Regenerative Medicine/Cardiac Cell Therapy: Adult/Somatic Progenitor Cells

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Abstract

Preclinical data suggested that somatic stem or progenitor cells derived induce and/or support endogenous repair mechanisms of the myocardium. Such cell populations were clearly shown to promote neovascularization in postischemic tissue, and some evidence also indicated transdifferentiation into cardiomyocytes. In the clinical setting, however, many attempts to regenerate damaged myocardium with a variety of autologous and allogeneic somatic progenitors have failed to generate the expected therapeutic efficacy. Currently, efforts are being made to select specific cellular subpopulations, modify somatic cells to augment their regenerative capacity, improve delivery methods, and develop markers selection of potentially responding patients. Cardiac surgical groups have pioneered and continue to advance the field of cellular therapies. While the initial excitement has subsided, the field has evolved into one of the pillars of surgical research and benefits from novel methods such as cellular reprogramming, genetic modification, and pluripotent stem cell technology. This review highlights developments and controversies in somatic cardiac cell therapy and provides a comprehensive overview of completed and ongoing clinical trials.

Keywords
- experimental
- heart failure
- stem cells
- ischemic heart disease
- angina
- angiogenesis

Introduction

In the early 1990s, the concept of replacing lost cardiomyocytes with cells injected into the infarcted myocardium was pioneered by a handful of surgical and basic-research groups.[1] In rodent models, transplanted cardiomyocytes from donor animals were shown to persist, integrate, and improve heart function, but there was no clinically usable source of human cells. Attention increased when skeletal myoblasts, readily available in man, were shown to engraft in cryoinjured rodent myocardium,[2] and excitement culminated when subpopulations of murine bone marrow (BM) progenitor cells of primarily hematopoietic lineage were shown to improve postinfarct heart function by inducing neovascularization[3] and presumably also by de novo formation of contractile tissue.[4] Compared with other completely novel therapeutic concepts, cardiac cell therapy then managed the transition from bench to bedside and commercialization attempts in an extremely short period of time.[5] Numerous clinical studies involving many thousands of patients have been, or are still being, conducted utilizing some form of cell transplantation into damaged myocardium. The first trials were done in small patient cohorts and showed feasibility, safety, and in some cases, evidence of efficacy.[6] However, the results of larger, double-blinded trials revealed a sobering outlook on the therapeutic efficacy of cell-based therapies. There were typically large discrepancies regarding the primary efficacy end points, and most meta-analyses of eligible trials could only detect small, if any therapeutic effects of somatic cell therapy.[7] Moreover, in one secondary analysis of published clinical trials, the number of detected numeric

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irregularities positively correlated with magnitude of the reported increase in heart function,[8] while publications with no or few discrepancies mostly reported little or no therapeutic effect. Although the philosophy of metascience has been questioned,[9] the publication raised serious questions about the quality of some of the clinical research conducted so far in this field.

Skeletal Myoblasts

Quiescent satellite cells are found beneath the basal lamina of skeletal myofibers. In response to injury, they re-enter the cell cycle and give rise to proliferating myoblasts, which in turn differentiate, fuse, and repair damaged muscle. In the 1990s, several groups reported that skeletal myoblasts transplanted into cryodamaged or infarcted rodent myocardium survive and induce an improvement of left ventricular (LV) function.[2] These findings led to clinical trials testing the safety of autologous skeletal myoblast transplantation in patients undergoing coronary artery bypass grafting (CABG) surgery after myocardial infarction (MI).[10,11] The results of these studies did not suggest a therapeutic benefit with regard to ejection fraction or survival. Instead, in the case of the MARS trial, an increased incidence of ventricular arrhythmias led to premature termination of the trial.[10] The cause was traced back to insufficient electromechanical coupling of transplanted myoblasts with host cardiomyocytes, attributable to a lack of expression of the gap junction protein connexin 43.[12] In animal studies, overexpression of connexin 43 in skeletal myoblast prior to transplantation was protected from ventricular arrhythmias, but clinical application of these modified cells has not been investigated.[13] Today, few groups still pursue the concept of using skeletal muscle progenitors for heart repair in experimental models, for instance, in the form of cell sheets applied to the surface of the heart.[14] Indeed, a recent clinical pilot trial in patients with ischemic cardiomyopathy suggested hemodynamic improvements after stand-alone epicardial implantation of skeletal myoblast sheets.[15]

Bone Marrow Mononuclear Cells/ Hematopoietic Progenitors

BM consists of a heterogenous population of hematopoietic stem/progenitor cells (HSC), usually CD45+, and plastic-adherent mesenchymal stromal cells (MSCs). The latter will be described in the next section. Inconsistencies in terminology may occasionally be misleading concerning which cell population was used. In general, there have been four approaches to generate cell-based products from BM: The primary nucleated cell population prepared without selection of specific cells (BM mononuclear cell [BM-MNC]), surface-marker purified subpopulations (e.g., CD34+ or CD133+ cells), cultivated adherent BM-MNCs, and manipulated/preconditioned BM cells.

