

Current Status and Perspectives in Stem Cell Therapy for Heart

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For most patients, the prognosis of heart failure remains poor despite therapeutic advancement in recent decades. The option of cardiac transplantation is high risk and limited by a shortage of donors. Traditionally, the heart had been considered a terminally differentiated organ incapable of regeneration. However, numerous preclinical and clinical studies have been performed since the first report of cell therapy in heart failure using skeletal myoblasts in 2001. These investigations looked at the promising potential and use of several kinds of stem cells, which could some day dramatically alter the understanding of the regenerative capacity of the heart.

To date, although there is no existing cardiac cell therapy that has been conclusively reported to be effective, stem cell-related cardiomyocyte regeneration strategies have become significant areas of research in modern cardiovascular medicine. In this review, we outline a variety of common cell sources, surface biomarkers of stem cells, and provide information related to cardiac cell therapy clinical trials.

Key Words: Clinical trial • Stem cell

INTRODUCTION

The prognosis of patients with heart failure (HF) remains poor, with a 5-year mortality rate of 50%, despite significant therapeutic advancement in recent decades.¹

The traditional concept that the mammalian heart is a terminally differentiated organ, with the number of cardiomyocytes remaining constant throughout life, has been challenged in the past two decades.

One study using ¹⁴C birth dating has suggested that about half of the heart's cardiomyocytes are replaced, and the other half lives throughout a person's lifetime with an annual cell renewal turnover rate of 1% at 25

years and 0.45% at 75 years of age.²

The capacity for cardiomyocyte regeneration associated with the age of the mammalian heart has been documented,³ and the capacity for continuous turnover of human cardiomyocytes has also been reported.⁴ Cardiomyocytes and coronary vessels with Y-chromosome have been noted in female donor hearts transplanted to male recipients, showing the possibility of recipient cells colonized in the donor heart after cardiac transplantation.⁵ However, the rate of myocyte loss was estimated to be 0.006% per day, which accounts for a 2.2% annual loss. However, the rate of myocyte apoptosis increases with age, and apparently cannot be compensated for by myocyte renewal - especially with HF.^{4,6}

Researchers have noted the remarkable ability of the human heart related to myocytes growth reserve, especially in acute myocardial infarction.⁷ In the last 10 years, notable progress has been made using several kinds of stem cells, involving variable approaches to enhance myocyte regeneration in ischemic heart disease.⁸

Despite the fact that no cardiac cell therapy has been conclusively proven effective since the first use of

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skeletal myoblasts in HF in 2001,⁹ the ability of various types of stem cells populations to improve cardiac function and reduce infarct size, either in ischemia induced HF or non-ischemic cardiomyopathy, has been reported in many preclinical and clinical studies.

The purpose of this review is to provide information to clinical cardiologists of the rapid progress in cardiac cell therapy clinical trials, and briefly mention the cell surface biomarkers used to identify various stem cell

types as applied to cardiac cell therapies.

Sources of stem cells for cardiac cell therapy (Table 1)

1. Embryonic stem cells

Embryonic stem cells (ESCs) derived from blastocysts are pluripotent, and can differentiate into all cell types including cardiomyocytes.¹⁰ However, the possibility of inducing an immune reaction and teratoma forma-

Table 1. Variable sources for cardiac cell therapy

Types of stem cells	Method	Capacity to differentiate into cells	Limitation/problem	Specific markers
Embryonic stem cells (ESCs)	Inner cell mass (blastocyst) -Enzymatic dissection	pluripotency (+) 3 germ layers: Ectoderm, Endoderm Mesoderm including cardiomyocytes	Tumorigenesis (+) Immune rejection (+) Ethical problem (+) Clinical trial (-)	Nkx 2.5 Gata 4 Mef 2C
Induced pluripotent stem cells (iPSCs)	Direct reprogramming of fibroblast/stromal cells to pluripotent status	pluripotency(+)	Tumorigenesis (+) Genetic instability (+) Low efficiency Clinical trial (-) Clinical trial (+)	Oct 4 Sox-2 c-Myc Klf 4
Bone Marrow-derived stem cells 1. BMMNCs 2. MSCs 3. HSCs 4. NHSCs 5. EPCs 6. VSELs	Bone marrow aspiration/biopsy -Surface markers -Side population	Multipotency (+): Adipocyte (+) Osteoblast (+) Skeletal muscle (+) Cardiomyocyte (±) Endothelial cells (±)		Table 2 for details
Cardiac stem cells (CSCs) 1. SPCs 2. Cardiospheres 3. Epicardial progenitor cells	Myocardial tissue/biopsy -Surface markers -Side population	Multipotency (+): Cardiomyocytes (+) Smooth muscle cell (+) Endothelial cells (+)	Clinical trial (+)	Table 2 for details
Skeletal myoblasts	Muscle biopsy-satellite cells under basal membrane of myofibers	Myotubes (+) Skeletal muscle-like graft (+) Extracellular matrix remodeling Paracrine effect Cardiomyocyte (-) Endothelial cells (-)	Ventricular arrhythmias Lack of gap-junction protein such as N-cadherin and connexin-43; lack of electro-mechanical coupling Clinical trial (+)	Table 2 for details
Adipose-derived MSCs	Surface markers; Adipose tissue	Multipotency (+): Adipocytes (+) Cardiomyocytes (±) Endothelial cells (±) Mesenchymal lineage cells (+)	Clinical trial (+)	Table 2 for details

