

## MISE AU POINT/IN-DEPTH REVIEW GENE AND CELL THERAPY IN HEART FAILURE

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**RÉSUMÉ : L'insuffisance cardiaque est devenue une cause majeure d'hospitalisation et de mortalité surtout dans les pays industrialisés. Les traitements conventionnels sont encore insatisfaisants. La meilleure compréhension de cette pathologie et les progrès en biologie moléculaire ont ouvert de nouvelles perspectives thérapeutiques notamment les thérapies génique et cellulaire.**

**Cet article s'adresse aux principes et accomplissements de ces deux thérapies. En plus, il décrit les vecteurs et différentes méthodes d'administration relatives à chacune de ces thérapies.**

**Une meilleure évaluation de la sécurité et de l'utilisation clinique de la thérapie génique et cellulaire est encore nécessaire. Les dernières études sont optimistes en ce regard.**

### I. INTRODUCTION

Heart failure (HF) has reached epidemic levels in the industrialized world, especially with the increasing life span of both men and women.

Conventional treatments for HF aimed mainly at protecting the surviving cardiac myocytes, maximizing their functioning and preventing new complications. These aims were achieved with vasodilators, coronary interventions, bypass surgery, special devices (Left ventricular assist device (LAVD) and pacemakers) and ultimately cardiac transplantation.

They were conceived based on the generally accepted twin notions that (1) in the adult heart all myocytes are terminally differentiated and, therefore, cannot be recalled into the cell cycle, and that (2) the myocardium lacks a stem cell population able to generate new myocytes.

With the advent of recombinant DNA technology, molecular biology and increasingly efficient gene transfer technology, gene-based therapy, which was originally envisaged as a treatment strategy for inherited disorders, and cell therapy, are emerging as promising therapeutic options in the setting of HF.

These two therapies are administered by using different vectors and a variety of interventional techniques in order to attain the targeted cardiac structure.

### II. VECTORS AND DELIVERY SYSTEMS FOR GENE AND CELL THERAPY IN HEART FAILURE

#### II.1. Vectors

They are divided into non-viral, recombinant viral and hybrid (Viral/Non-Viral) systems. Table I shows the subtypes and characteristics of each system.

Recombinant adenovirus and adeno-associate virus (rAAV) are the most commonly used viral gene transfer systems due to the ability of these vectors to transduce, *in vitro* and *in vivo*, vascular endothelial and smooth muscle cells and cardiomyocytes.

The drawbacks of adenoviral use is that the commonly used human serotypes 2 and 5 vectors are also the most frequent etiological agents in naturally occurring infections, and as a result a high proportion of the population have preexisting neutralizing antibodies reducing vector efficacy. In addition, these agents can provoke intense immune and inflammatory reactions. Recombinant AAV has reduced immunogenicity, and the capacity for stable long-term transgene expression. Wild type AAV has been shown to integrate in chromosome 19, however, recombinant AAV has been shown to be episomal. Retrovirus vectors exhibit two major constraints : modest viral titre and inability to transduce non-dividing cells (unlike lentivirus vectors).

Once the suitable vector system has been selected for a specific application, the next consideration is expression cassette design which encompasses promoter/enhancer elements, the gene(s) of interest and an appropriate polyadenylation signal.

In gene therapy, the retention and effect of vectors carrying genes are revealed as the transduction efficiency and the functional effect of the gene product, respectively. In addition, in contrast to cell therapy, gene therapy needs not only to settle into the injected area, but also to cross the membrane of targeted cells and transduce genes.

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**TABLE I**  
MAIN VECTOR TYPES, SUB-TYPES AND THEIR MAJOR ADVANTAGES AND DISADVANTAGES

VECTORS	SUB-TYPES	Advantages	Disadvantages
VIRAL	ADENOVIRAL	Broad tropism	<i>Limited DNA insert capacity</i>
	ADENO-ASSOCIATED	Relatively efficient gene transfer	<i>Immunogenicity</i>
	RETROVIRAL	Variable capacity for long-term	<i>Replication risk</i>
	LENTIVIRAL	transgene expression	
NON-VIRAL	PLASMID DNA	Large DNA insert capacity	<i>Relatively inefficient gene transfer</i>
	OLIGONUCLEOTIDES	Biological safety	<i>Transient expression</i>
	LIPOPLEXES	Ease and reproductibility of production	
	POLYPLEXES		
HYBRID (VIRAL/NON-VIRAL)	HAEMAGGLUTINATING VIRUS OF JAPAN (HVJ)-LIPOSOME	Relative biological safety	<i>Immunogenicity</i>

## II.2. Delivery systems

They are the same for gene therapy and cell therapy and categorized into two major methods : injection using the coronary circulation [intracoronary injection (ICI) and retrograde injection via coronary sinus (RCI)], and direct injection (DI) including epicardial approach and endocardial approaches. All systems have the same potential risk, namely, untoward effects on non-target organs.