The assumption that BM stem or progenitor cells possess plasticity sufficient to regenerate contractile myocardium in terms of both neovascularization and cardiomyocyte differentiation was largely based on the article by Orlic et al in 2001,[4] where green fluorescent protein (GFP)-labeled lin-/c-kit+ murine BM cells were injected in a mouse model of MI. Although the reproducibility of the experimental approach was later questioned (Murry et al[16] and Balsam et al[17]), these findings sparked an immense interest in BM-MNCs, and within few months of the initial publication, the first results from human trials were published.[5] A variety of clinical phases II and III trials were followed with varying and sometimes contradictory results. For example, the REPAIR-AMI[18–20] trial showed a beneficial effect on cardiac function and a reduction of cardiac events, but the TIME and LateTIME studies[21,22] could not detect any recruitment of transplanted cells to the infarcted myocardium or improvement in ventricular function when investigating different time points and concentrations of BM-MNC injections postMI. In the majority of the trials, BM-MNCs were delivered intracoronary in patients with (sub) acute MI, but the specifics regarding cell preparation, dose, and timing vary greatly (►**Table 1**). Several meta-analyses of those trials suggested an overall slightly higher LV ejection fraction (LVEF) at follow-up in cell-treated patients, but, as in any meta-analysis, publication bias cannot be ruled out, and the association between incremental changes in contractility and improvements in heart failure symptoms and survival remains unclear.[23] Disconcertingly, the meta-analysis by Nowbar et al showed that the increase in ejection fraction after BM cell therapy positively correlates with the number of discrepancies found in the published reports.[8] Furthermore, the studies with no or few discrepancies were not able to show any benefit regarding the efficacy end points of their respective trials. In 2012, investigators at the Queen Mary University London initiated an European Union (EU)-founded, investigator-driven multicenter, double-blinded phase III trial utilizing BM-MNCs in the setting of acute MI, aiming at enrolling 3,000 patients (BAMI, NCT01569178). The current recommendation of the task force of the European Society for Cardiology is to await the results of BAMI before initiating more trials or routinely incorporating BM-MNC therapy in the treatment of acute MI.[24]

Fewer trials were performed in patients with chronic ischemic heart disease (ischemic cardiomyopathy), but those are probably of higher relevance to the cardiac surgical community. Here, intracoronary, catheter-based intramyocardial and open surgical intramyocardial cells have been used, but the majority of groups lately focus on the latter two routes (►**Table 1**). Of the major trials investigating the effect of intracoronary or transendocardial injection of BM-MNCs, the ESPCAPE trial did report significant improvement of survival, improvement of cardiac function, and symptomatic relieve in patients receiving BM-MNCs.[25] Currently, the investigators of the REPAIR-AMI trials are recruiting for a phase III trial with 600 patients where the effect of repeated injections of BM-MNCs in ICM is investigated (REPEAT trial, NCT01693042, ►**Table 1**). Due to the regulatory complexity and costliness of cardiac cell therapy, where cell products are considered advanced therapy medicinal products (European Community Regulation No 1394/2007), conducting such investigator-initiated trials has become extremely challenging and is only possible with significant third-party support.