BMMNCs, bone marrow mononuclear cells; EPCs, endothelial progenitor cells; HSCs, hematopoietic stem cells; MSCs, mesenchymal stem cells; NHSCs, non-hematopoietic stem cells; SPCs, cardiac side population cells; VSELs, very small embryonic-like cells.

tion, as well as legal issues and ethical concerns have limited the use of ESCs in human trials to date.

2. Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) derived from somatic fibroblasts can be reprogrammed to an embryonic-like state by overexpression of a defined set of transcription factors (Oct4, Sox2, c-Myc, Klf4, Nanog, and Lin2).^{11,12}

Direct reprogramming of infarcted area cardiac fibroblasts into cardiomyocyte-like cells by introducing Gata4, Mef2c, Tbx5, and/or Hand2, using viral vectors has been shown possible with a mouse model.^{13,14} Cardiac fibroblasts can also be directly reprogrammed into cardiomyocyte-like cells through miRNAs regulating posttranscriptional gene expression by base-pairing to be partially complementary to a sequential target mRNA.^{15,16} However, the use of oncogenic reprogramming factor c-Myc related tumor formation,¹⁷ which to date involves a low efficiency mechanism of direct reprogramming,¹⁸ random integration of viral vectors, and possible gene instability all remain major concerns which hinder progress involving clinical trials of cardiac cell therapy using iPSC-derived cells.

3. Bone marrow derived stem cells

Bone marrow contains unfractionated bone-marrow stem cells, bone marrow-derived mononuclear cells (BMMNCs), mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), non-hematopoietic stem cells, endothelial progenitor cells (EPCs) and very small embryonic-like cells (VSELs). Bone-marrow derived cells were reported to have the capacity to differentiate into chondrocytes, adipocytes, endothelial cells and cardiomyocytes.^{19,20} The randomized TIME (Timing In Myocardial infarction Evaluation) trial, and the Late-TIME trial both showed significant left ventricular function improvement.^{21,22}

The FOCUS-CCTRN (First Mononuclear Cells injected in the United States conducted by the CCTRN) trial, where autologous bone-marrow first mononuclear cells were injected into patients with chronic heart failure and conducted by the Cardiovascular Cell Therapy Research Network in the United States²³ showed no significant improvement in left ventricular (LV) ejection fraction (EF), nor improvement of LV end-systolic volume,

maximal oxygen consumption, and reversibility on single-photon emission tomography (SPECT). However, the report did show that it is feasible and safe to trans-endothoracically inject autologous bone marrow-derived cells into the heart. The report also suggested that higher CD34⁺ and CD133⁺ cell counts are associated with a higher increase in LVEF (ejection fraction of left ventricle). Cells expressing CD34 or CD133 and vascular endothelial growth factor receptor 2 (VEGFR2), namely CD34⁺/VEGFR2⁺ or CD133⁺/VEGFR2⁺ cells, are likely EPCs which can be isolated from BMMNCs, and have the potential to differentiate into endothelial cells as reported by Assmus et al. and as observed in the TOPCARE-AMI trial.²⁴ However, the results of preclinical studies using bone-marrow derived cells have been shown to be both multifactorial and controversial.²⁵

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are an abundant source of stem cells, and can differentiate into adipocytes, chondrocytes, and cells of mesodermal lineage. Although earlier studies did report some controversy, there was some evidence that suggested possible cell differentiation into cardiomyocytes.²⁶⁻²⁸

Because MSCs are relatively hypoimmunogenic, the allogeneic transplantation of stem cells into patients with ischemic cardiomyopathy in the POSEIDON randomized pilot study (Comparison of allogeneic versus autologous bone marrow-derived MSC therapy) reported both allogeneic and autologous MSCs were safe, although computed tomography (CT) did not show LVEF improvement.²⁹