In gene therapy, DI shows significant transfection and gene expression with limited extent of gene expression surrounding injection sites, while the transfection efficiency of ICI is low except for few studies [1-2] reporting the injection at the aortic root level only after clamping transiently both the aorta and pulmonary artery. The clamping reduces ventricular filling and therefore, elevations in ventricular wall tension allowing the sub-endocardial coronary circulation to remain open. A difference in the transfection efficiency between DI and ICI is that the latter requires breaching the endothelial barrier to transduce cardiomyocytes. This is supported by the result that adeno-associated virus (18-26 nm in diameter), which is smaller than adenovirus (approximately 70-100 nm in diameter) and endothelial vesicles (< 50 nm), showed better transfection efficiency with ICI [3]. Regarding optimal conditions for gene delivery by ICI, the study using explanted hearts by Donahue et al. [4] indicates that the critical factors for the transfection efficiency were (1) washing blood out in the heart by perfusing crystalloid solution, (2) high coronary flow rate with high pressure in coronary arteries, (3) long exposure time, (4) high virus concentration and (5) body temperature (37°C). Furthermore, it is consistent with the results of RCI, which can reach cardiomyocytes without passing through endothelial barriers, and showed significant transgene expression without any assistance [5-6]. Some attempts to improve ICI efficiency are mainly aimed at enhancing endothelial permeability by using ultrasonic and pharmacological pretreatment

(histamine, serotonin, bradykinin, vascular endothelial growth factor, and low calcium solution). We reported in the meeting of Heart Failure 2003 that the efficacy of gene transfer via the combination of sonoporation and adenoviral vectors was 7-fold higher than that of DI.

In cell therapy, there is no significant difference between delivery methods in experimental studies. The cells however are larger than viruses and their permeation in case of coronary delivery may be harder. However in case of direct injection into the myocardium they may be able to settle within the myocardial space in a stable fashion. The survival of grafted cells can be influenced by physical factors and biological factors. The former may include cell loss during injection due to technical errors and cell death due to high pressure injection. In addition, washing-out by coronary flow and contractile force within injected area may affect the survival of grafted cells. The latter encompasses inflammatory reaction, environmental hypoxia and apoptosis.

## III. GENE THERAPY IN HEART FAILURE

Considering the limitations of the conventional treatment of HF, i.e. donor shortages for cardiac transplantation, along with the advances in the understanding of the molecular defects that underlie the clinical syndrome [7-8], gene-based therapeutic approaches have begun to emerge [9].

Gene therapy is a technology by which genes are delivered to human cells, tissue or organs to correct a genetic defect, or to provide new therapeutic functions for the purpose of preventing or treating diseases. This technology affects specific somatic cells unlike genetically modified organisms in which there is a permanent modification of the inheritable genome.

The cardiac components targeted by gene therapy include the myocardium, the coronary vasculature and the cardiac conducting system. In HF, most studies address the myocardium.

Gene transfer, aiming at enhancing cardiac function in the setting of HF, has targeted Calcium homeostasis, -adreno-receptor signalling, cardiomyocyte resistance to ischemia and apoptosis and myocardial protection from inflammation and immune reaction as shown in Figure 1.

### III.1. Modulating calcium homeostasis

Calcium is the “currency” of the excitation-contraction coupling in cardiomyocytes. Indeed, when calcium enters the cytoplasm through voltage-dependent calcium channels in the sarcolemma, it triggers the release of larger amount of calcium from the sarcoplasmic reticulum (SR) via the Ryanodine receptor2 (RYR2). The free calcium in myocytes interacts with Troponin C, facilitating the actin-myosin interaction and eventually inducing contraction and force production. The afterwards removal of free calcium from the cytosol induces myocyte

relaxation and diastole. The removal occurs by calcium re-uptake into the SR via the energy dependent calcium pump named the Sarcoplasmic Endoreticulum Calcium ATPase (SERCA2a) and by calcium extrusion to the extracellular space via the sarcolemmal sodium-calcium exchanger (NCX). SERCA2a function is inhibited by unphosphorylated Phospholamban (PLB). Phosphorylation of Phospholamban via the G-protein, c-AMP and protein kinase A pathway, relieves the inhibitory effect that this molecule has on SERCA2a.