Table 1 Bone marrow mononuclear cell preparations

Cells	NCT identifier	Study name/PI[PMID]	No. of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
BM-MNC	NCT01074099	Patel et al[T]	5	RaSC, SB, nP	IMI	CABG	Harvest Tech.
	NCT00615394	STEMDILCARD[C+ 18553028]	6	SGA	IMI + PCI	DCM, LVEF <35%, NYHA III–IV	
	NCT00933621	Blatt et al[SU]	8	SGA	ICI	AMI	
	NCT00128258	Patel et al[T]	10	SGA	IMI	LVAD	
	NCT02479776	N/A[C]	10	Ra, SB, CO, PC	ICI	LVEF <45%, age 1–16	
	NCT02549625	Nelson et al[R]	10	SGA	ICI	ES-HF, refusal LVAD, LVEF <40%	
	NCT01061580	Trehan et al[T]	20	nR, DE, OL, nP	IMI	CABG, LVEF <40%	Harvest Tech.
	NCT00235417	Diedreichsen et al[C+ 20233136]	25	SGA	ICI	ICM, NYHA II–III, LVEF <40%	
	NCT00869024	ASSURANCE[C]	25	Ra, DB, PC	IMI	LVAD	
	NCT00874354	REVITALIZE[C]	27	SGA	ICI	3–14 d after AMI	
	NCT00284713	TOPCARE-DCM[C+ 23362308]	30	RaSC, OL, nP	ICI	DCM, LVEF <40%	
	NCT01354678	IMPI[ANR]	30	Ra, DB, PC	TEndo + PCI	AMI	
	NCT00743639	SDILCM[C]	30	SGA	IMI + PCI	DCM, NYHA III–IV, LVEF <35%	
	NCT00711542	REPAIR-ACS[T]	31	Ra, DB, PC	ICI		
	NCT02256501	Aghdami et al[R]	34	RaSC, SB, nP	ICI	LVEF <45%, age 1–16	
	NCT00199823	ASTAMI[C+ 21414223]	50	Ra, SB, PC	ICI	5–7 d post-AMI	
	NCT02033278	Conde et al[R]	51	Ra, DB, PC	ICI	DCM, LVEF <40%	
	NCT00418418	Harjula et al[SU 25142068, 24656645]	60	Ra, DB, PC	IMI	CABG + LVEF 15–45%	
	NCT01299324	REVIVE[C+ 26217065]	60	Ra, OL, CO, PC	RICI	ICM, NYHA III–IV	Harvest Tech.
	NCT01302171	REGEN-DCM[C 21749209]	60	Ra, DB, PC	ICI	DCM, NYHA II–III	
	NCT00224536	BOOST[C+ 15246726]	60	RaSC, OL, nP	ICI	5–7 d post-AMI	
	NCT00264316	Leuven-AMI[C+16413875]	67	Ra, DB, PC	ICI	1 d post-AMI	
	NCT00363324	FINCELL[C+ 20961630, 18845667]	80	Ra, DB, PC	ICI	2–6 d post-AMI	
	NCT00684060	Late-TIME[C+ 20844613, 22084195]	87	Ra, DB, PC	ICI	14–21 d post-AMI	
	NCT01150175	END-HF[SU+ 25079593]	90	Ra, DB, PC	IMI + PCI	ICM NYHA II–III, LVEF <35%	
	NCT00824005	FOCUS[C+ 22447880, 20691824]	92	Ra, DB, PC	ICI	ICM, CHF NYHA II–III, LVEF < 45%	
	NCT01234181	CHINA-AMI[C+25755063]	22	RaSC, OL, nP	ICI	AMI	
	NCT00326989	Cellwave CHF[C+ 23592107]	103	Ra, DB, DE, PC	Shockwave + ICI	ICM, NYHA II–IV, LVEF <50%	
	NCT00747708	REGEN-IHD[C]	148	Ra, DB, PC	ICI	HF	
	NCT02323620	RACE-STEMI[NA]	200	RaSC, OL, nP	ICI	AMI + LVEF <45%	
	NCT00279175	REPAIR-AMI[C+ 19996415, 1924942]	204	Ra, DB, PC, MC	ICI	AMI	
	NCT00841958	ESCAPE[C+ 20560030]	250	RaSC, SB, nP,MC	TEndo + PCI	ICM, NYHA III–IV, LVEF <35%	
	NCT00350766	EMRTCC[SU 8598362]	300	Ra, DB, PC	ICI	AMI, LVEF <50%	
	NCT01693042	REPEAT[R]	676	Ra, OL, nP	ICI 2x	ICM, NYHA II–III, LVEF <45%	
	NCT00962364	BMC registry[17379833, 23362308]	1,500	–	FU		
	NCT01569178	BAMI[R]	3,000	Ra, DB, PC, MC	ICI	AMI	
MNC vs. EPCs	NCT00383630	Naka et al[T]	1	RaSC, DB	IMI	LVAD	

Table 1 (*Continued*)

Cells	NCT identifier	Study name/PI[PMID]	No. of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
Cocktail	NCT00810238	C-CURE[C+ 23583246]	240	RaSC, SB, nP, MC	TEndo + PCI	ICM, LVEF 15–40%, NYHA III–IV	Celyad
	NCT01768702	CHART-1[ANR 26662998]	240	Ra, DB, PC	TEndo + PCI	ICM, LVEF <35%, NYHA III–IV	Celyad
	NCT02317458	CHART-2[ANR]	400	Ra, DB, PC	TEndo + PCI	ICM, LVEF <35%, NYHA III–IV	Celyad
Anti-Stro	NCT02032004	DREAM HF-1[R]	600	Ra, DB, PC	TEndo + PCI	HF > 6 mo	Mesoblast Ltd.

Abbreviations: +, study results posted/published; AMI, acute myocardial infarction; ANR, active, not recruiting; BM-MNC, bone marrow mononuclear cell; C, trial completed; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CHF, congestive heart failure; CO, cross-over study design; DB, double blinded; DE, dose escalation; EPCs, endothelial progenitor cells; HF, heart failure; ICI, intracoronary injection; ICM, ischemic cardiomyopathy; IMI, intramyocardial injection; LVEF, left ventricular ejection fraction; MC, multicenter study; Np, no placebo; NYHA, New York Heart Association; OL, open label; PC, placebo controlled; PCI, percutaneous intervention; Pts, patients; Ra, randomized; RaSC, randomized against standard care; SB, single blinded; SGA, single group assignment; SU, status of trial unknown for more than 2 years; T, trial terminated; TEndo, transendocardial injection.

Note: This table lists all registered clinical trials involving bone marrow mononuclear cell preparations. "Cocktail" is defined as a proprietary "priming" cocktail for cardiomyocyte differentiation.