VSELs (very small embryonic-like cells) are found in the bone marrow and peripheral blood in both mouse and human.³⁰ VSELs can differentiate into the cells of 3 germ layers (endoderm, mesoderm and ectoderm), including cardiomyocytes and vascular endothelial cells in vitro. VSELs expressing embryonic stem cell markers such as Oct-4, Nanog and SSEA-1, can be isolated by fluorescence-activated cell sorting and the expression of Sca-1 (in mice), CXCR4 and CD133 (in humans) on VSELs also express early markers of cardiac and endothelial lineages (Gata4, Nkx2.5, VE-cadherin, and the von Willebrand factor).^{31,32} The report of using intramyocardial injection of VSELs into an infarcted murine heart showed an improvement of LVEF and a decrease of myocardial hypertrophy, suggesting that it was possibly due to the paracrine effect.³⁰ BM-MSCs are also known as bone

marrow stromal cells, which are subsets of multipotent non-hematopoietic cells, capable of differentiation into chondrocytes, adipocytes, osteoblasts, skeletal muscle cells, and possibly endothelial cells and cardiomyocytes.³¹⁻³³

Furthermore, MSCs are positive expression of CD73, CD90, CD105 and STRO-1, and the negative expression of CD14/CD11b, CD19, CD34, CD45, CD79 α , and HLA class II; all have the potentials of differentiation into mesenchymal lineages including osteocytes, adipocytes, and chondrocytes.³⁴ The beneficial effects for MSCs in cell therapies depend on their potency of secretion of growth factors and cytokines for tissue repair, regeneration, immunomodulation, and the potential differentiation capacities for damaged organs including the heart.³⁵

Aldehyde dehydrogenase - bright cells are subpopulations of hematopoietic cells, from human bone marrow and peripheral blood. The positive expression of CD34, CD105, CD117, CD133, and CD166, including CD34/CD38 - primitive cells (Table 2), in small size clinical trials using these cells in ischemic and non-ischemic heart failure patients showed a decreased of LV end-systolic volume.^{36,37} Larger studies are necessary to elucidate the role of MSCs in cardiac cell therapy.³⁸

4. Cardiac stem cells (Table 3)

The origins of adult cardiac progenitor cells (CPCs) remain controversial. However, evidence has shown that CPCs provide stem cells with cardiomyogenic potential that can home in on the injured heart. This was in part proven through sex-mismatched cardiac transplantation in which the female donor heart contained vascular cells

and cardiomyocytes with a Y-chromosome apparently from the male recipient.⁵ Although CPCs were reported to have the expression of markers commonly used to identify hematopoietic stem cells from bone marrow, such as c-kit and Sca-1,³⁹ CPCs remain controversial and continue to be the subject of isolation and potential cardiac cell therapy application.

In rodents, side population (SP) cells contain vascular endothelial cells, smooth muscle cells, and mesenchymal progenitor cells including cardiomyogenic precursors.⁴⁰ However, no clinical trial has been reported using side population cells to date.

C-kit⁺ CPCs

The stem cell infusion in patients with ischemic cardiomyopathy (SCIOPIO) clinical trial in post myocardial infarction patients receiving coronary bypass graft (CABG) surgery, using c-kit⁺ hCPCs harvested during CABG, was thereafter expanded and intracoronarily infused back to patients 4 months post CABG, this was reported to significantly increase LVEF, and decrease infarct size without hCPC-related advance effect.⁴¹

Cardiosphere/Cardiosphere-derived stem cells

In the prospective, randomized cardiosphere-derived autologous stem cells to reverse ventricular function (CADUCEUS) trial,⁴² there were reported cardiospheres (CSs) harvested and expanded from the endomyocardial biopsies of patients with 2 to 4 weeks post myocardial infarction and low LVEF. Additionally, there were no complications noted after intracoronary infu-

Table 2. Cell surface biomarkers of stem cells for cardiac cell therapy

Bone-marrow mesenchymal stem cell	CD44 ⁺ , CD73 ⁺ , CD90 ⁺ , CD105 ⁺ , CD106 ⁺ , CD166 ⁺ , Stro-1 ⁺ , CD11b ⁻ , CD14 ⁻ , CD19 ⁻ , CD34 ⁻ , CD45 ⁻
Bone-marrow side population cells	CD34 ⁺ , CD43 ⁺ , CD45 ⁺ , c-Kit ⁺ , Sca-1 ⁺
Hematopoietic stem cells	CD31 ⁺ , CD34 ⁺ , CD45 ⁺ , CD133 ⁺ , CD14/11b ⁺
Bone marrow and blood-derived endothelial progenitor cells	CD14 ⁺ , CD34 ⁺ , CD133 ⁺ , Fek-1 ⁺
Cardiac side population cells	CD34 ⁺ , CD45 ⁺ , c-Kit ⁺ , Sca-1 ⁺ , CD31 ⁻
Cardiospheres = c-Kit ⁺ /Lin ⁻ /Sca-1 ⁺ /CD31 ⁺ cells	CD31 ⁺ , CD34 ⁺ , CD90 ⁺ , CD105 ⁺ , CD133 ⁺ , c-Kit ⁺ , Sca-1 ⁺
Aldehyde-dehydrogenase-bright cells (from bone marrow peripheral blood)	CD34 ⁺ , CD105 ⁺ , CD117 ⁺ , CD133 ⁺ , CD166 ⁺ , CD34 ⁺ , CD38 ⁻
Skeletal myoblasts	Myo D, Myf 5, PAX 7 ⁺
Adipose-derived stem mesenchymal stem cells	CD13 ⁺ , CD29 ⁺ , CD44 ⁺ , CD49D ⁺ , CD49e ⁺ , CD51 ⁺ , CD73 ⁺ , CD90 ⁺ , CD105 ⁺ , CD166 ⁺ , HLA-ABC ⁺ ; CD3 ⁻ , CD4 ⁻ , CD11c ⁻ , CD14 ⁻ , CD15 ⁻ , CD16 ⁻ , CD19 ⁻ , CD31 ⁻ , CD33 ⁻ , CD34 ⁻ , CD38 ⁻ , CD45 ⁻ , CD56 ⁻ , CD62p ⁻ , CD104 ⁻ , CD106 ⁻ , CD133 ⁻ , CD144 ⁻