Intracellular calcium homeostasis is, therefore, maintained by these calcium handling proteins and the abnormality of the failing heart is associated to alterations in protein level or activity. Although the precise role of these proteins in HF is still controversial, in failing heart NCX is often found to be up-regulated [10], whereas SERCA2a activity is reduced [11-12]. Hence, in the presence of up-regulated NCX and impaired

## Targets in human cardiomyocytes

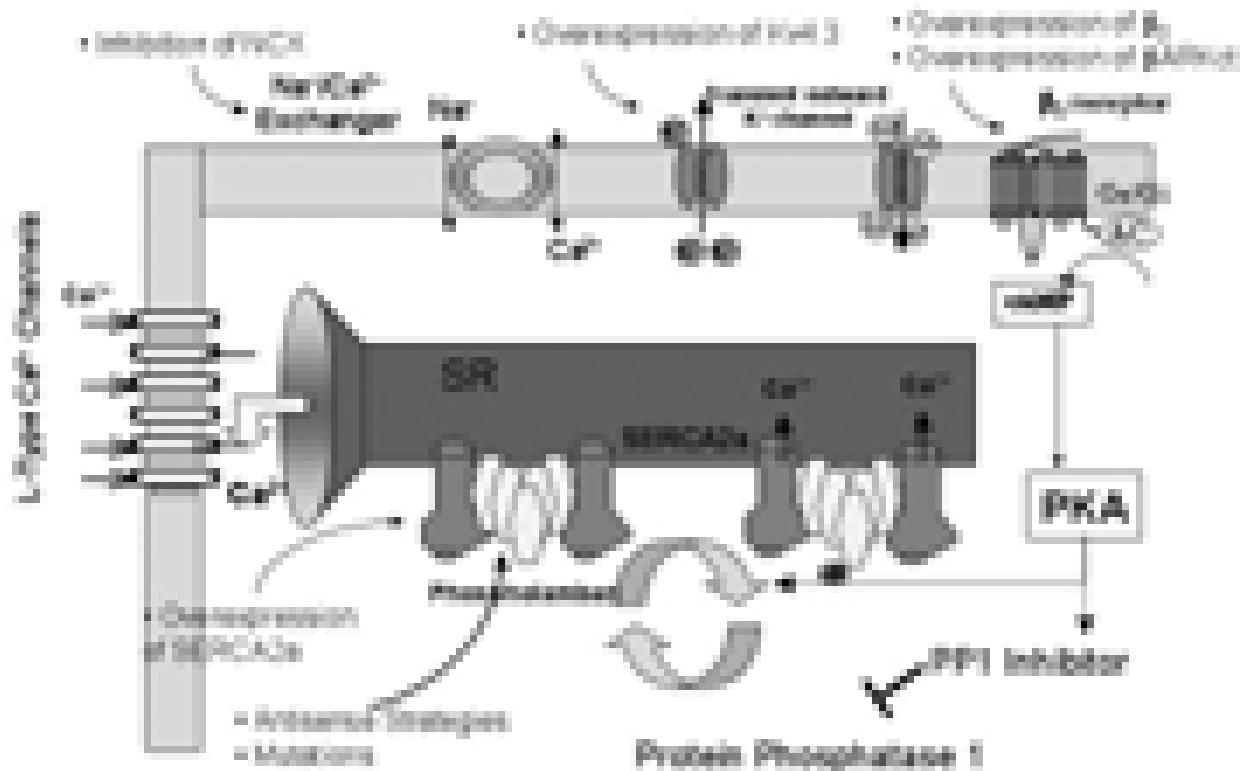


FIGURE 1

The various scientific steps of excitation-contraction coupling offer targets for genetic manipulation

- Increasing SERCA2 would enhance SR calcium uptake in failing hearts and improved relaxation and contraction.
- Inhibiting phospholamban by antisense strategies would enhance SR calcium uptake in failing hearts and improved relaxation and contraction.
- Overexpression of I-1 decreases phosphatase 1 activity making phospholamban phosphorylated and thereby decreasing inhibition to SERCA2a leading to improved relaxation and contraction.
- Failing hearts have prolonged action potential duration secondary to a decrease in Kv4.3 expression. Overexpression of Kv4.3 would decrease the action potential duration.
- Enhancing β-adrenergic stimulation through the overexpression of β<sub>2</sub> receptors or β-ARKct would increase contractility.
- In failing hearts, there is an increase in NCX that may overtake the SERCA2a activity which is depressed in heart failure.

SERCA2a, calcium store in the SR is reduced. Furthermore, early reports of calcium transients observed in cardiomyocytes of failing hearts describe a prolonged transient with reduced peak systolic and elevated diastolic calcium relative to normal controls [13-14]. In this regard, gene transfer to modulate calcium homeostasis (restoration of SERCA2a level and/or activity, reduction of NCX) might be predicted to rectify the cardiac dysfunction.

Hajjar et al. [15] and Giordano et al. [16] have initially demonstrated that overexpression of SERCA2a in isolated myocytes using adenoviral gene transfer resulted in increased contractility and a faster decay of the cytosolic calcium transient. A similar result has been also confirmed in human ventricular myocytes from patient with end-stage HF [17]. In addition to these promising results, there was some concern about the possibility of arrhythmogenicity with this therapy related to its inotropic effect. Indeed it is recognized that inotropic interventions which raise cAMP via stimulating  $\beta$ -adrenoreceptor or inhibiting phosphodiesterase activity increase arrhythmogenicity. But, unlike ordinary pharmacological inotropic intervention, which increases intracellular calcium concentration due to a raise in cAMP, overexpression of SERCA2a leads to a decrease in diastolic calcium concentration in cardiomyocytes, which underlines its anti-arrhythmogenic and anti-apoptotic effect as shown by several studies. In a rat failure model created by ascending aortic banding, gene transfer of SERCA2a improved survival rate after 4 weeks (63% vs. control 19%) and cardiac function [18].