Several commercial providers of BM-MNC–based cell products or respective equipment have been or are still being involved in clinical trials for chronic ischemic disease. For instance, Celyad evaluated the benefit of preconditioning BM-MNCs with a proprietary cocktail for cardiogenic priming in patients with ischemic cardiomyopathy (C-CURE, CHART-1, and CHART-2 trials [►**Table 1**]).[26,27] Initially, C-CURE demonstrated feasibility, safety, and improvement of LV function and symptoms compared with the placebo group,[26] but the phase III studies CHART-1 and CHART-2 failed to meet their respective end points.[28] Vericel Corp. uses a proprietary bioreactor system for expansion of BM-MNC, yielding a cell product with enhanced proportions of CD90+ stromal cells and an enrichment of "alternately activated CD45 + CD14+ autofluorescent macrophages."[29,30] The initial IMPACT-DCM (surgical cell injection) and CATHETER-DCM (NOGA-guided catheter delivery) trials in patients with ischemic and nonischemic cardiomyopathies were followed by the IxCell-DCM trial (►**Table 2**). Here, a reduction in a composite end point of cardiovascular events was recently reported, but there were no significant changes in LV contractile function compared with placebo,[31] so that the cause of the lower event rate remains elusive.

Another concept is the intramyocardial transplantation of BM cells enriched for HSC markers CD34 or CD133 (►**Tables 2** and **3**). Here, antibodies attached to magnetic beads are used for cell isolation. In the surgical setting, autologous CD34+ BM cells were transplanted intramyocardially at the time of bypass surgery in small-scale trials, but little is known about phase III–equivalent follow-up studies.[32] Similarly, catheter-based injection of naïve CD34+ cells has largely been abandoned and only one randomized placebo-controlled study was able to demonstrate reduction in refractory angina after transendocardial injections.[33] A larger body of data are available on intramyocardial transplantation of CD133+ cells, an antigen that was considered to reflect a more immature population of HSCs with greater plasticity (►**Table 3**). A dose-escalation pilot study demonstrated feasibility and safety of CD133+ cell injection in conjunction with CABG surgery,[34] and controlled trials showed a significantly higher gain in LVEF after CABG and CD133+ cell transplantation than after CABG alone.[35] However, a subsequent randomized, placebo-controlled, double-blinded trial failed to show any evidence of improved global LV function.[36] Nevertheless, the concept was still pursued in the surgical community, with a good clinical practice (GCP)-standard multicenter trial in Germany being terminated early.[37] Meta-analyses including clinical cell therapy trials in chronic ischemic heart disease underscored the heterogeneity in outcome,[38] and are prone to publication bias due to preferential reporting of "positive" outcomes.

Mesenchymal Stromal Cells

MSCs and Immune System

MSCs are the most extensively studied candidate for cell therapy and can be isolated from wide variety of adult tissues. Although it has become clear that MSC properties vary greatly depending on their source tissue and ex vivo culture conditions, minimal defining characteristics have been established. These include the expression of CD90, CD105, CD73, and CD44, colony formation in cell culture, and the capacity to differentiate into adipocytes, chondrocytes, and osteocytes in vitro.[39] In vivo, MSCs appear to arise from cells in the vicinity of small blood vessels (perivascular cells).[40] A host of biologic functions has been attributed to MSC. It is well established that they interact with immune cells by secreting cytokines such as interleukin-6, indoleamine 2,3 dioxygenase, prostaglandin E2, Granulocyte-macrophage colony-stimulating factor, and transforming growth factor-β2.[41] Their capability in inhibiting macrophage polarization,[42] modulating neutrophil activity and function,[42] reducing the sensitivity of natural killer cells to major histocompatibility complex (MHC) epitopes,[43] and influence activation, differentiation, and proliferation of T-cells[44] best describes their suppressive and activating impact on immune responses. At the same time, their lack of expression of MHC-class II molecules[45] allows MSCs evade immune activation when transplanted from allogenic donors.

Table 2 Cardiac and endothelial progenitor cell preparations

Cell	NCT identifier	Study name/PI[PMID]	No. of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
CPC	NCT00893360	CADUCEUS[C+ 24036024]	31	RaSC, OL, DE, nP	ICI	AMI with LVEF 25–25%	EMMES Corp.
	NCT00474461	SCIPIO[C+22965994, 22088800]	33	RaSC, OL, nP	ICI	ICM, LVEF <40%	
	NCT02293603	DYNAMIC phase 1[ANR]	14	SGA, OL	ICI	DCM, LVEF <30%	Capricor Inc.
		DYNAMIC Phase 2[NA]	28	Ra, DB, PC			
	NCT01458405	ALLSTAR[ANR]	134	Ra, DB, PC	ICI	AMI in past 12 mo	Capricor Inc.
CPC + FGF	NCT00981006	ALCADIA[C+ 19038683]	6	SGA	IMI	CABG + LVEF <15%	
EPC	NCT00629018	Amione et al[C+ 21440864]	110	RaSC, SB, nP	ICI	DCM, LVEF <35%, NYHA III–IV	
	NCT00300053	ACT34-CMI[C+ 27151378]	167	Ra, DB, PC	IMI	Refractory angina	Baxter
	NCT02248532	REMEDIUM[R]	80	Ra, DB, nP	Single vs. repetitive IMI + PCI	DCM, NYHA II–III, LVEF 20–40%	
	NCT00316381	REGENT[C+ 19208649]	200	RaSC, OL, nP	ICI + PCI	AMI	
Ixmyelocel-T	NCT01020968	CATHETER-DCM[C+ 25142002]	22	RaSC, OL, nP	IMI + PCI	DCM, LVEF <35%, NYHA III–IV	Vericel Corp.
	NCT00765518	IMPACT-DCM[27,C+ 25142002]	39	RaSC, OL, nP	IMI + PCI	DCM	Vericel Corp.
	NCT01670981	IxCELL-DCM [ANR]	109	Ra, DB, PC	TEndo + PCI	DCM	Vericel Corp.