Table 3. Cardiac cell therapy clinical trials (bone marrow stem cells and cardiac stem cells)

Trial	Patient condition	Cell types	Delivery route/method	End-point evaluation method	Follow-up duration	Results	Adverse effect
TIME	ST-elevation AMI 3 vs. 7 days post primary PCI	BMMNCs	IC	Echo	6 mo	LVEF↔ Regional wall motion↔ (the timing has no significant effect)	No major adverse event
FOCUS-CCTRN	Heart failure	BMMNCs	IM	Echo SPECT	6 mo	LVEF↔ Angina score↓ Perfusion↑	No major complication
TOPCARE-CHD	Chronic ischemic cardiomyopathy	BMMNCs	IC	Echo SPECT MRI	3 mo	LVEF↑ NYHA class↓	Ventricular arrhythmias, 5 deaths in circulating
ABCD	Non-ischemic dilated cardiomyopathy	BMMSCs	IC	Echo	3 mo	LVEF↑ LVESV↓ NYHA class↓	No major complication
SCIPIO	Ischemic cardiomyopathy 4 months post CABG	CSCs	IC	Echo MRI	4 mo and 12 mo, to 24 mo	LVEF↑ Infarct size↓ NYHA class↓	No major complication
CADUCEUS	1.5-3 mo after AMI	CDCs	IC	MRI	6 mo and 12 mo	LVEF↔ LV volume↔ Regional function↑ Scan mass↓	Serious adverse event in 4 of 17 treated patients

AMI, acute myocardial infarction; BMMNCs, bone marrow mononuclear cells; CABG, coronary bypass graft surgery; CDCs, cardiosphere-derived cells; CSCs, cardiac stem cells; Echo, echocardiography; IC, intracoronary injection; IM, intramyocardial injection; LV, left ventricular; EF, ejection fraction; MRI, magnetic resonance imaging; PCI, percutaneous coronary intervention; SPECT, single photon emission computed tomography.

sion of 12.5-25 million cardiosphere-derived stem cells (CDCs) 4 to 5 weeks later. There was a significant reduction of scar mass, an increase of viable heart mass, and regional contractility and regional systolic wall thickening in the 17 treated patients as compared with 8 control patients. However, no change in LVEF, LV end-diastolic and end-systolic volume was noted. CSCs/CDCs from mice and humans are clonogenic, heterogeneous, express c-kit, Sca-1, CD31, and CD34, and can differentiate into cardiomyocytes and vascular cells.⁴²

Epicardium-derived CPCs

These progenitors are marked by transcription factors Wilms tumor 1 (Wt 1) and T-box,⁴³ and can differentiate into cardiomyocytes, smooth muscle, and endothelial cells. They also express Nkx 2.5 and Isl-1, possibly similar with multipotent Nkx 2.5 and Isl-1⁺ progenitor cells⁴⁴ as reported in a mouse model. Nevertheless,

whether similar findings can be demonstrated in humans remains to be studied.

5. Skeletal myoblasts (Table 1, Table 2 and Table 4)

Several small non-randomized clinical trials using skeletal myoblasts as the source for cardiac cell therapy have shown positive results in improving LV function. However, due to the high risk of developing ventricular arrhythmias and the negative results reported in the large randomized, placebo-controlled, double blind MAG2C trial, the skeletal myoblast does not appear to be an ideal source of cardiac cell therapy.⁴⁵⁻⁴⁹

Adipose-derived mesenchymal stem cells (Table 5)

Adipose-derived regenerative cells (ADRCs) are multipotent, able to replicate as undifferentiated cells, capable of differentiation into mature adipocytes and other mesenchymal lineage cell types.