In addition to improved calcium handling, SERCA2a overexpression also shortens cardiomyocyte action potential duration [19] adding another beneficial effect to the treatment of HF.

The second approach to improving calcium handling involves the targeting and reduction of Phospholamban function. In experimental studies, the ablation of PLB, which theoretically restore SERCA2a activity, has been achieved by the knockout transgenic approach and the virally-mediated antisense or dominant negative strategy for PLB. In the comparative study between PLB-knockout and PLB-overexpressing transgenic mice, the hearts from PLB-knockout mice exhibited increased contractility of the left ventricle compared to wild type mice, whereas PLB-overexpressing mice showed the more severely depressed contractility even though the level of SERCA2a activity remained normal [20]. The study shows, therefore, that the ratio of PLB to SERCA2 is a critical regulator of cardiac function and could be a potential target to treat HF.

This result is also supported by studies of *in vivo* gene transfer of the antisense message for PLB. He et al. [21] first demonstrated the significant effect of mutant and antisense RNA of PLB on SERCA2a activity. The reduced expression of PLB resulted in increased calcium affinity of the SR calcium pump. In this study, PLB level was not significantly affected in adult rat

myocytes by the antisense strategy. This is probably because in adult myocytes having high abundance of endogenous PLB, more effective antisense oligonucleotide of PLB could be required to reduce PLB protein levels. In other *in vitro* study [22], the antisense strategy using adenoviral vectors restored the frequency response to normal positive force-frequency relationship and enhanced SR calcium release in failing human myocytes from 9 patients with end-stage HF. We [23] et al. reported that *in vivo* gene transfer of the antisense of PLB had a significant stimulatory effect on the cardiac function in normal rat heart and cardiomyopathic hamster models, respectively. The *in vivo* study demonstrated that the functional effect of PLB-antisense in myocytes isolated from the heart was similar to our previous result *in vitro*, SERCA2a showing a significant increase in the contractility and reduction of relaxation time [24].

Therefore, the antisense strategy for PLB as well as *in vivo* SERCA2a-overexpression has potential to treat human HF.

### III.2. Manipulating $\beta$ -adrenoreceptor signaling

The  $\beta$ -adrenergic receptor (BAR) signaling cascade is a major modulator of normal cardiac function [25-26]. Agonists of BAR induce second messenger signaling via Guanine nucleotide-binding (G) protein association and subsequent stimulatory G protein  $\alpha$ -subunit ( $G_s$ ) dissociation. The consequent modulation of the effector molecule adenylyl cyclase results in activation of protein kinase A via increased cyclic AMP production. Protein kinase A dependent phosphorylation of targets such as troponin I, PLB, and L-type calcium channels results in downstream effects such as increased inotropy (contractility), chronotropy (heart rate), lusitropy (diastolic relaxation), and dromotropy (cardiac conduction) [25].

A number of alteration in this signaling cascade have been described in the context of HF [7, 27]. The chronic stimulation of BAR by the large sympathetic outflow of norepinephrine leads to receptor desensitization and to the aggravation of cardiac dysfunction. The desensitization is mainly characterized by down regulation of myocardial BAR, BAR uncoupling and up-regulation of the BAR kinase [28]. Hence, these three factors could be targets for manipulating BAR signaling, which increases cyclic AMP levels and improves the contractile function: To date, it has been reported that in human failing heart, the  $\beta_1$ -AR contribution was reduced following prolonged sympathetic activation, while  $\beta_2$ -AR numbers were preserved. In addition, inhibitory G-protein ( $G_i$ ) was increased and acted to suppress the contractile effects of BAR stimulation [29]. Therefore, restoration of  $\beta_1$ -AR was seemingly a main target. In contrary to the expectation, transgenic studies showed that overexpression of  $\beta_1$ -ARs was pathological and involved in the development of cardiomyopathy and apoptosis with catecholamines [30], whereas overexpression of  $\beta_2$ -AR works protectively. Milano et al. [31] demonstrated that transgenic overexpression of  $\beta_2$ -ARs enhanced cardiac

function and importantly, these mice showed normal lifespan and minimum negative effects. In a study on isolated myocytes, the stimulation of  $\beta_2$ -AR signaling was anti-apoptotic, ameliorating the pro-apoptotic effects of I/Gs activation in adult rat heart [32].

Another alternative is inhibition of BARKinase1. Koch et al. reported that overexpression of BARKct, which competitively inhibits  $\beta_2$ -ARK1, could result in enhanced inotropic responsiveness [33]. The efficacy of BARKct has been also investigated in several heart failure animal models [34-35] by demonstrating the preventive effects on the contractile function.