Abbreviations: +, study results posted/published; AMI, acute myocardial infarction; ANR, active, not recruiting; C, trial completed; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CHF, congestive heart failure; CPC, cardiac progenitor cells; DB, double blinded; DE, dose escalation; DI-CMP, drug-induced cardiomyopathy; HF, heart failure; ICI, intracoronary injection; ICM, ischemic cardiomyopathy; IMI, intramyocardial injection; LVEF, left ventricular ejection fraction; MC, multicenter study; Np, no placebo; NYHA, New York Heart Association; OL, open label; PC, placebo controlled; PCI, percutaneous intervention; Pts, patients; Ra, randomized; RaSC, randomized against standard care; SB, single blinded; SGA, single group assignment; SU, status of trial unknown for more than 2 years; T, trial terminated; TEndo, transendocardial injection.
Note: This table lists all registered clinical trials involving cardiac and endothelial progenitor cell preparations.

MSCs and Myocardium

MSCs secrete angiogenic cytokines such as vascular endothelial growth factor (VEGF) promoting neovascularization and form vascular-like networks in in vitro assays and in vivo models.[46] In models of MI, MSCs exert an antiapoptotic effect,[47] promote angiogenesis,[48] and influence the homing of circulating endothelial progenitor cells via the secretion of (stromal cell-derived factor 1) SDF-1.[49] Other potential mechanisms are the activation of endogenous cardiac stem cells[50] and the prevention of the adverse ventricular

Table 3 CD133-selected subpopulation of bone marrow mononuclear cell preparations

Cells	NCT identifier	Study name/PI[PMID]	No of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
CD133	NCT03043742	Sekela et al[R]	10	SGA	IMI + TMR	ICM, LVEF 30–45%	
	NCT00529932	SELECT-AMI[T]	19	Ra, DB, PC	ICI + PCI	AMI	
	NCT01467232	IMPACT-CABG[33,C]	40	Ra, DB, PC	IMI	CABG + ICM, NYHA II–IV, LVEF 25–25%	Miltenyi
	NCT02870933	Soetisna et al[R]	30	RaSC, OL	Tepi	CABG + LVEF <35%	
	NCT00462774	Cardio133[C+ 17320570]	60	Ra, DB, PC	IMI	CABG + LVEF <35%	Miltenyi
	NCT01337011	AlsterMACS[R]	64	Ra, DB, nPC	IMI+ PCI or ICI	HF NYHA II–IV	Miltenyi
	NCT00950274	PERFECT[T 22747980]	81	Ra, DB, PC, MC	IMI	CABG + LVEV 25–50%	Miltenyi
CD133 vs. MNC	NCT01748383	ESTABOMA[SU]	85	RaSC, OL, nP	ICI	AMI	
	NCT01187654	Ghassemi et al[C]	80	Ra, SB, PC	ICI	AMI, LVEF 20–45%	
	NCT01167751	Ahmadi et al[C+17691968]	90	Ra, SB, PC	IMI	AMI and CABG	

Abbreviations: +, study results posted/published; AMI, acute myocardial infarction; ANR, active, not recruiting; C, trial completed; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CHF, congestive heart failure; CO, cross-over study design; DB, double blinded; DE, dose escalation; HF, heart failure; ICI, intracoronary injection; ICM, ischemic cardiomyopathy; IMI, intramyocardial injection; LVEF, left ventricular ejection fraction; MC, multicenter study; MNC, mononuclear cell; Np, no placebo; NYHA, New York Heart Association; OL, open label; PC, placebo controlled; PCI, percutaneous intervention; Pts, patients; Ra, randomized; RaSC, randomized against standard care; SB, single blinded; SGA, single group assignment; SU, status of trial unknown for more than 2 years; T, trial terminated; TEndo, transendocardial injection.
Note: This table lists all registered clinical trials involving bone marrow mononuclear cell preparations. "Cocktail" is defined as a proprietary "priming" cocktail for cardiomyocyte differentiation.