Table 4. Cardiac cell therapy clinical trials (skeletal myoblast)

	Patient condition	Study design/ numbers of patient (n)	Delivery route/ method	End-point evaluation method	Follow-up duration	Results	Adverse effect
Herreros et al.	Non-acute MI	Non-randomized uncontrolled study n = 12	IM during CABG	Echo PET	12 mo	LVEF↑ Regional contractility↑ Myocardial viability and perfusion↑ NYHA class↓	No major complication
Ince et al.	MI	Non- randomized, case-controlled study n = 6; controls = 6	Transendocardia I IM	Echo	12 mo	LVEF↑ Walking distance↑ NYHA class↓	Early non- sustained ventricular arrhythmias (vent. arrhyth. in two patients)
Dib et al.	MI	Non-randomized uncontrolled study n = 30	IM during CABG ²⁴ and LV assist-devices (6)	Echo PET	12 mo	LVEF↑ Tissue viability↑ LVEDV and ESV↓ NYHA class↓	CABG: ventricular arrhythmias (4/24), 1 death, 1 MI LVAD: vent. arrhyth. (2/6) 3 deaths
CAuSMIC by Dib et al.	MI	Randomized, placebo- controlled double-blind study n = 12; controls = 11	Transendocardia I IM	Echo	12 mo	LVEF↑ Regional wall motion↑ viability↑ LV dimension↓ NYHA class↓	Vent arrhyth in 6/12 patients
POZNAN by siminiak et al.	HF	Non-randomized uncontrolled study n = 10	Percutaneous transcoronary venous	Echo	6 mo	LVEF↑ (3-8%) NYHA class↓	No major complication
MAGIC by Menasché et al.	MI	Randomized, placebo- controlled double-blind study n = 97; controls = 30	IM during CABG High dose Low dose	Echo	6 mo	LVEF↔ Regional wall motion↔ LVEDV and ESV↓	Vent arrhyth in 9 (5 high dose, 4 low dose) 9 deaths (4 high dose, 5 low dose)
SEISMIC by Duckers et al.	HF	Prospective, randomized, open label study n = 26; controls = 14	Transendocardia I IM	MUGA	6 mo	LVEF↔ 6 minutes walk distance↓ NYHA class↓	Vent arrhyth in 12/26 patients, 1 death

CABG, coronary bypass surgery; Echo, echocardiography; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HF, heart failure; IC, intracoronary; IM, Intramyocardial; LV, left ventricular; MI, myocardial infarction; MUGA, multi-gated acquisition scan; NYHA, New York Heart Association; PET, positron emission tomography.

Table 5. Cardiac cell therapy clinical trials (bone marrow mesenchymal/stromal cells, MSCs; adipose-derived mesenchymal stem cells, ADMSC)

Trial	Patient condition	Cell types	Delivery route	End-point evaluation method	Follow-up duration	Results	Adverse effect
POSEIDON	Ischemic cardiomyopathy (ISCM)	MSC	IM	Computed tomography	12 mo	LVEF↔ Physical performance↑ LVEDV↓	Hospitalization of heart failure in one patient
PROMETHEUS	Ischemic cardiomyopathy (ISCM)	MSC	IM		NCT 00587990	Active not recruiting	NCT00587990
Perin et al.	ISCM	MSC CD34+/ CD133+	Transendocardial IM	Echo SPECT	6 mo	LVEDV↓ Maximal oxygen consumption↓	No major complication
Vrtorec et al.	Non-ischemic dilated cardiomyopathy (DCM)	MSC CD34+	IC	Echo	5 years	LVEF↑ 6 minutes walk distance↑	27 of the 110 studied (55 treated 55 control) patients cardiac deaths 7 heart transplant
APOLLO phase I/IIa NCT00442800	Acute ST-elevation MI (AMI) within 24 hours after PCI	ADRCs (adipose-derived regeneration cells) freshly isolated by liposuction	IC	Echo SPECT	6 mo	Global LVEF↑ Percentage of infarcted area↓	2 in 14 treated patients with significant bleeding events: excluding use of glycoprotein IIb/a inhibitor 1 in 14: target lesion revascularization
PRECISE NCT00426868	Ischemic cardiomyopathy (ISCM)	ADRCs (adipose-derived regeneration cells) freshly isolated by liposuction	IC	Echo SPECT MRI	36 mo	LVEF↔ LV mass↑ Wall motion score index↑ Maximal oxygen consumption↓	No major adverse events
Mystromal cell phase II NCT01449032	Chronic myocardial ischemia (ISCM) + refractory angina	VEGF-A stimulated ADRCs	IC	CT MRI PET	3 mo 6 mo 1,2,3 year	Angiogenic factors do not change with patient age Proangiogenic factor secretion decline from aged patients	No major adverse events
SWISS-AMI phase II NCT00355186	5-7 days vs. 3-4 weeks post AMI after successful PCI	BMMNCs	IC	MRI	4 mo	No study results posted (2006-2012)	No study results posted
ALCADIA Phase I NCT00981006	Ischemic cardiomyopathy (ISCM)	Autologous human cardiac-derived stem cells (CDCs) harvested during CABG	IM + bFGF(basic fibroblast growth factor) Hybrid therapy	Safety and efficacy (gelatin-hydrogel sheet)	12 mo	No study results posted (2009-2013)	No study results posted

BMMNCs, bone marrow mononuclear cells; Echo, echocardiology; EDV, end-diastolic volume; EF, ejection fraction; IC, Intracoronary; IM, intramyocardial injection; LV, left ventricular; MRI, magnetic resonance imaging; PCI, percutaneous coronary intervention; SPECT, single-photon emission computed tomography.

