# Heart Regeneration: More Hope, Less Hype

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**Biosketch:**

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**Purpose & Overview:**

The purpose of this lecture is to highlight recent advances in the field of heart regeneration, a field notorious for being highly polarized and contentious. I will discuss the various strategies that have been commonly used in an attempt to regenerate the human heart, the current consensus in the filed, and the emerging regeneration strategies.

**Educational objectives:**

1) Understand the underlying progressive nature of cardiomyopathy and the current available treatment options.
2) List the most widely used strategies for heart regeneration, and outline the major findings of recent clinical trials that tested these strategies.
3) Understand the role of cardiomyocyte proliferation in endogenous heart regeneration, and the potential therapeutic value of this approach in treatment of cardiomyopathy.

## Heart failure:

Heart failure is a costly and deadly disease, affecting over 23 million patients worldwide, half of which die within 5 years of diagnosis. The syndrome of heart failure is defined as reduced cardiac output, most frequently results from systolic dysfunction (cardiomyopathy). The pathophysiological basis of cardiomyopathy lies in the inability of the adult heart to regenerate lost or damaged myocardium. Instead of regenerating new cardiomyocytes, the adult heart heals through fibrotic scar formation. This is followed by a cascade of deleterious events, termed *myocardial remodeling*, which result in further cardiomyocyte loss, progressive dilatation and weakening of the remaining myocardium, all of which underpins the progressive nature of heart failure. Consequently, current therapies for cardiomyopathy are focused on either prevention of remodeling and preserving the remaining myocardium, or complete pump replacement in terminal cases. These limited options for



**Figure 1.** Myocardial Remodeling

treatment of cardiomyopathy have sparked an intense interest in regenerative therapies, which has reached a precipice in the past decade. While initial strategies have focused on stem cell therapies for heart regeneration, the focus has now shifted to a more in depth mechanistic understanding of the endogenous regenerative properties of the myocardium, and how they can be harnessed to cure heart failure.
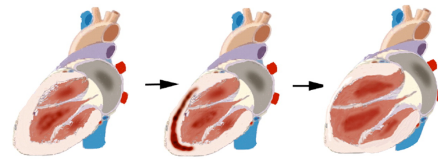
## History of Stem Cell therapy for heart failure:

In 2001, a report published in Nature claimed that bone marrow-derived stem cells could regenerate the myocardium and restore cardiac function following myocardial infarction. The authors demonstrated by genetic fate mapping that bone marrow-derived cells are capable of acquiring a cardiomyocyte fate, resulting in myocardial regeneration and normalization of cardiac function[1]. Although this report was highly contentious, it sparked a global frenzy of animal studies and clinical trials aiming to identify the stem cells best suited for heart regeneration. In fact, within a few years of the initial report, there were over 1000 clinical trials worldwide investigating the role of stem cells in heart regeneration. Everything from allogeneic unfractionated bone marrow mononuclear cells (MNCs), to autologous mesenchymal stem cells (MSCs), skeletal muscle and cardiac-derived stem cells were tested in various scenarios of cardiac dysfunction. Although the results varied widely, it became clear that none of these cells actually trans-differentiate into cardiomyocytes, and that only minimal effects, if any, were noted, which are attributed to an unidentified paracrine effect of these cells. In the following sections, I will discuss various cell therapy types, and the corresponding clinical trial results.
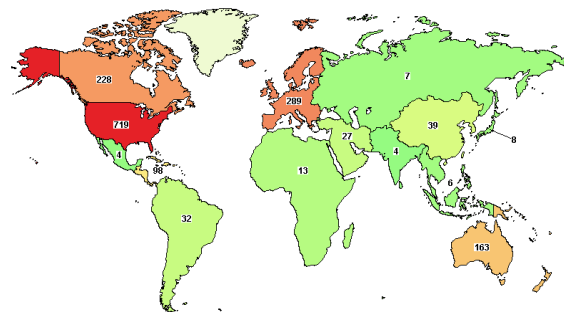


**Figure 2.** Cell Therapy for Heart Regeneration clinical trials in 2010 (from *clinicaltrials.gov*)

## Types of cell therapy:

The rationale for stem cell therapy for heart failure was built on the speculation that the plasticity of these cells results in their trans-differentiation into cardiomyocytes to regenerate the injured myocardium. Although this premise proved to be false, one thing that cell therapy clinical trials have shown is that these cells are safe, with the exception of skeletal myoblasts, and may have a modest effect on cardiac function
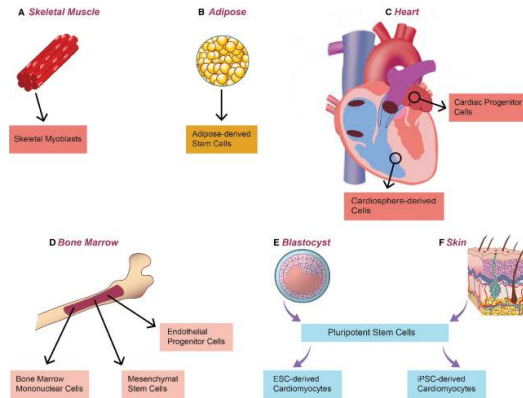


**Figure 3.** Sources of exogenous cell

### 1) Non-cardiac Cells:

### A) Skeletal myoblasts

One of the first studies using a cell-based strategy for ischemic heart disease relied on skeletal myoblasts. The hypothesis was based on the ability of skeletal myoblasts, which are specialized muscle stem cells, to regenerate skeletal muscle. Advantages of using this cell type include easy *ex vivo* expansion, and the ability to use an autologous source. Initial preclinical studies demonstrated a potential for intra-myocardial injection of skeletal myoblasts to improve LV function. However, several clinical trials