remodeling.[51] MicroRNAs and exosomes have been also hypothesized to play a role in the trophic potential of MSCs.[52] In small animal models of MI, MSC-derived exosomes induced a similar reduction as MSCs themselves,[53] and the biologic mechanisms are the subject of current investigations.[54] A matter of long-standing discussion was the capacity of MSCs to give rise to de novo cardiomyocytes. Despite several encouraging reports,[55] efforts have largely failed to show differentiation of un- or minimally modified MSCs into mature cardiomyocytes in vitro or in vivo. In vitro, expression of late cardiac markers has been reported under exposure to the DNA methylation inhibitor 5-azacytidine or under coculture conditions with neonatal cardiomyocytes.[56] However, it is generally acknowledged that MSCs are not a reliable source of de novo contractile cells. Nevertheless, the promising results from various preclinical models of MI sparked numerous clinical trials with allogeneic and autologous BM-MSCs in patients with acute MI, chronic ischemic heart failure, or dilated cardiomyopathy (►**Table 4**). In addition, there have been commercial activities around the isolation and delivery systems for BM-MSCs. Most clinical data have so far been collected in smaller randomized trials where BM-MSCs were injected through either intracoronary or transendocardial injections. As with BM-MNCs, the great heterogeneity of routes of administration and different cell sources (allogenic or autologous) makes it difficult to compare the outcomes between studies (►**Table 4**). Interestingly, the TAC-HF study was able to demonstrate that in direct comparison between BM-MSCs and BM-MNCs, the cultured MSCs perform significantly better in regard to improvement of LVEF and reverse remodeling.[57] There are currently two larger randomized and placebo controlled trials underway (CONCERT-HF and CardiAMP Heart Failure Trial). It remains to be seen whether the results will be as disappointing as those of trials using BM-MNCs.

Table 4 Bone marrow mesenchymal stem cell preparations

Cells	NCT identifier	Study name/PI[PMID]	No. of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
BM-MSC	NCT02460770	MESAD[R]	4	SGA	IMI	LVAD	Thoratec Corp.
	NCT00587990	PROMETHEUS[C+ 24565698]	9	Ra, DB, PC	IMI	CABG + LVEV 15–50%	EMMES Corp.
	NCT01076920	MESAMI[C+ 26901787]	10	SGA	IMI + PCI	ICM, NYHA II–IV, LVEF <35%	
	NCT02467387	Stardal et al[C+ 27856497]	23	Ra, SB, PC	IV	NICM, LVEF <35%, NYHA II–III	CardioCell LLC
	NCT01442129	Ascheim et al[C+ 24682346]	30	Ra, DB, PC	IMI	LVAD	
	NCT02013674	TRIDENT[ANR]	30	Ra, DB, DE nP	DOSE ICI	AMI + LVEF <50%	EMMES Corp.
	NCT01087996	POSEIDON[C+ 23117550]	31	Ra, DB, PC	Tendo + PCI	ICM, LVEF 20–50%	EMMES Corp.
	NCT01392625	POSEIDON-DCM[ANR 25354998]	36	Ra, DB, PC	Tendo + PCI	DCM, LVEF <40%	
	NCT02408432	Olson et al[R]	45	RaSC, SB, nP	IV	DI-CMP, LVEF <40 (anthracycline)	
	NCT01394432	ESTIMATION[R]	50	Ra, DB, PC	IMI + PCI	AMI + LVEF <50%	
	NCT00114452	Hare et al[C+ 19958962]	53	Ra, DB, DE, PC	Dose IV	AMI	Mesoblast
	NCT00644410	MSC-HF[C+ 25926562, 22980293]	59	Ra, DB, PC	IMI + PCI	HF, NYHA II–III, LVEF <45%	
	NCT02962661	Olson et al[ANR]	72	RaSC, OL, nP	ICI 4x in 4 wk	Patients with new-onset HF	
	NCT01720888	Maskon et al[ANR]	80	RaSC, OL, nP	ICI	DCM, LVEF <50%	Cytopeutics
	NCT01392105	SEED-MSC[C+ 24431901]	80	RaSC, OL, nP	ICI	AMI	FCB-Pharmicell
	NCT02362646	Gelijns et al[R]	159	Ra, DB, PC	IMI	LVAD	
	NCT00877903	Golden et al[C]	220	Ra, DB, PC, MC	IV	AMI + LVEF 20–45%	Mesoblast
	NCT02438306	CardiAMP[R]	250	Ra, DB, PC	ICI	CHF, NYHA II–III, LVEF 20–45%	BioCardia Inc.
MSC vs. CPC	NCT02503280	TAC-HFT-II[NA]	55	Ra, SB, PC	Tendo + PCI	ICM, LVEF <50%	
	NCT02501811	CONCERT-HF[R]	144	Ra, DB, PC	IMI + PCI	ICM, NYHA II–III, LVEF <35%	
MNC vs. MSC	NCT00768066	TAC-HFT[C+ 24247587, 21392602]	65	Ra, DB, PC	TEndo + PCI	ICM, LVEF <50%	EMMES Corp.

Abbreviations: +, study results posted/published; AMI, acute myocardial infarction; ANR, active, not recruiting; C, trial completed; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CHF, congestive heart failure; CPC, cardiac progenitor cells; DB, double blinded; DE, dose escalation; DI-CMP, drug induced cardiomyopathy; HF, heart failure; ICI, intracoronary injection; ICM, ischemic cardiomyopathy; IMI, intramyocardial injection; LVEF, left ventricular ejection fraction; MC, multicenter study; MNC, mononuclear cell; MSC, mesenchymal stromal cell; Np, no placebo; NYHA, New York Heart Association; OL, open label; PC, placebo controlled; PCI, percutaneous intervention; Pts, patients; Ra, randomized; RaSC, randomized against standard care; SB, single blinded; SGA, single group assignment; SU, status of trial unknown for more than 2 years; T, trial terminated; TEndo, transendocardial injection.