Clinical trials using a liposuction procedure obtaining freshly isolated ADRCs in patients with acute ST-elevation myocardial infarction reported an improvement in global LVEF, and a reduced infarcted percentage at 6 months follow-up in the APOLLO trial.⁵⁰ Transendocardial injection of isolated ADRCs from adipose tissue harvested by liposuction in “no option” patients with ischemic cardiomyopathy also was reported to be safe and feasible, and may preserve ventricular function, myocardial perfusion and exercise capacities in patients in the PRECISE trial.⁵¹ The MyStromalCell Trial is a prospective, randomized, double-blind placebo-controlled study using culture-expanded adipose-derived mesenchymal stromal cells (ADMSCs) for chronic myocardial ischemia. The ADMSCs were cells obtained from abdominal adipose tissue and stimulated with VEGF-A the week before treatment.⁵²

Adipose tissue is of mesodermal origin, the stromal cells interposed between mature adipocytes composed of endothelial cells, smooth muscle cells, fibroblasts, leukocytes, macrophages, and preadipocytes.⁵³ On the ADRC surface are positive expression of CD13, CD29, CD44, CD49e, CD51, CD73, CD90, CD105, CD166 and HLA-ABC markers; low level expression of CD9, CD34, CD49d and CD105; the initial low expression of CD13, CD29, CD44, CD73, CD90, and CD166, significantly increases successively and with stability after passage of P2.^{54,55} As for the expressions of CD34 and CD106, the

results are contradictory.⁵⁴

Potential mechanisms of cardiac cell therapy (Table 6)

Paracrine effects

Myocardial repair induced by transplanted cells promotes variable restorative processes such as activation of neovascularization, inhibition of myocytes apoptosis, inhibition of cell hypertrophy, activation of endogenous cardiac stem cells differentiation and proliferation, and creates favorable survival surrounding conditions through release of cytokines, chemokines, various types of growth factors, and microparticles or exosomes causing alteration of the extracellular matrix. These actions can improve LV contractile function, increase myocardial perfusion and enhance myocardial repair both in the infarcted and non-infarcted regions.⁵⁶ Various types of growth factors including hepatocyte growth factor and insulin growth factor-1 were reportedly able to stimulate cardiac stem cells to migrate, proliferate and differentiate into vascular structures and cardiomyocytes.^{56,57}

Stem cells induce neovascularization through the action of secreting chemokines such as stromal cell-derived factor-1, and proangiogenic factors such as vascular endothelial growth factors (VEGF), fibroblast growth factor, hepatocyte growth factor (HGF), tissue growth factor- β , and angiotensin-1.^{58,59} By improving coronary perfusion through the action of secreting endothe-

Table 6.

Possible mechanisms of cardiac cell therapy	Continuing controversies in cardiac therapy
<ol style="list-style-type: none"> 1. Further differentiation of transplanted cells into cardiac cells after injection in infarcted/non-infarcted myocardium. 2. Fusion of injected progenitor cells with host cardiomyocytes. 3. Secretion of growth factors and cytokines by the engrafted stem cells: the paracrine effect; through its neovascularizational and antiapoptotic effects; known growth factors such as: VEGF (vascular endothelial growth factor) HGF (hepatocyte growth factor) ILGF (insulin-like growth factor) HIF (hypoxia inducible factor) known of cytokines such as: TGF-β (transforming growth factor beta) Angiopoietin-1 4. Stimulate endogenous cardiac progenitor cell, expressing receptors for growth factors (as above), migrating into sites of injury, proliferate and differentiate into tri-lineage cells (cardiomyocytes, smooth muscle, endothelial cells). 	<ol style="list-style-type: none"> 1. Which types of cells are the best candidates? 2. Does a combination of more than two types of cells have an additive or synergistic effect? For example: Bone marrow derived mesenchymal stem cells combined with human c-kit + cardiac progenitor cells are synergistic to either type alone (proven in swine). 3. Direct reprogramming of fibroblast or stromal cells into cardiomyocytes using TFs (Gata4, Han2, Mef2c, Tbx5). 4. Biomaterials to improve engraftment and differentiation. 5. Pre treatment of cells with survival factors, pre hypoxia, and gel-suspension to prolong local stem cell retention and survival.