**Figure 4.** Skeletal myoblasts have no effect on LV function (MAGIC)

including the MAGIC[2] and MARVEL studies, as well as their long-term followup, have since showed lack of efficacy in improving LV function. Moreover, subsequent analysis showed that the injected cells do not integrate electromechanically with the surrounding myocardium and have a propensity to induce arrhythmias (especially dangerous ventricular tachyarrhythmias). Given the lack of clinical improvement and the potential arrhythmogenic side effects, skeletal myoblasts have fallen out of favor as a therapeutic candidate for cellular therapy.
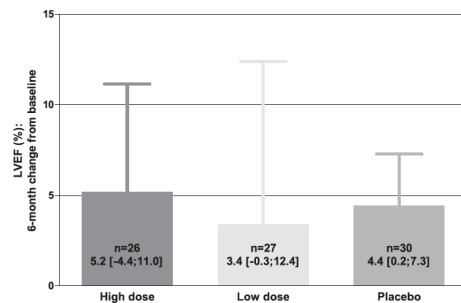
| | Placebo (n=34) | Low Dose (n=33) | High Dose (n=30) | HR (95% CI) | P* | EuroSCORE-Adjusted HR (95% CI) | P† |
|---|---|---|---|---|---|---|---|
| Six-month MACE,‡ n (%) | 7 (21) | 13 (39) | 6 (20) | 1.6 (0.7; 3.8) | 0.29 | 1.5 (0.6;3.6) | 0.69 |
| Six-month mortality, n (%) | 2 (6) | 5 (15) | 4 (13) | 2.6 (0.6;12.1) | 0.20 | 2.5 (0.5;11.4) | 0.25 |
| Six-month ventricular arrhythmias, n (%) | 2 (6) | 4 (12) | 5 (17) | 2.7 (0.6; 12.6) | 0.18 | 2.8 (0.6; 12.8) | 0.19 |
| Sustained VF or polymorphic VT | … | 1 | … | … | … | … | … |
| Sustained monomorphic VT | 1 | 2 | 4 | … | … | … | … |
| Sustained monomorphic VT and sustained VF or polymorphic VT | 1 | 1 | 1 | … | … | … | … |

**Table 1.** Skeletal myoblast intramyocardial injection increases the number of ventricular arrhythmias (MAGIC)

## B) Bone marrow cells

Bone marrow cells are the most widely tested cell population as a source of cell therapy for heart regeneration. This may be due to the association with the initial *Nature* report outlining their regenerative potential, as well as the relative ease of allogeneically harvesting them in large numbers. While unselected bone marrow MNCs have been the most widely tested in pre-clinical and clinical trials for cardiac therapy.



**Figure 5.** BOOST trial results showing no change in left ventricular function at 18 months followup.

Referring to MNCs as a stem cell preparation is a misnomer because true stem cells comprise well below 0.1% of the total mononuclear cell population. Unfractionated MNCs mainly consist of a heterogeneous population of hematopoietic cells including monocytes, committed myeloid progenitor cells and lymphocytes, and a small population of hematopoietic and mesenchymal stem cells.
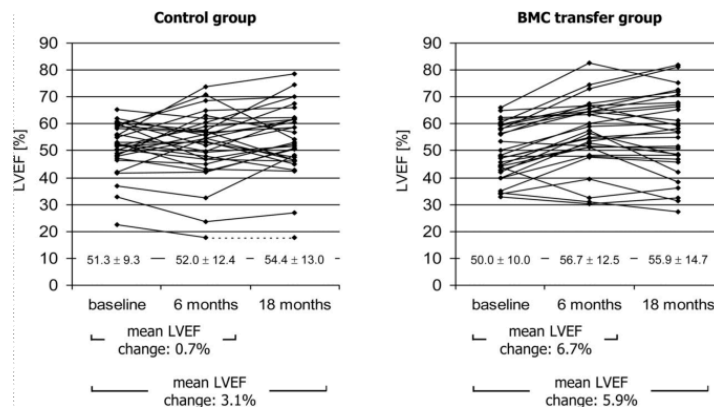
Most of the early MNC studies enrolled acute MI patients with ST-segment elevation and a modest decline in LVEF and they reported functional improvement after treatment. One such study was the BOOST trial in which autologous MNCs (which have < 1% CD34$^+$ cells) were isolated from patients and delivered by intracoronary infusion to the infarct-related artery the same day. No serious adverse events were reported in either group, and cardiac magnetic resonance imaging (MRI) at 6 months indicated a significant increase in LV ejection fraction after cell treatment (compared to placebo control). However in longer-term follow-up, the control group showed a "catch-up" phenomenon, where the benefits of MNCs could no longer be demonstrated[3].

Another initial study that demonstrated some benefit of bone marrow MNCs is the REPAIR-AMI trial, which reported a 5.5% increase in LV ejection fraction at 4 months after intracoronary infusion of MNCs compared to a 3.0% improvement in controls. While the results of this study were hindered by the use of quantitative LV angiography to assess function as
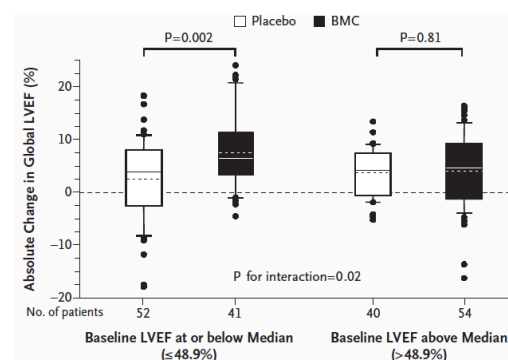


**Figure 6.** REPAIR-AMI Trial showed a modest improvement of LVEF following MNCs injection.

opposed to cardiac MRI, the enrollment of over 200 patients made this the largest MNC trial at the time and set the standard for expected systolic improvement, albeit a modest increase, after cell therapy[4]. The same group reported that the functional improvement persists up to 5 years post-treatment in a subset of patients. However, the use of ventriculogram for assessment of LVEF, instead of echocardiography or MRI, was widely viewed as weakness of this study.

Despite these and other studies reporting functional improvement after MNC treatment, larger trials employing greater degrees of randomization, placebo controls, and blinding conducted in the years following have not replicated these results. The Cardiovascular Cell Therapy Research Network (CCTRN) was designed to facilitate cell-based therapies in the United States and sponsored the FOCUS-CCTRN trial, which was one of the first trials to target patients with



chronic LV dysfunction who had not qualified for revascularization therapy post-MI. Enrolled patients had a mean baseline ejection fraction of 30–32% and New York Heart Association (NYHA) class of 2 or 3. This study found no difference in endpoints including chamber dimensions, LVEF, MVO2, or myocardial viability.
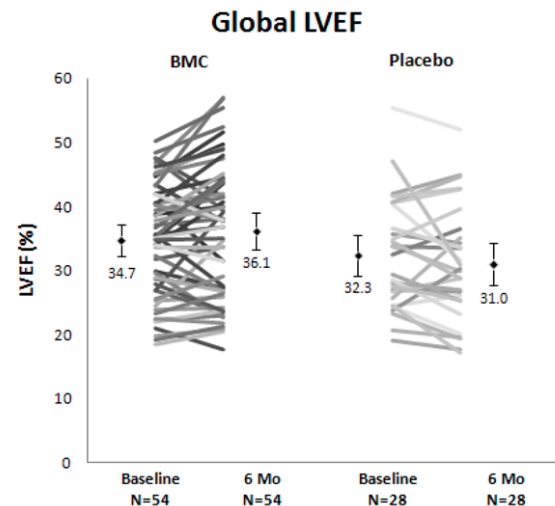
**Figure 7.** FOCUS-CCTRN Trial showed no improvement of LVEF following MNCs injection.