### III.3. Augmenting cardiomyocyte resistance to apoptosis

Apoptosis is a programmed cell death in which the cell and the nucleus shrink, condense and frequently fragment into membrane-neighboring cells. In the process of apoptosis, there is no leakage of cytosolic components and no inflammatory response. In a theory, apoptosis is associated with tissue where cells progress through the cell cycle. It was once thought, therefore, that no apoptosis could occur in terminally differentiated cells, such as cardiomyocytes and brain cells. However, recent studies have indicated that myocardial renewal occurs throughout life in the heart and it is partly involved in cardiac homeostasis [30, 36]. Condorelli et al. [37] reported that apoptosis increased with the progression of the contractile dysfunction in pressure-overload heart failure rat models and that dysfunction was accompanied by high levels of the pro-apoptotic protein Bax and a reduction ratio of the anti-apoptotic protein Bcl-2/Bax. Other studies also indicated that the decrease in the Bcl-2 to Bax ratio was responsible for the increased susceptibility of stretched myocytes to undergo apoptosis [38]. Overexpression of BCL-2 may, therefore, be a target to increase resistance to apoptosis. This concept is supported by several *in vivo* studies from both transgenic approaches [39] and gene transfer using adenoviral vectors [40]. In rabbit ischemia-reperfusion models, despite no effect in acute stage, Bcl-2 overexpressing rabbit showed better heart function and smaller ventricular size with greater preservation of ventricular free-wall thickness six weeks after reperfusion injury. Now it is widely accepted that continued ventricular dilatation occurred after myocardial infarction because thinning of ventricular wall occur not only in the infarcted zone, but also in the border zone region between ischemic segment and non-ischemic viable myocardium by increased tension load. Therefore, this study may indicate the possibility that overexpression of Bcl-2 prevents mechanical stretch-induced apoptosis in the border zone. However, it should be mentioned that overexpression of Bcl-2 can prevent the heart function from further deteriorating due to apoptosis of viable myocytes, but not restore the contractile function and it is uncertain whether blocking apoptosis in such a cell may prove to be detrimental since the cell may go on to necrosis instead of apoptosis. Recent studies have indicated another

possibility that cytokines may be a crucial mediator of the transition from hypertrophy to apoptosis in response to biomechanical stress. In particular, gp130, which is a common receptor of IL-6, is thought to play an important role in the transition [41]. This study demonstrated that in the ventricular muscle-specific gp130 knockout mice having normal cardiac structure and function, pressure-overload by aortic banding rapidly led to dilated cardiomyopathy with massive cardiac apoptosis. Hence, overexpression of gp130-dependent signaling may be an additional therapeutic option to prevent heart failure.

### III.4. Myocardial protection from inflammation and immune reaction

The transcription factor  $\text{NF-}\kappa\text{-B}$  can modulate the expression of several inflammatory genes [42] and mediates injury associated reperfusion of ischemic myocardium. Decoy oligonucleotides packaged in HVJ liposomes, targeting  $\text{NF-}\kappa\text{-B}$ , have been reported to result in a reduction of infarct size following transcatheter delivery in a rat model of myocardial infarction [43]. Furthermore, the same targeting improved cardiac graft preservation [44]. In a murine model of autoimmune myocarditis, HVJ-liposome packaged  $\text{NF-}\kappa\text{-B}$  oligonucleotide decoys reportedly resulted in improved histological and functional outcomes compared with mice receiving scrambled oligonucleotides [45].

Over-expression of immunosuppressive cytokines (i.e. interleukin-10, Interferon A6) and blockage of T-cell co-stimulatory signals (B7 and CD40) by gene transfer in a rodent model of autoimmune myocarditis have been reported [46-47] to reduce immune response and improve pathological findings.

## IV. CELL THERAPY IN HEART FAILURE

Although targeting cell therapy (known as cellular cardiomyoplasty) has spread to non-ischemic cardiomyopathy, the basic concept of cell therapy is to replace post-infarction scar tissue with contractile cells and to restore the contractile function in the area. As shown in Figure 2, there are distinct approaches, such as (1) reinduction of residual cardiomyocytes to a mitotic cycle, (2) transformation of in-scar fibroblasts into contractile cells, and (3) injection of exogenous contractile cells into the scar [48].