lial synthase, and by using inducible isoforms of nitric oxide synthase to promote endothelial cells proliferation there can be improved cardiac function in patients with ischemic cardiomyopathy.⁶⁰

Transdifferentiation and cell fusion

There are reports which note that transplanted skeletal myoblasts can differentiate into skeletal muscle fibers yet do not express cardiac-specific genes.⁶¹ Fusion of bone marrow cells with resident cardiomyocytes were suggested to be the responsible mechanism instead of highly controversial reports of bone marrow cells that can transdifferentiate into cardiac myocytes.⁶² Controversy also remained concerning peripheral blood CD34⁺ cells transdifferentiating into cardiomyocyte and vascular smooth muscle,^{61,62} as well as in bone marrow mesenchymal stem cells.⁶²

Mesenchymal stem cells, adipose derived cells, CD34⁺ cells, and cardiac stem cells transplanted to ischemic but viable myocardium have been reportedly able to differentiate into new blood vessels.⁶³ C-kit⁺ bone marrow cells differentiating into coronary arteries and myocytes were also reported to be independent of cell fusion.⁶⁴ However, the concept of cell fusion as an important mechanisms responsible for the beneficial effects of cardiac cell therapy has waned in recent years.

Growth factors released by transplanted stem cells can inhibit cardiomyocyte apoptosis, leading to improved cardiac function.^{56,64} Studies involving the reduction of survival myocyte hypertrophic response, through the action of matrix collagens, and reduced peri-infarct region fibrosis by the transplanted stem cells in post myocardial infarction models have been reported.^{64,65}

Cardiac stem cell self-renewal

The recent recognition of the potential capacity of adult human cardiac cells to possess the ability of self renewal, with resident cardiac stem cells having the ability to differentiate into endothelial cells, smooth muscle cells and cardiomyocytes has generated a dramatic shift in the history and future of cardiac biology.^{66,67}

Continued controversies in cardiac cell therapy (Table 6)

There currently remains substantial questions as to several important questions revolving around the evolu-

ing field of stem cell therapy, including: 1) what is the best stem cell type to select, 2) whether the combination of two or more types of stem cells can have a synergistic effect, 3) what would be the optimum route and best timing for administration, and with what frequency, 4) how to optimize cell preparation and what cell numbers are proper for most effective dosage, and 5) how to improve stem cell survival and engraftment.⁶⁷⁻⁶⁹

CONCLUSIONS

Although it might appear that more questions remain than answers after 15 years of intensive research into the field, we are still in the early stages of regenerative medicine investigation. What we know with some certainty is that current cardiac cell therapy appears safe, and there have not been any reports of serious adverse effect of stem/progenitor cells applications.

The precise mechanisms of action, the exact pathophysiology in facilitating cardiac regeneration, and many other practical issues related to cardiac cell therapy have not been clearly resolved. However, it is important to realize that an abundance of tremendously valuable work in cell therapy-related research has been ongoing for the past two decades, both in experimental animal models and human clinical trials.

Despite recently reported disappointing clinical trials involving the use of autologous bone marrow stem cells for cases of heart failure,⁶⁷⁻⁶⁹ we recognize that more careful consideration is needed with a rational investigation on a larger scale, with an appropriately designed confirmatory basic study to translational clinical researches/trials. Hopefully, this can be used to advance cell-based therapies to become a potent future reality in the clinical treatment of patients with heart failure.

CONFLICTS OF INTEREST

None declared.