Other studies that were also sponsored by The CCTRN were the TIME and LateTIME trials, which assessed the effect of delivery timing of bone marrow on LV function. Each of these double-blinded and placebo-controlled trials enrolled MI patients and delivered $150×10^6$ autologous BMMNCs by intracoronary perfusion either at day 3 or 7 (TIME) or at 2–3 weeks (LateTIME) after MI. Neither study detected any functional benefit by cardiac MRI at 6 months after cell treatment, regardless of delivery timing. Collectively, these studies strongly challenge earlier reports of functional improvement following bone marrow MNCs injection, and should be collectively viewed as a conclusive result, especially given their double-blinded, placebo controlled study design.

*One large ongoing European study that is worth mentioning is the BAMI trial, which is enrolling 3000 patients (clinical trial identifier NCT01569178), although it is not expected that this study will yield significantly different results.*

## C) Mesenchymal stem cells

Another source for allogeneic cell therapy consists of mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells or colony forming unit-fibroblasts. These were first isolated from bone marrow stroma and described by Friedenstein *et al*[5] more than 40 years ago, and have been shown in the intervening decades to be a multi-potent source of mesoderm (as well as some non-mesoderm) derived tissues including osteoblasts, chondrocytes, adipocytes, skeletal muscle, hepatocytes and even neurons *in vitro*. The ability of MSCs to differentiate into cardiomyocytes is heavily in dispute, with some studies demonstrating transdifferentiation of MSCs to cardiomyocytes, while most others showing very limited cardiomyogenic potential. Despite this controversy, MSCs have been eagerly pursued as a cell-based source for cardiac repair, because of their many other favorable properties including their immunomodulatory properties and their easy isolation and amplification from an allogeneic source, which facilitates their commercialization. Mesenchymal stem cells have been isolated from many different tissue types including bone marrow, adipose tissue, lung tissue, umbilical cord blood and peripheral blood, but are most easily harvested from the bone marrow and adipose tissue. In particular, adipose-derived mesenchymal stem cells (ADCs) have the attractive feature of being easily harvested and isolated from an allogeneic source through liposuction with a high yield. Thus, most pre-clinical and clinical studies have focused on delivery of MSCs isolated from these two sources.
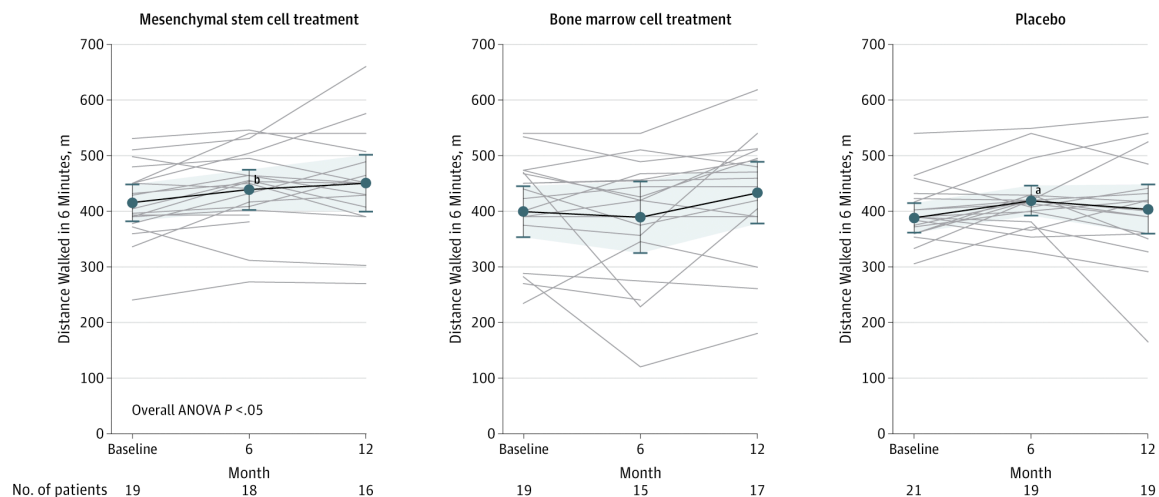


**Figure 8.** TAC-HFT trial compared bone marrow MNCs to MSCs injection and showed that while there was no change in ventricular function, there was a mild improvement in the 6-minute walk test with MSCs injection.

Several studies have directly compared the safety profile and efficacy of bone marrow MNCs to MSCs, of which the TAC-HFT trial[6] is the largest. In this study, chronic MI patients received a transendocardial injection of MNCs or MSCs. While there was no difference between groups in the 1-year serious adverse

event rate or LV ejection fraction, patients receiving MSCs showed some improvement in exercise capacity.

It is important to note here that there are two types of MSCs that have been used in clinical trials; autologous (as in TAC-HFT) and allogeneic, which are used as an "off-the-shelf" product. These two types of MSCs have been compared head to head in the POSEIDON trial[7]. Chronic MI patients received a dose of 20, 100, or 200 million autologous or allogeneic MSCs, injected into the myocardium via a transendocardial catheter, and the study concluded that neither cell source stimulated a significant adverse immune response, and that they have a modest effect on LV function and MI size.
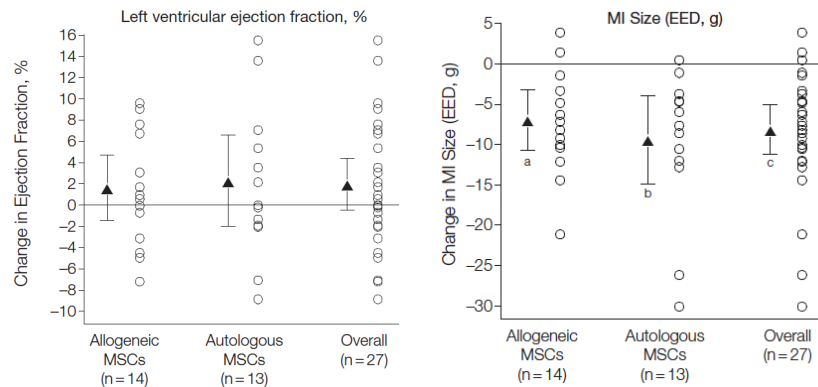


**Figure 9.** POSEIDON trial showed mild improvement of LV function and mild decrease in infarct size

In summary, MSCs seem to have a minimal effect on myocardial regeneration and improvement of left ventricular systolic function. Although they may be tangentially better than bone marrow MNCs, their effect of both cell types is modest at best. Moreover, the mechanism by which these cells act remains largely unknown, although it is well accepted now that they do not transdifferentiation into contractile cardiomyocytes. Some efforts are now focused on identifying the mechanism of their paracrine effect, which may be mediated by secreted microvesicles known as exosomes.