### IV.1. Reinduction of residual cardiomyocytes to a mitotic cycle

A well validated morphometric study showed an increase in myocyte number from birth to adulthood [49], therefore confirming the concept that cardiac homeostasis is not static and that myocyte renewal occurs throughout life. Thus, most adult myocytes are terminally differentiated and irreversibly withdraw from the cell cycle, but there are some myocytes able to re-enter the cell cycle especially in certain pathological conditions [50]. It is known that progression of mam-

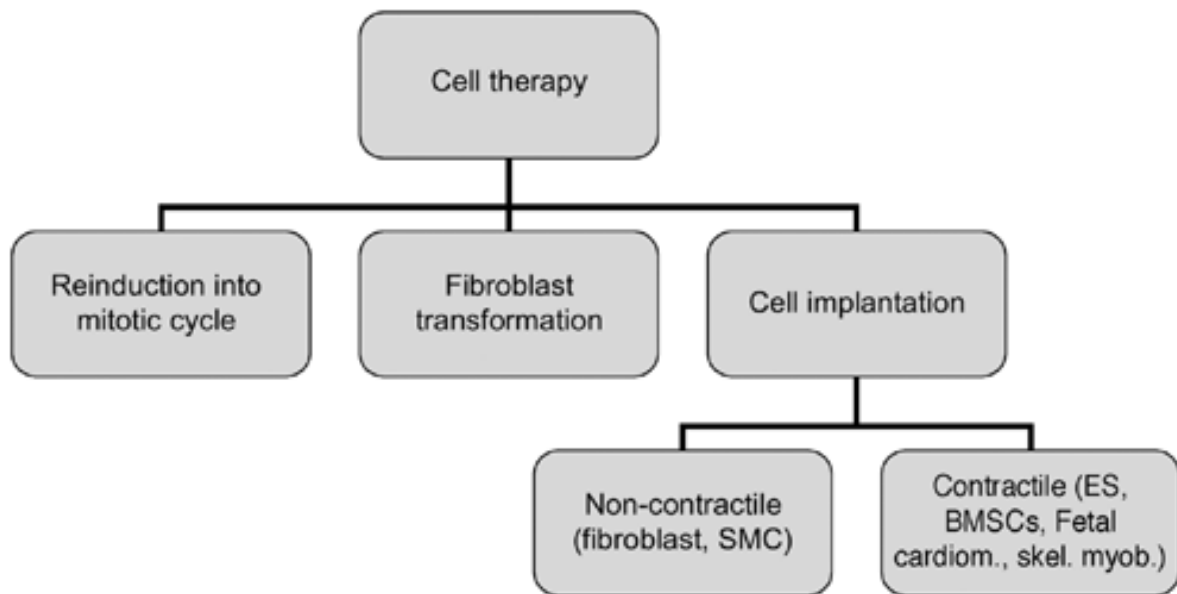


FIGURE 2  
Schematic of the various scientific avenues being taken by investigators for cardiac regenerative medicine.

malian cell cycle is regulated by a family of cyclins and cyclin-dependent kinases (CDKs). In particular, the cyclin D1 plays an important role for promoting cell cycle of G1-to-S phase progression by inactivating the action of the retinoblastoma protein (Rb) through phosphorylation. Tamamori-Adachi et al. [51] showed that the nuclear import of the cyclin D1/CDK4 complex was tightly inhibited in postmitotic cardiomyocytes and the nucleocytoplasmic transport machinery of cyclin D1 played a critical role for determining proliferative capacity of cardiomyocytes. This study also demonstrated that dual gene transfer *in vivo* with D1 and CDK4 induced cell cycle progression leading to cell division of neonatal cardiomyocytes. Although the nuclear import of cyclin D1/CDK4 certainly shows the possibility for induction of the cell cycle reentry of cardiomyocytes in the adult heart, this strategy raises major safety issues because of the stimulation of cell cycle by gene expression of viral oncoproteins. Moreover it is uncertain whether the reentry into cell cycle is due to the stimulatory effect on cardiomyocytes that already have a proliferative capacity or a direct effect to promote cell cycle reentry by other cardiomyocytes. A recently reported study by Beltrami et al. [52] showed that the adult rat heart contains undifferentiated cells that are self-renewing, clonogenic and multipotent, giving rise to a minimum three differentiated cell types: myocytes, smooth muscle and endothelial vascular cells. In addition, when injected into an ischemic heart, they reconstitute a well-differentiated myocardial wall that encompasses up to 70% of the left ventricle. The origin of these multipotent cells, whether within the heart itself or by homing from another locus, is still unknown. Data from sex-mis-

matched heart transplant [53] show that cells from the host, that are not endothelial progenitor cells (EPC), reach the heart and differentiate into the cardiac cells. Another study by Badroff et al. [54] showed that EPCs from healthy volunteers and CAD patients can transdifferentiate *in vitro* into functionally active cardiomyocytes when co-cultivated with rat cardiomyocytes suggesting the possible therapeutic use of these cells in the settings of ischemic disease and consequent HF.