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SUPPLEMENT

1. More cardiac cell therapy clinical trials are registered at clinicaltrials.org and can be freely viewed. For example: PROMETHEUS (phase I/II, MSC, NCT0058 7990), TAC-HFT (phase I/II, MSC, NCT00768066), REVASCOR (phase II, MSC, NCT00721045), NOGADCM (phase II, BM-HSCs-CD34⁺ cells, NCT01350310), CHART-1 (phase III, BM-derived mesenchymal cardiopoietic cells-C3BS-CQR-1, NCT01768702), and ALLSTAR (phase I/II, CDCs, NCT01458405), etc.
2. Transcription factor (TF) is a protein also called a sequence-specific DNA-binding factor, containing one or more DNA-binding domains to specific DNA sequences, thereby controlling and regulating the transcription of genetic information from DNA to messenger RNA. TFs perform their function alone or with other proteins in a complex, by promoting or blocking the recruitment of the enzyme, RNA polymerase; that is, as an activator or repressor to the transcription of genetic information from DNA to RNA to specific genes.
 - TFs for direct reprogramming of human fibroblasts toward a functional cardiomyocyte-like state: Gata 4, Mef 2c, Tbx 5, (these three are called GMT combination), and Hand 2.
 - TFs expressing in cardiac stem cells/progenitor cells: Nkx 2.5, Mef 2c, Gata 4, Tbx 5, Isl-1; Myo D Myf 5 (myogenic, skeletal muscle TFs)
 - Regulatory genes coding a TF also function as TFs (also can be defined as TFs): Oct 3/4, Sox 2, Klf 4 and C-Myc.
 - Human-induced pluripotent stem cells (iPSCs) are similar to human embryonic stem cells in morphology, proliferation, surface antigen, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity.
 - Embryonic stem cells derived from inner cell mass (ICM) of mammalian blastocytes have the ability to grow indefinitely while still maintaining pluripotency.
3. Cell surface markers: Cluster of differentiation (CD) molecules are markers on the cell surface, and are recognized by specific sets of antibodies, used to identify the cell type, stage of differentiation, and activity of a cell. For example:
 - **c-Kit**: is a protein found on the cell surface of many different types of cells, and is the tyrosine kinase receptor which can bind to stem cell factor (SCF), initially used for isolation of bone marrow-derived hematopoietic stem cells, c-kit⁺ cardiac progenitor cells injected into ischemic heart of a rat model, were reportedly able to differentiate into a tri-lineage of cardiac cells in vitro, namely cardiomyocytes, endothelial cells and smooth muscle cells, and thus reducing infarct size and improving cardiac function. C-kit is also recognized as a proto-oncogene, and encodes a transmembrane kinase (tyrosine kinase receptor; KIT) which is related to colony-stimulating factor type-1 (CSF-1), and platelet-derived growth factor (PDGF), as well as to the immunoglobulin superfamily Kit gene. C-kit⁺ human cardiac progenitor cells (hCPCs) can divide into vascular and myocytes progenitor cells. C-kit⁺ 45⁺ co-expressing CD31⁺, CD34⁺ and Flk-1⁺ were designated as endothelial progenitor cells. The c-kit⁺ cardiac stem cells have the capacity for self-renewal, clonogenicity, and multipotency in vivo, in vitro and in humans.
 - **CD117**: is an important cell surface marker used to identify certain types of hematopoietic progenitors in the bone-marrow. CD117 is a receptor tyrosine kinase type III, and binds to stem cell factors (SCF), causing

certain types of cells to grow. CD117 is also known as C-kit ligand.

- **CD105:** In the CADUCEUS trial, the CDCs applied were of CD105+ mesenchymal nature cardiac-derived cells, in the form of self-adherent clusters and able to differentiate into tri-lineage cells.
- **CD34:** is a transmembrane protein, a sialomucin protein, and a cell surface glycoprotein which functions as a cell-cell adhesion factor and may also mediate the attachment of stem cells to bone-marrow extracellular matrix or directly attach to stromal cells. CD34 expression cells are found in the umbilical cord and bone marrow, and can function as hematopoietic cells, subsets of mesenchymal stem cells, endothelial progenitors and endothelial cells of blood vessels. CD34 is also the name of the human gene that encodes the protein.
- **Sca-1:** is a member of Ly-6.2 (lymphocyte antigen 6.2), expressed as one of the surface markers of early hematopoietic stem cells. Sca-1⁺ CPCs coexpress CD31⁺, CD105⁺ and c-kit⁺, but CD34⁻ and CD45⁻ have high growth potential. These cells also express Isl-1, Nkx 2.5, Gata 4 and Mef 2 by reverse transcriptase-polymerase chain reaction in murine hearts. Sca-1⁺ CPCs from human heart was reported to form beating cardiomyocytes when stimulated with transforming growth factor- β (TGF- β) in vitro, without the need of co-culturing with neonatal rat cardiomyocytes. To date, no clinical trial

using Sca-1⁺ CPCs has been noted.

- **Islet-1 (Isl-1):** Islet-1⁺ CPCs are resident neonatal cardiac progenitor cells, where Isl-1 is a transcription factor expressed during fetal development in humans by the second heart field (atria, right ventricular and out-flow tract) progenitor cells. In rat, these cells are negative for Sca-1, c-Kit, and CD31, and their distribution suggests that Isl-1⁺ CPCs are embryonic precursors, and are remnants of the fetal progenitor population having multipotency to all 3 cardiovascular lineages. Isl-1 is not expressed on the cell surface, making it difficult to purify and isolate Isl-1⁺ cells for therapeutic purpose. The Isl-1 CPC cell population declines rapidly after birth, also limiting their clinical applicability in the adult patients. No clinical trial using Isl-1⁺ CPCs have been reported to date.

Other cardiac progenitor cells, including Sca-1⁺ CSCs are positive expression of CD31 and cardiogenic transcription factors Gata 4, Mef 2c and Mef-1, and negative expression of hematopoietic stem cell markers (CD34, CD45), c-kit, vascular endothelial cadherin, von Willebrand factor, Flt-1 and Flk-1. The Isl-1⁺ cells are endogenous cardiac progenitor cells, and have been reported to have mature cardiomyocyte calcium dynamics and action potentials capable of differentiating into cardiomyocytes, conduction systems, smooth muscle cells and endothelial cells.