead cardiomyocyte pool is the target. To address the ischemic component of cell death, establishment of an adequate blood and nutrient supply is mandatory. This can be accomplished by the interventional or surgical revascularization of the transplanted area whenever feasible. Cell genetic engineering to induce increased expression of angiogenic proteins has been experimentally successful, but its implementation in clinics would raise some safety and practicality issues, thereby adding another layer of regulatory complexity. A more realistic option is probably to co-deliver (by injection of patch seeding) cells featuring an intrinsic angiogenic potential such as MSC derived from the bone marrow or the adipose tissue [56]. A second important factor to keep cells alive is to maintain both their connexions and their anchorage to a matrix. If delivery is based on needle injections, it is probably useful to embed cells into a biomaterial intended to reconstitute a three-dimensional niche inside which they can grow appropriately and survive [57]. In a surgical setting, a better option is to use a scaffold which does not only provide a better early retention of cells but also enhances their survival [55] through maintenance of their cohesiveness and anchorage to a self-secreted matrix. The inflammatory component of cell death can be addressed by minimizing the invasiveness of cell delivery. The last death-promoting factor is the immune response to allogeneic cells. Several strategies can then be considered, which include customized drug immunosuppression, immunological matching and induction of tolerance. We have previously outlined the potential advantages of the latter approach over drugs whose side-effects are well

recognized and immune cell matching with requires more logistically complex and expensive banking.

Of note, a thorough assessment of techniques aimed at improving cell engraftment requires the ability to follow cell fate in a reliable, nontoxic, longitudinal and clinically relevant fashion. Although none of the current imaging modalities meets all these requirements, the most sensitive of them appears to be reporter gene imaging [58] whereby the cells are genetically engineered to produce a protein which, upon exposure to its ligand, will emit a signal that can be imaged by magnetic resonance imaging or positron emission tomography (PET). So far, the major limitation of this technique has been that its still limited spatial resolution only allowed to use it in small animals, primarily mice. However, the potential for its clinical applicability stems from the recent report of the first human case in which transduction of CD8<sup>+</sup> T lymphocytes with a reporter gene has allowed to track them by PET scan after the gene-encoded protein had reacted with its systemically administered radiolabelled ligand [59].

## 6. Selection of patients

In patients with acute myocardial infarction, there is mounting evidence that the greatest benefits of cell therapy are seen in those who present the most severely depressed LV functions at baseline [60]. The challenge here is to prevent an adverse LV remodelling which may still develop despite a successful early reperfusion of the culprit coronary vessel. The future of cell therapy in this indication will therefore likely depend on its ability (or not) to achieve this objective, which should be known from long term follow-up studies but first implies optimization of several procedural factors including the cell type (unpurified bone marrow mononuclear cells, CD34<sup>+</sup>, CD133<sup>+</sup> or endothelial progenitors, MSC, adipose-derived stromal cells), the place of allogeneic cells to overcome the often defective functionality of bone marrow cells in atherosclerotic patients [61], dosing, timing of delivery, use of single versus repeated infusions, adjunctive role of cytokine-induced bone marrow cell mobilization, technique of delivery and enhanced homing of cells which, in this context of acute infarction are necessarily administered by intravascular (systemic or intracoronary) routes.

The relief of ischemic symptoms is the primary objective of cell therapy in patients with refractory angina selected on the basis of the failure of conventional treatments. This goal should be more easily obtained by the paracrine, and particularly the angiogenic effects of the grafted cells.

Likewise, cell therapy should be discussed in heart failure patients who have exhausted common drug- and device-based therapeutic options, are not (or no longer) candidates for heart transplantation and are deemed at high risk of short term adverse outcomes based on predictive scores [62]. Because these patients are treated under elective conditions, there is a greater flexibility in the choice of the delivery route but, as previously mentioned, there is compelling evidence that the greatest degrees of engraftment are achieved by direct intramyocardial cell transfer by catheter or surgically [43,60].

Alternatively, the intracoronary route can be reasonably considered because of its more limited invasiveness; in this case, enhanced homing may be an issue because signals that may facilitate cell trans-endothelial trafficking at the acute stage of infarction have gone at the later stage of scar. Rather than attempts at genetically re-establishing these homing signals (for example by induced over-expression of SDF-1 or CXCR-4 in the cells [63] or the target tissue [64]), physical methods like low-energy shock wave [65] or magnetic targeting of iron-labelled cells [42] might be easier to implement clinically. As previously discussed, the common cells' paracrine angiogenic and anti-apoptotic effects are unlikely to be powerful enough to reverse failure of extensively scarred hearts which may rather require the exogenous supply of cells able to generate new cardiomyocytes directly or through the activation of self-regenerating endogenous pathways. This distinction impacts on the design of clinical trials. Namely, whereas cells which already have a well-documented safety record, primarily bone marrow cells, should now be tested in randomized placebo-controlled confirmatory phase III trials focusing on hard clinical end points, the new generation of cells endowed with a cardiomyogenic potential and which are at the early stage of investigation first requires to be tested in cautious safety and feasibility studies with efficacy as a secondary end point assessed by surrogate markers. In all cases, because of the multiplicity of the mechanisms potentially causing arrhythmias, it is mandatory to carefully monitor these events, particularly if the success of survival-enhancing strategies allows a greater number of cells to engraft in a sustainable fashion.

Finally, in the context of cardiac surgery, a specific indication could be patients under LV assist devices as stem cell transplantation during unloading has been shown experimentally to better preserve LV geometry when loading conditions are resumed [66] and, consequently, might contribute to a successful device removal.

It is now timely appropriate to draw lessons from the first wave of cell therapy clinical trials conducted over the last decade and, along with laboratory findings that have been accumulated in parallel, to use them as a building block for developing more effective strategies with regard to cell type, delivery, engraftment, tracking and clinical assessment. It is equally important to remind that other biologics other than cell-based therapies are being developed for treating heart failure, such as gene therapy [67] or microRNAs [68]. One of the challenges of the forthcoming years will be to determine the respective role of each of these strategies and to assess their risk-to-benefit and cost-to-benefit ratios in patients suffering from acute or chronic cardiac diseases.

### Disclosure of interest

The author declare that he has no conflicts of interest concerning this article.

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