#### IV.2. Transformation of in-scar fibroblasts into contractile cells by overexpression of MyoD

So far, no specific transcription factor for cardiac myogenesis exists. Meanwhile, in skeletal muscle, the MyoD family of basic helix-loop-helix proteins are well known to function as master genes for induction of the skeletal muscle differentiation program [55]. Therefore, studies about the transformation of scar tissue have focused on the conversion into skeletal muscle. Tam et al. [56] first indicated the feasibility that gene expression of MyoD gene *in vitro* may convert cardiac fibroblasts into skeletal muscle cells showing myotubes, myosin heavy chain (MHC) and myocyte-specific enhancer factor 2 (MEF2). Although the *in vivo* conversion of fibroblasts to skeletal muscles required high dose of adenoviral vectors, MyoD gene transfer induced skeletal muscle differentiation in the scar formation of infarcted heart [57]. However, in canine myocardial infarction models, the transfection of MyoD was limited and the converted cells showed the expression of skeletal MHC, but no morphological myotubes [58]. Therefore further investigation of specific cardiac myogenic factors must be required for successful transformation strategy.

### IV.3. Injection of exogenous cells into the scar

The candidate for implanted cells can be broadly divided into non-contractile cells, such as fibroblasts along with contractile cardiomyocytes and vascular smooth muscle cells (VSMCs). Non-contracting cells are implanted for the purpose of preventing the deterioration of post-infarct diastolic function, while contracting cells are expected to improve both systolic and diastolic function [59]. Furthermore, contractile cells are divided into two cell types, namely naturally contractile cells and potentially contractile cells. The former includes fetal and neonatal cardiomyocytes, and skeletal myoblasts. The latter is represented by embryonic stem (ES) cell and bone marrow cells (also known as adult stem cell) [48].

#### IV.3.1. Non-contracting cell transplantation

##### a) Fibroblast cell as a cell source for cell transplantation

Fibroblast cells are an attractive cell source for cell transplantation as well as the target for transformation strategy. This is because fibroblasts are autologous, abundant, and easily expandable. It is also easy to harvest from several organs including skin and pericardium. These properties are particularly important for elderly patients because of their limited availability of adult stem cells and myoblasts. Etzion et al. [60] reported the possibility of two-staged procedure (MyoD-based therapy) composed of *ex vivo* transformation of cardiac fibroblasts with MyoD and cell transplantation.

##### b) Smooth muscle cell (SMC) transplantation

Mickle's group demonstrated that SMC transplantation improved both systolic and diastolic function in rat MI model and in hamster dilated cardiomyopathy models because implanted cells increased wall tension and elasticity [61-62]. SMCs have the following technical and biological advantages: the possibility of auto-transplantation, the ability to proliferate more easily than that of skeletal myoblasts, the elastic property, long-term steadiness of the contractile property (termed tonus contraction), and the hyperplastic response of SMC to the increased stretch.

#### IV.3.2. Naturally contractile cells

##### a) Fetal and neonatal cardiomyocytes

In cellular cardiomyoplasty, the primary candidate was fetal and neonatal myocytes because these cells have the ability to differentiate into an adult matured cardiomyocyte phenotype, but at the same time, they still retain a certain ability to proliferate [63]. Soonpaa et al. [64] first demonstrated that grafted fetal cardiomyocytes were detected over two months in normal mouse hearts. Interestingly, in this fundamental study, grafted fetal cardiomyocytes in normal mice hearts showed nascent intercalated disks connected with the host myocardium. Similarly, Murry's group indicated that both grafted fetal and neonatal cardiomyocytes formed matured myocardium in rat hearts two months after the injection. In some cases in this study, graft cells formed gap and adhesion junctions with host cardiomyocytes, suggesting electro-mechanical coupling [65]. Theoretically, successful cell

transplantation, which contributes to the improvement of cardiac function, depends crucially on integration into the host and differentiation toward the adult phenotype. In this regard, fetal and neonatal cardiomyocytes seem to be quite promising candidates. It must be mentioned, however, that as far as clinical perspective is concerned, ethical issues could severely hinder the application with other hurdles to be overcome, including, availability and immunogenicity.