## 2) Cardiac-Derived Cells:

### A) C-Kit Cells

Several types of putative 'cardiac progenitor cells' (CPCs) have been reported, with the shared definition that they are clonal and multi-potent cells capable of self-renewal and differentiation into the three major cardiac cell types. The most clinically relevant of these cells have been the c-kit$^+$ cell and the cardiosphere-derived cell (CDC), while other cells like Sca-1$^+$ cells, Isl-1$^+$ cells, SSEA-1$^+$ cells, and side-population cells have not been adequately tested clinically. It is important to note that none of these cells have demonstrated in vivo multipotentiality by rigorous methods such as genetic fate mapping. Most researchers now believe that these cells, like bone marrow cells and MSCs,

show minimal long-term engraftment or cardiac differentiation and instead work principally through paracrine mechanisms. Nevertheless, many of these cells have proceeded to clinical trials despite lack of sufficient mechanistic evidence of their multipotentiality. The three leading trials using CDCs to date are described below.

The SCIPIO trial was the first trial using CDCs focused on cells expressing the surface antigen c-kit, which were first isolated and characterized in the rat. Similar to bone marrow-derived cells, initial animal studies suggested that c-kit$^+$ cells gave rise to cardiomyocytes;
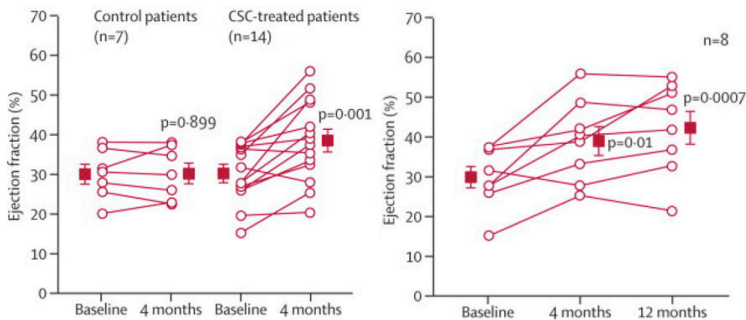


**Figure 10.** SCIPIO trial showed a mild improvement of LV function following c-kit cells injection. However serious ethical concerns have been raised.

however, lineage tracing studies have determined that these cells show minimal long-term engraftment and only extremely low rates of cardiac differentiation in the adult heart. The SCIPIO (cardiac *S* tem *C* ell *I* nfusion in *P* atients with *I* schemic *C* ardi *O* myopathy) trial enrolled 33 heart failure patients with chronic MI (mean ejection fraction of 27.5% at baseline), who underwent a right atrial appendage biopsy during coronary bypass surgery. This atrial tissue was used to isolate a putative cardiac progenitor cell that expressed the surface antigen c-kit and was negative for other lineage markers. After 4 months of in vitro expansion, 0.5–1 million cells were injected through the coronary arteries supplying the ischemic myocardium of 20 patients, while 13 patients remained as controls. Analysis of heart function by 3D echocardiography or cardiac MRI showed an 8.2% and a 12.3% improvement in LV ejection fraction at 4 and 12 months, respectively, and, somewhat surprisingly, a reduction in infarct size in a subset of patients.

*It is important to note however, that results from this study have been flagged with an "expression of concern" by the editors of the Lancet relating to an ongoing investigation pertaining to data integrity.*

## B) Cardiosphere-Derived Cells

Another trial worth discussing is he CADUCEUS trial of CDCs involved "cardiosphere-derived cells", a mesenchymal cell population obtained by explant culture of endomyocardial biopsies, followed by expansion as cellular spheroids. Cardiosphere-derived cells are heterogeneous by surface markers but are primarily CD105$^+$/CD45$^-$. In the Phase I CADUCEUS (*CA* rdiosphere- *D* erived A *U* tologous stem *CE* lls to reverse ventric *U* lar dy *S* function) trial, patients with a

mean LV ejection fraction of 39% undergoing primary angioplasty 2–4 weeks after MI had a right ventricular biopsy removed to expand autologous cells. After a 1- to 3-month expansion period, 25 million cardiosphere-derived cells were delivered as an intracoronary infusion into the infarct-related artery. Although the primary endpoint was safety, cardiac MRI at 6 and 12 months after cell delivery revealed a reduction in infarct size (identified as a reduced region of delayed gadolinium enhancement) and an increase
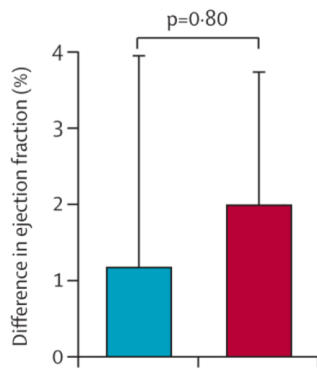


**Figure 11.** CADUCEUS trial showed that Cardiosphere-Derived Cells can decrease myocardial scarring

in viable myocardium. Although there was no significant change in global ejection fraction, cell-treated patients showed improved regional systolic wall thickening that was maintained from 4 months to 1 year after treatment. The authors interpreted the increase in viable myocardium seen by MRI as proof of regeneration, but pathological hypertrophy of preexisting cardiomyocytes cannot be ruled out as an alternate explanation. Although not statistically significant, cell-treated patients experienced higher levels of nonsustained ventricular tachycardia and serious adverse events at the 1-year follow-up, which will require closer evaluation in future trials.
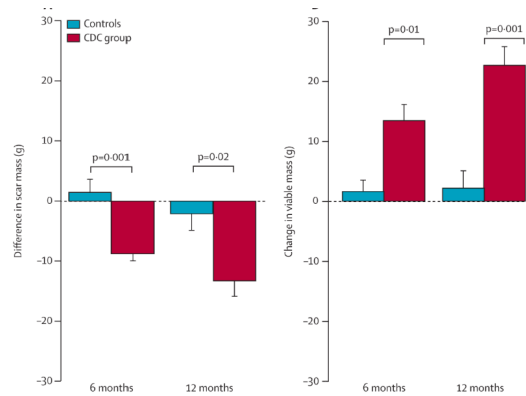


**Figure 12.** CADUCEUS however showed no improvement of LV function.

Although the SCIPIO and CADUCEUS demonstrated relative safety, data manipulation in the SCIPIO trial and lack of functional improvement of the CADUCEUS trial have raised concerns about the validity of cardiac-derived cells for myocardial regeneration.