Note: This table lists all registered clinical trials involving bone marrow mesenchymal stem cell preparations.

Adipose Tissue Derived Cells

MSCs can readily be isolated from adipose tissue by plastic adherence followed by expansion in cell culture. Alternatively, freshly isolated cells, usually called the stromal vascular fraction (SVF), can be used for cell therapy. SVF also contains CD34+ cells and endothelial progenitors, suggesting a greater proangiogenic potential.[45] SVF can also be cultured and expanded, leading to a loss of CD34.[58] The differences between BM and adipose tissue MSCs have been discussed intensely. The frequency of colony forming unit cells was shown to be five times higher in adipose tissue compared with BM.[45] Like BM-MSCs, A-MSCs have been shown to secrete proangiogenic and anti-apoptotic factors such as VEGF and hepatocyte growth factor, further enhanced by hypoxia.[59] CD34 + /CD31-AT cells differentiate into endothelial cells[60] and promote angiogenesis via SDF-1/CXCR4.[61] They also differentiate into smooth muscle cells.[62] Again, certain in vitro differentiation protocols induced the expression of individual cardiac markers such as myosin heavy chain, troponin-I, and α actinin,[63] but reports on the formation of functioning cardiomyocytes usually involve error-prone coculture or in vivo labeling experiments. Nevertheless, there have been several murine and porcine models of MI in which transplantation of A-MSCs[64] lead to significant improvement of LV function and reduction in scar size. The majority of clinical studies have been sponsored by Cytori Therapeutics Inc., who produces an AT cell processing system (►**Table 5**). These studies include the ATHENA I and II, ADVANCE and APOLLO trials of which only the ATHENA II and the PRECISE trials posted results (►**Table 5**). The larger phase III trial ADVANCE and the ATHENA trial were terminated. The cause for termination for the ATHENA trial was given as non-ARDC (non-A-MSCs)-related complications and the PRECISE trial fell short of its initial goal of more than 200 enrollments. Published results indicate safety and some functional improvement, but no definitive conclusions can be drawn.[65,66]

Neonatal Cells

Autologous somatic cells from older patients have been subject to disease and age-related impairments of the transplanted cells,[67] which may in part explain the negative outcome of clinical trials. Neonatal cells with mesenchymal properties have been isolated from cord blood, Wharton's jelly, amniotic membrane, and amniotic fluid,[47] and displayed higher proliferative potential[68] and expression of stem cell markers than "adult" MSCs.[48] Some neonatal cells do not meet the minimal MSC criteria, but are capable of modulating the immune response and promote neovascularization in a similar fashion as BM-MSCs and A-MSCs.[49,69] While angiogenesis support by neonatal cells is undisputed, the evidence of cardiomyogenesis induction is as controversial as that of adult MSCs.[70] There have been very few clinical attempts to use cord/cord blood-derived MSCs for cardiac cell therapy.[71] Hematopoietic-lineage CD133+ cord blood cells were also shown to promote angiogenesis after MI in mice,[72] but the low cell dose and lack of an autologous source preclude clinical application.

The placenta is also a source of large doses of neonatal cells with high proliferation capacity, doubtless immunomodulatory, and proangiogenic, but controversial myogenic capacity. Epithelial cells from the fetal side of the amniotic membrane were believed to retain embryonic stem cell characteristics (OCT-4, Nanog, TRA-1–60, TRA-1–81, and SSEA-3 expression), and did show some cardioregenerative effects in experimental models,[73] as did MSCs derived from amniotic membrane stroma, both with potential allogenic applicability.[74] Outcomes of systematic clinical trials, however, have not been reported.

Resident Cardiac Progenitor Cells

The dogma of the mammalian heart having no intrinsic cardiomyocyte regeneration capacity has been challenged by reports that adult cardiomyocytes are capable of mitosis and the identification of resident cardiac progenitor cells (CPCs).[75]

Table 5 Adipose tissue derived cell preparations

Cells	NCT identifier	Study name/PI[PMID]	No. of Pts	Study design	Intervention	Inclusion criteria	Commercial sponsoring
A-MSCs	NCT01709279	Kaneko et al[SU]	6	SGA	ICI	ICM	
	NCT02387723	CSCC_ASC[C]	10	SGA	IMI + PCI	ICM, NYHA II–III, LVEF <45%	
	NCT00442806	APOLLO[C]	14	Ra, DB, PC	ICI	AMI + LVEF 30–45%	Cytori
	NCT01216995	ADVANCE[C]	23	Ra, DB, PC	ICI	AMI	Cytori
	NCT00426868	PRECISE[C+ 24952864]	27	Ra, DB, DE, PC	TEndo + PCI	CAD	Cytori
	NCT01556022	ATHENA[T+ 27148802]	31	Ra, DB, PC	IMI + PCI	CAD + LVEF <45%	Cytori
	NCT02052427	ATHENA II[T+ 27148802]	3				
SVF	NCT01502514	Parcero et al[C+ 27255774]	10	SGA	TEndo + PCI	ICM, NYHA II–IV, LVEF <40%	

Abbreviations: +, study results posted/published; AMI, acute myocardial infarction; C, trial completed; CAD, coronary artery disease; DB, double blinded; DE, dose escalation; ICI, intracoronary injection; ICM, ischemic cardiomyopathy; IMI, intramyocardial injection; LVEF, left ventricular ejection fraction; MSCs, mesenchymal stromal cells; PC, placebo controlled; PCI, percutaneous intervention; Pts, patients; Ra, randomized; SGA, single group assignment; SU, status of trial unknown for more than 2 years; SVF, stromal vascular fraction; T, trial terminated; TEndo, transendocardial injection.