##### b) Skeletal myoblasts

Skeletal myoblasts, are myogenic precursors and normally exist in a quiescent state under the basal membrane of skeletal muscle fibers. These cells are rapidly mobilized into the injured area and proliferate and fuse to regenerate the damaged fibers. Skeletal myoblasts have some advantages as a source for cell transplantation, such as (1) autologous origin, (2) relatively easy to multiply to large numbers from a small biopsy, (3) low tumorigenicity because of well-differentiated myogenic lineage and (4) high resistance to ischemia. In experimental studies, it has been detected that grafted myoblasts differentiated into typical multinucleated myotubes and substituted the postinfarct fibrosis. However, so far no studies have shown reliable evidence of trans-differentiation of injected myoblasts into cardiomyocytes, despite some forms of phenotype (slow myosin pattern), which can adapt to the myocardial environment. In addition, most studies of skeletal myoblast transplantation failed to show the existence of gap junction of injected cells with host myocytes *in vivo* [66, 57]. Menasché's group has shown some conclusive results that skeletal myoblasts retained the typical electrical membrane properties and functional and electrophysiological independence after transplantation. In contrast to these negative results of the graft-host coupling, the improvement of cardiac function after skeletal myoblast transplantation has been continuously demonstrated in many animal models and even clinical cases in both the short and long term [67-68]. This is due to first, the elastic properties of implanted cells may provide a scaffold strengthening the ventricular wall and subsequently limiting postinfarct scar expansion in a similar way to the SMC implants [61]. In this regard, it is consistent that the grafted skeletal myoblasts have a protective effect against excessive remodeling. Second, intrinsic contractile function of skeletal myoblasts may be directly involved in the improvement of failing ventricular systolic function. Last, the engrafted myoblast may act as a source of growth and/or angiogenic factors. Menasché et al. [69-71] reported in a trial with a major dual purpose—the feasibility and safety of autologous skeletal myoblast transplantation in ten patients with severe ischemic cardiomyopathy, one early death and one noncardiac death during the follow-up, but no perioperative complication related to the cell preparation and the transplantation, except for sustained ventricular arrhythmia in four cases requiring internal defibrillator. Now the clinical trial has moved to the Phase 2 aiming at assessing the safety and efficacy with placebo-control group in multiple centers.

### IV.3.3. Potentially contractile cells

#### a) Embryonic Stem (ES) cells

They are derived from the inner cell mass of blastocyst-stage embryos and characterized by the ability to proliferate and to be pluripotent, which means the capability to differentiate into every somatic cell type of the adult organism. In rat heart infarction models, implanted ES cells survived and differentiated into mature cardiomyocytes six weeks after the transplantation and as a result of that, cardiac function was improved [72]. Moreover, the recent study reported the improvement remained in the long-term (32 weeks) [73]. In 2001, Kehat et al. [74] reported the differentiation of cardiomyocytes from human ES cell and electrical coupling and synchronous contraction of the human stem cell-derived cardiomyocytes with rat cardiomyocytes in coculture [75]. However, a number of major issues should be overcome for clinical application of human ES cells. First, the allogeneic origin of these cells raises immunological problems. Second, the immortality of undifferentiated stem cells, which can form cell types other than cardiomyocytes, is a huge benefit, but also may cause tumorigenicity. Last but not least, like fetal and neonatal cardiomyocytes, moral and ethical issues could be the greatest obstacle to clinical application.

#### b) Bone marrow stem cells (BMSCs) or adult stem cell

BMSCs are also expected to have the capacity of unlimited, undifferentiated proliferation and to be able to develop into different types of cells including cardiomyocytes. In contrast to ES cell, BMSCs appear to be an ideal cell source for cardiac repair because these cells having great plasticity can be collected from patients without evoking ethical and moral questions, and creating problems of immunological reaction. Although the precise angiogenic and myogenic effects after the transplantation of BMSCs are still unclear, most experimental studies showed the differentiation into cardiomyocytes with the improvement of global cardiac function in acute or chronic infarcted heart models [76-78] and even in non-ischemic heart [79]. The recent study by Fukuhara and Tomita [80] showed that only mouse BMSCs co-cultured with neonatal rat cardiomyocytes showed the synchronous contraction with cardiomyocytes and cardiac-specific proteins including connexin 43 and troponin I. Therefore, direct cell-to-cell interaction may be one of the most important factors for BMSCs to differentiate into cardiomyocytes. To date, several results of clinical phase-1 trial of autologous bone marrow cell transplantation have been published [81-83]. These trials indicated that this procedure was safe and appeared to improve symptoms and myocardial perfusion. In particular, it is noteworthy that in contrast to clinical studies of skeletal myoblast transplantation, these studies reported no patients with ventricular arrhythmia including sustained VT. Still, it should also be mentioned that the condition and cell types of these clinical studies are various and long-term follow-up must be done. Recent reports suggest that the main effect of bone

marrow cell transplantation on the improvement of regional heart function is angiogenesis by the paracrine effect of bone marrow cells, not direct effects on contraction. It is certain that the paracrine effects are likely to trigger the differentiation of adult stem cells into cardiomyocytes, stimulate host myocytes to re-entry of a cell cycle, and induce a cardiomyogenic lineage of bone marrow cells or circulating progenitor cells to injured sites.

## V. CONCLUSION AND FUTURE PROSPECTS

This review highlighted the basic concepts and potentials of gene and cell therapies in heart failure. These treatments bring a new hope of curativity to this disease when more conventional treatments were more palliative except for heart transplantation which unfortunately, beside its procedure related risk of mortality, faces the problem of donor shortage.

A progress ought to be achieved within vectors and delivery systems so that efficiency and safety would be optimal. We are awaiting more pre-clinical and human clinical studies addressing these issues.

The precise mechanism of arrhythmia after cell transplantation should be further investigated since it could be a major constraint in the future use of such therapy.

Finally, even when gene and cell therapy are still at the basic level of development, much is awaited from these procedures in the near future with a new revolution in the treatment of heart failure and many other diseases.

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