*In summary, adult stem or progenitor cells, derived from either cardiac or extracardiac sources do not appear to have significant capacity to transdifferentiate into cardiomyocytes, and have failed to demonstrate significant functional improvement.*

Another source of cell therapy for treatment of cardiomyopathy is pluripotent stem cell-derived cardiomyocytes. Although the field
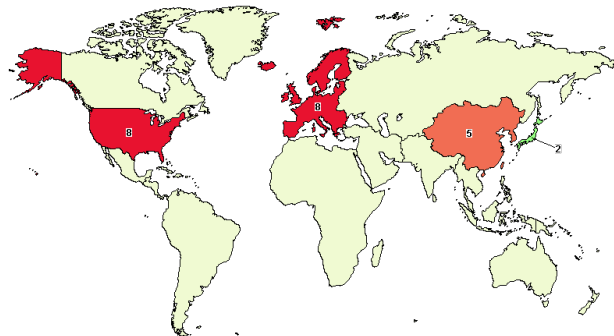


**Figure 13.** The poor results of somatic stem cell therapy has markedly decreased enthusiasm for this approach (23 studies compared to over 1000 studies 5 years ago)

of pluripotent-derived cells in regenerative medicine has faced ethical concerns surrounding the use of human embryonic stem cells (hESCs), the seminal discovery of induced pluripotent stem cells (iPSCs) by Yamanaka in 2006[8] has revolutionized the field, and effectively removed much of the ethical concerns surrounding the use of hESCs.

## 3) Pluripotent Stem Cells

Pluripotent stem cells have the ability to differentiate into all cell lineages, and hence offer novel treatment options for many intractable diseases including end-stage heart failure. hESCs have been investigated as a source of cells for cardiac repair through *ex vivo* differentiation into either cardiac progenitors or into mature cardiomyocytes. However, limitations include the inability to isolate pure tissue-specific progenitors capable of robust engraftment and regeneration, potential risk of teratoma formation from residual undifferentiated cells, and ethical concerns with their generation. In addition, it is uncertain that hESCs can functionally engraft and electromechanically couple into the surrounding myocardium. These concerns have limited the clinical translation of hESCs for cardiac therapy.

The report by Yamanaka in 2006 that terminally differentiated murine fibroblasts could be 'reprogrammed' to a primitive embryonic stem cell-like state through introduction of four specific transcription factors (Oct3/4, Sox2, c-Myc and Klf4) brought new hope to cardiac regenerative medicine. These iPSCs may bypass the ethical concerns associated with ESCs, and serve as a potentially unlimited source of cells for transplantation. While murine studies reported engraftment of iPSCs into infarcted myocardium, concerns for tumourgenicity have greatly limited further investigation using direct transplantation of iPSCs.

The most promising application of PSCs in cardiac regenerative medicine has been their use as a cell source for derivation of adult cardiomyocytes for transplantation. While early protocols for differentiating ESCs into cardiomyocytes generated less than 1%
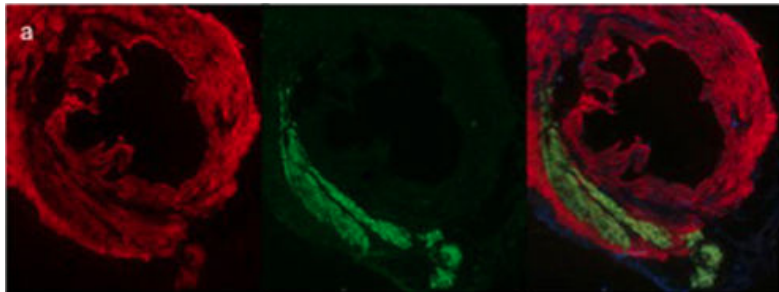


**Figure 14.** Human hSCs-derived cardiomyocytes integrate into the primate heart

yields, more recent differentiation protocols have achieved yields of up to 70%. Further enrichment for ESC-derived cardiomyocytes can be accomplished through use of a cardiac-specific promoter for expression of a fluorescent protein, sorting for cell surface markers or sorting *via* Raman spectroscopy.

*In vivo* studies utilizing PSC-derived cardiomyocytes have been promising, with early rodent studies in acute and chronic infract models demonstrating improvement in ventricular contractile function. More recently, hESC-derived cardiomyocytes have been shown in a primate model of ischaemia-reperfusion injury to engraft into infarcted host tissue, 'remuscularize' the infarct region, and electromechanically couple to surrounding host cardiomyocytes. However, the presence of arrhythmias were reported in animals receiving cell therapy, highlighting the potential problem with the arrhythmogenicity of transplanted cell Whether these cells are inherently arrhythmogenic or serve as a nidus to induce arrhythmias is still not entirely clear. Future translation of this approach will require further understanding to eliminate the arrhythmogenicity inherent in transplanted cardiomyocytes before human clinical studies can be initiated.

In the previous section, we discussed cell-based therapy for treatment of cardiomyopathty, which has been the prevalent approach over the past two decades. However, the tide has turned, and focus is now shifting towards generating cardiomyocyte in vivo, and whether this can be used as an effective strategy for heart regeneration.

## 4) Direct Reprogramming:

Shortly after Yamanaka's report of reprogramming of somatic cells to iPSCs the ability of these cells to differentiate into functional cardiomyocytes was readily demonstrated. However, as mentioned earier, the utilization of iPSC-derived cardiomyocytes raised a number of concerns such as potential differentiation towards alternative cell fates and teratoma formation once introduced to the heart. Direct reprogramming of fibroblasts to cardiomyocytes bypassing the pluripotent



**Figure 15.** Direct programming of fibroblasts generates cardiomyocytes.

state and provides hope in bypassing these hurdles. The Olson group and others showed that the combination of several transcription factors, GATA4, Hand2, MEF2C and TBX5 (referred to as GHMT) was able to convert mouse fibroblasts into cardiomyocyte-like cells, termed induced cardiomyocytes (iCMs)[9]. iCMs exhibited a gene expression profile similar to native cardiomyocytes while the fibroblast gene program was silenced, and a small fraction was able to spontaneously contract in vitro. More recent studies demonstrated that direct viral injection into the myocardium resulted in in vivo reprogramming of cardiomyocytes and improved left ventricular systolic function following myocardial infarction. In addition, other studies identified a cocktail of small molecules that is capable of inducing direct reprogramming of fibroblasts into cardiomyocytes.
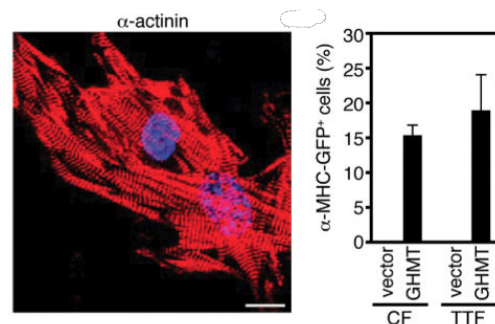
Consistent with the findings in mice, recent studies have demonstrated the conversion of human fibroblasts to cells with cardiomyocyte characteristics. Although human cells have been proven to be more challenging, numerous combinations of transcription factors and miRNAs (GATA4, HAND2, TBX5, myocardin and the miRNAs miR-1 and miR-133, GMT, together with Myocardin, ZFPM2 and TGF-β, and GMT in addition to Mesp1 and Myocd) have successfully produced human cardiomyocyte-like cells. Studies are now focused on larger animals models, as well a small molecules instead of viruses, which are required to explore the true therapeutic potential of this technique.

## 5) Regenerating the Heart from Within:

### Cardiomyocyte turnover in the human heart:

While is well established that the adult human heart is incapable of any meaningful regeneration following cardiomyocyte loss, recent evidence suggests that the human heart is not a "*terminally differentiated organ*", and that limited but measureable cardiomyocyte turnover does in-fact occur in the human heart. The landmark study on this topic came from the Frisen lab at the Karolinska Institute where they used an ingenious method to determine the age of cardiomyocytes in cadaveric adult human hearts [10]. They measured carbon-14 ($^{14}$C) that was generated from aboveground nuclear

**Figure 16.** $^{14}$C dating of cardiomyocyte age was used to demonstrate that slow but measurable cardiomyocyte turnover occurs in the human heart

bomb tests in genomic DNA of human cardiomyocytes, which allows retrospective birth dating. $^{14}$C concentrations in the atmosphere remained relatively stable until the Cold War, when aboveground nuclear bomb tests caused a sharp increase. After the Limited Nuclear Test Ban Treaty in 1963, the $^{14}$C concentrations dropped exponentially due to diffusion from the atmosphere (not due to radioactive decay). Because DNA is stable after a cell has undergone cell division, the concentration of $^{14}$C in DNA serves as a date mark for when a

cell was born. The authors used this method following purification of human cardiomyocyte DNA. They found that the annular turnover rate was relatively constant throughout life between 0.2 and 2% annually, with a very clear age-depending decline in the rate of cardiomyocyte turnover. Despite this surprising ability of the human heart to generate new cardiomyocytes, this endogenous capacity for cardiomyocyte turnover is not
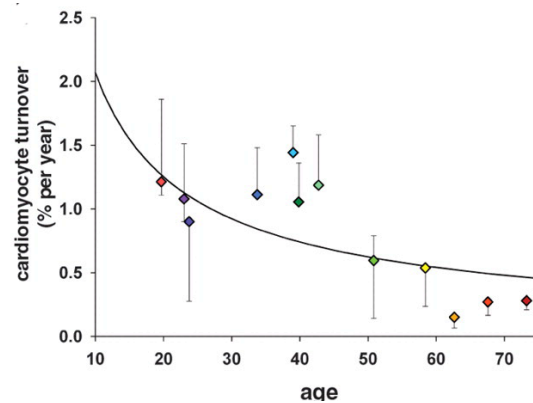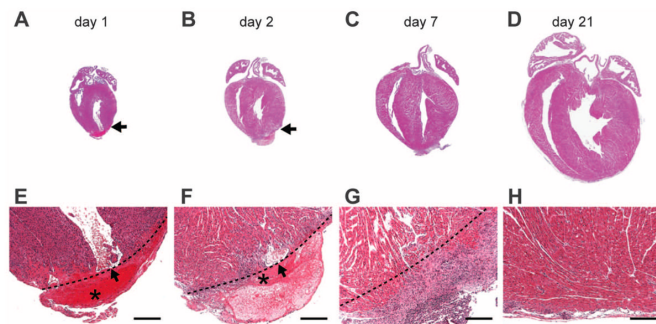
**Figure 17.** Apical resection in neonatal mice results in complete regeneration within 21 days

translated into any meaningful regenerative ability. In fact, there is progressive cardiomyocyte loss, rather than gain, in cardiomyopathy.

## Endogenous Heart Regeneration in Mammals:

In contrast to the adult heart, the neonatal mammalian heart is capable of substantial regeneration following injury through cardiomyocyte proliferation[11,12], not unlike urodele amphibians[13-16] or teleost fish[17-19]. Our group recently demonstrated that resection of the ventricular apex or induction of myocardial infarction in neonatal mice is followed by a



**Figure 18.** Myocardial infarction in neonatal mice results in complete regeneration within 21 days

robust regenerative response, which restores the lost myocardium within 21 days. Genetic fate mapping demonstrated that this regenerative response is mediated by proliferation of pre-existing cardiomyocytes rather than a stem or progenitor population. However, this regenerative capacity is lost by postnatal day 7[11,12], which coincides with cardiomyocyte binucleation and cell cycle arrest[20]. After this postnatal cell cycle arrest, cardiomyocyte growth occurs through hypertrophic increase in cardiomyocyte size[21-22].
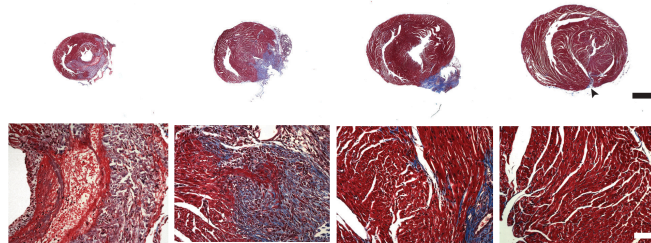
## Oxygenation, Metabolism and Myocardial Regeneration:

One of many factors shared by organisms that are capable of heart regeneration is the oxygenation state. For example, the zebrafish's stagnant and warm aquatic environment has $1/30^{th}$ oxygen capacitance compared to air, and is prone to poor oxygenation[23,24]. Moreover, the zebrafish circulatory system is relatively hypoxemic, as it has a primitive two-chambers heart, which results in mixing of arterial and venous blood. Similarly, the mammalian fetal circulation is shunt-dependent with significant



**Figure 19.** The transition from the hypoxic intrauterine environment to the hyperoxic postnatal environment induces oxidative DNA damage in cardiomyocytes

mixing of arterial and venous blood. Although blood in the umbilical vein going to the fetus is 80%-90% saturated with a $PaO_2$ of 32-35mmHg, the saturation of the blood ejected from the left ventricle is only 65% saturated with a $PaO_2$ of 25-28mmHg[25], which is quite hypoxemic compared to the postnatal circulation with a saturation above 95% and a $PaO_2$ of 100 mmHg. Therefore, both the zebrafish and mammalian fetal hearts reside in a relatively hypoxic environment, however the transition from embryonic- to postnatal-circulation soon after birth drastically changes the oxygenation state of cardiomyocytes.
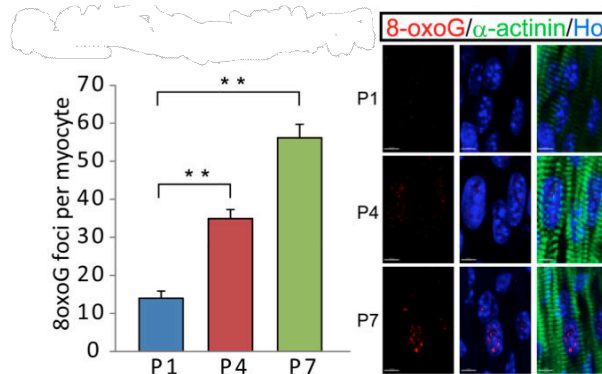
## Reactive Oxygen Species and DNA Damage Response (DDR) in the Heart:

The heart is the most energy demanding organ in the body, accounting for > 30% of total oxygen consumption at rest. It therefore not surprising that cardiomyocytes have the highest mitochondrial content and activity compared to any other cell type. Mitochondrial oxidative phosphorylation produces 18 times as much ATP as cytoplasmic glycolysis [26,27]. However, the energy advantage of mitochondrial oxidative phosphorylation over glycolysis is not without deleterious consequences, as



**Figure 20.** Scavenging mitochondrial ROS prolongs the postnatal window of cardiomyocyte proliferation.

the mitochondrion is considered the major source of free radical production [28-32]. Mitochondrial ROS are generated as a consequence of electron leak by the electron transport chain [33,34] and can cause various forms of DNA damage, such as oxidized base, single- or double-strand breaks, resulting in cell cycle arrest and cellular senescence [35-37]. Our group has recently demonstrated that the primary upstream mechanism that results in cell cycle arrest of the majority of postnatal cardiomyocytes is oxidative DNA damage. We showed that the metabolic switch that occurs in early postnatal cardiomyocytes, from glycolytic to mitochondrial oxidative metabolism induces an increase in mitochondrial-

Clinical Characteristics

| Patient Study# | Age (years) | Sex | Race | Current Vital Status | Etiology of CM | History of CABG? | Duration on LVAD (months) |
|---|---|---|---|---|---|---|---|
| 12 | 60 | F | W | Alive | Chemotherapy | N | 2 |
| 13 | 40 | F | W | Dead | Nonischemic | N | 13 |
| 16 | 38 | M | W | Alive | Neuromuscular | N | 1 |
| 18 | 60 | F | W | Alive | Familial | N | 7 |
| 20 | 60 | M | W | Dead | Ischemic | Y | 14 |
| 23 | 48 | M | W | Alive | Nonischemic | N | 25 |
| 37 | 71 | M | W | Alive | Ischemic | ? | 7 |
| 47 | 65 | M | W | Alive | Nonischemic | N | 6 |
| 85 | 30 | M | AA | Alive | Nonischemic | N | 14 |
| 109 | 66 | M | W | Alive | Ischemic | N | 7 |

**Figure 21.** Matched samples were collected from LVAD patients at the time of VAD implantation (core) and subsequently (whole heart) at the time of heart transplantation.

derived ROS production, which results in oxidative DNA damage and cell cycle arrest of cardiomyocytes through DNA damage response. Intriguingly, scavenging mitochondrial ROS, or inhibition of DDR markedly prolonged the postnatal regenerative window in mice.
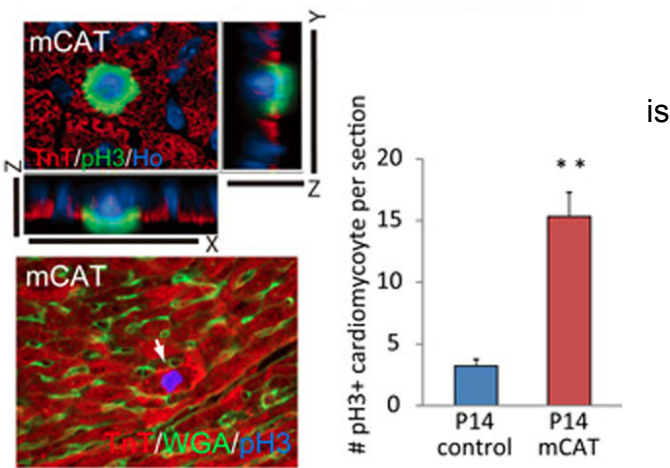
*In summary, loss of the endogenous regenerative properties of the mammalian heart is mediated by the highly oxidative nature of cardiomyocytes. Therefore, we hypothesized that mechanical unloading of the human heart decreases reliance on oxidative metabolism, and reactivates cardiomyocyte proliferation.*

## Human Ventricular Unloading and Myocardial Regeneration:



**Figure 22.** Human ventricular unloading decreases mitochondrial content, and cardiomyocyte size.

In patients with heart failure, persistent pressure or volume overload results in progression of the underlying cardiomyopathy. Although this cardiac remodeling can be slowed or sometimes reversed by intense pharmacological therapy, this process is often progressive. In advanced heart failure patients, left ventricular assist devices (LVADs) result in improved cardiac output, systemic perfusion, and end-organ function, which have led to an exponential increase in their implantation over the past decade. Intriguingly, myocardial recovery allowing for LVAD explantation has been reported in small subsets of patients[38], and is thought to result from functional recovery of viable myocardium due to a combination of ventricular unloading and pharmacological therapy.



**Figure 23.** Cardiomyocyte mitosis and cytokinesis in the unloaded human heart.

Given our recent findings outlining the role of DDR in regulation of arrest in postnatal cardiomyocytes, we reasoned that mechanical unloading might reverse the metabolic cascade that results in cell cycle arrest of cardiomyocytes. In that respect, human LVAD hearts provide the unique opportunity to perform histological analysis in the same patient in 2 drastically variable physiological states (core ventricular sample at the time of LVAD implantation, and whole heart at the time of heart transplantation). Therefore we conducted a study to test the effect of mechanical unloading on mitochondrial mass, DDR, and cardiomyocyte proliferation
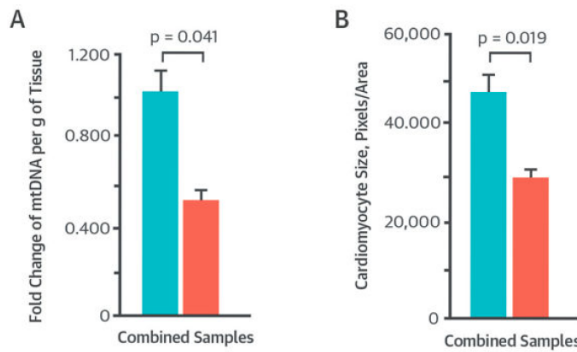


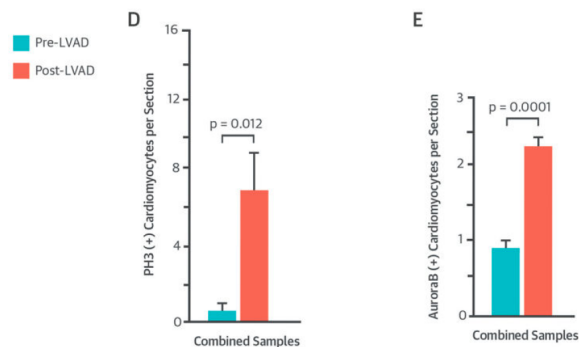**Figure 24.** Human ventricular unloading induces cardiomyocyte proliferation.

in patients who received LVADs. We found that prolonged mechanical unloading (for more than 6 months) resulted in cardiomyocyte atrophy, and marked reduction in mitochondrial content, with subsequent de-activation of DDR in cardiomyocytes. Intriguingly, we also found that prolonged mechanical unloading of the human heart resulted in a robust increase in markers of cardiomyocyte mitosis and cytokinesis[39]. These results indicate that loss of the endogenous regenerative capacity of the human heart may be the result of a tradeoff to achieve superior energy efficiency through oxidative phosphorylation.

**Future Studies:**

My group is interested in understanding the molecular mechanisms that mediate cell cycle arrest of cardiomyocytes in mammals, and therefore we are exploring these mechanisms as potential therapeutic targets for human heart regeneration. Given our recent results, we are encouraged that we may have identified a pathway to reactivate the endogenous regenerative potential of the human heart. Although mechanical unloading is a drastic measure reserved for terminal heart failure patients, our results indicate that it might be a therapeutic tool. It is important to note here that we are certainly not the first to propose that mechanical unloading can be used therapeutically in what it termed "Bridge to Recovery", however, we are the first to uncover its potential regenerative role, as well as the mechanism of this effect. Therefore, we plan to expand on these results further and examine the role of mechanical unloading on human heart regeneration.

The Center for Regenerative Science and Medicine (CSRM) at UT Southwestern is funding clinical trials aimed at determining the role of mechanical unloading on myocardial viability and bioenergetics. The first of these trials "**Role of Left Ventricular Assist Devices in Myocardial Regeneration**" is a



**Role of Left Ventricular Assist Devices in Myocardial Regeneration**

**Principal Investigator:** Hesham Sadek MD, PhD
**Co-PIs:** Pradeep Mammen MD and Mark Drazner, MD
**Co-Investigators:** Vlad Zaha MD, PhD and Jainy Savla, MD
**Funding Sponsor:** Hamon Center for Regenerative Science and Medicine

**Patient selection:**

**Inclusion criteria:**

1. Patients with dilated or ischemic cardiomyopathy
2. Age 18-70
3. LVEF $\leq$ 40% at the time of LVAD implant
4. Patient who underwent LVAD implantation within the past 30 days

**Exclusion criteria:**

1. Hypertrophic cardiomyopathy
2. Radiation-induced cardiomyopathy
3. End stage renal failure
4. Documented cirrhosis
5. Patients requiring RVAD support
6. Patients with post VAD complications of pump failure or VAD thrombosis requiring exchange, Or right heart failure requiring chronic inotropes
7. Pregnancy

**Intervention:**

Serial FDG-PET viability scans will be performed within 1 month of VAD implantation and at 6 months intervals. Studies will be concluded after performance of 4 serial viability studies, or at the time of heart transplantation, whichever comes first.

single center randomized clinical trial where serial FDG-PET scans will be performed to assess the changes in myocardial viability following mechanical unloading. In addition to myocardial viability, we aim to perform additional studies to determine the effect of mechanical unloading on cardiac mitochondrial function in vivo using $^{11}$C acetate.

**Conclusions:**

The past two decades have witnessed a revolution in regenerative medicine in general, and particularly in the field of cardiac regeneration. Although the initial focus was on cell therapy utilizing what was believed to be adult stem cells, recent reports suggest that these cells are not retained in the myocardium, do not differentiate into cardiomyocytes, and have minimal effects on functional recovery. Nevertheless, there are still some efforts focused on understanding the potential beneficial "paracrine" effects of these cells on the injured myocardium.

As the cardiac regeneration field moves away from adult cell therapy, several alternative approaches which stronger basic science roots have emerged. These include the use of cardiomyoyctes derived from pluripotent stem cells, direct reprogramming of fibroblasts into cardiomyocytes, and reactivation of cardiomyocyte proliferation in vivo.

The field of heart regeneration research has been fraught with controversies and ethically questionable practices, which have markedly slowed its progress and cast doubt on the validity of this approach for treatment of heart failure. However, the tides are changing, and the new frontiers that are emerging provide a great deal of hope that this seemingly far-fetched goal of curing heart failure may in fact be within our reach.

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