Note: This table lists all registered clinical trials involving adipose derived stem cell preparations.

They have been identified in niches within the myocardium but may predominantly derive from epicardial cells.[76] CPCs can be isolated from enzymatically dissociated atrial of ventricular myocardium or by outgrowth of cells from myocardial samples. Depending on the species and individual CPC subpopulation, they are enriched by cell sorting for plasma membrane markers such as c-kit or Sca-1, by formation of cell aggregates in vitro (cardiospheres) or by applying specific culture conditions.[77,78] One important hallmark of a "true" CPC is the ability to differentiate toward mature, beating cardiomyocytes, either spontaneously, in dedicated differentiation media, or under the influence of WnT signaling inhibitors. In small animal models of MI, transplantation of ex vivo expanded CPCs resulted in improved cardiac performance even though retention and survival of the transplanted cells in the heart was low.[79] CPC-derived cardiomyocytes could be found in the heart after 3 months, and neovascularization was also supported.[80] Effect sizes in large animal studies are significantly lower and are more representative of the expected effect sizes in human trials.[81] Two clinical pilot trials were conducted in North America using autologous CPCs that were isolated from endomyocardial biopsies or surgical right atrium samples. C-kit + cells (SCIPIO) and cardiosphere-derived cells (CADUCEUS) were injected intracoronary[82] (►**Table 2**). There were no safety issues, and some improvements in regional contractility and/or global LV function were reported. However, the design of one of the trials has been subject to criticism.[34] Another interesting approach is the combination of CPCs and MSCs in the CONCERT-HF study that is currently recruiting patients (►**Table 2**) because preclinical data suggested that a synergetic effect in improvement of cardiac remodeling can be achieved.[83]

In our institution, a different cell product obtained from endomyocardial biopsy tissue was developed. The "CardAP" cells are isolated via outgrowth followed by expansion in dedicated media.[84] They have no cardiomyocyte differentiation potential but exert potent immunomodulatory effects and have been designed for use in nonischemic, dilated of inflammatory heart disease.[85] Clinical pilot studies are currently being prepared.

Exosomes

Exosomes are microvesicles secreted from various kinds of somatic cells that contain numerous proteins and nucleic acids, that is, miRNA. They are collected from the media of naïve or stimulated cells and enriched by centrifugation. In various experimental settings, exosomes were shown to be as effective in preventing postinfarct deterioration of heart function as their viable and intact source cells.[86] Using exosomes instead of viable cell products may simplify therapeutic applications and lower regulatory hurdles. In a recent large animal study, exosomes were only therapeutic when injected directly into the myocardium, while intracoronary exosomes when ineffective.[87] Current research aims at producing standardized exosome-based products from universal source cells, so that an off-the-shelf product with defined characteristics can be applied clinically.

Comment

For approximately 20 years, cell-based applications for ischemic and other kinds of heart disease have been considered the future of heart failure therapy. A lot has been learned about somatic progenitor cell biology, myocardial cell behavior, and interactions between heart and BM. Rather than truly regenerating, that is, recreating, de novo contractile cells, somatic cell therapy was found to act via a wide range of secondary mechanisms such as supporting neovascularization, increasing host cardiomyocyte resistance to ischemia, modulation of tissue inflammation, and influencing fibrous tissue remodeling. Those are most likely mediated by secreted proteins, nucleic acids, or microvesicles containing both. A tremendous amount of resources went into this field, but little was achieved in terms of robust, relevant, and reproducible benefit for patients. More than 15 years after the first BM cell transplantations in the infarcted heart, clinical trials that showed no impact on LV function are still considered to successfully demonstrate feasibility and safety. Many of the initial proponents of cardiac cell therapy have entirely abandoned that field of research, while others argue that better patient selection, tailored approaches instead of "one size fits all," modification of cells or combination with drugs, biomaterials, or other cells will finally bring clinical success. Commercial providers of cell therapies for heart disease (and many other diseases) have flourished (i.e., as Cytori Therapeutics, Osiris Therapeutics). Many have redirected their activities, but others continue to offer their products to patients with little, if any scientific basis. In most of the industrialized countries, tightened regulations such as the EU Regulation No 1394/2007 have put a stop to many of those merely profit-oriented activities. On the contrary, the same regulations have made it extremely difficult to organize and conduct serious investigator-initiated clinical research in this field. Despite findings suggesting that postnatal mammalian myocardium has both baseline and inducible myocyte turnover,[75] the adult human heart is an organ that refuses to regenerate much. A lot more than simply injecting cells derived from other tissues will be needed to change that.

References

1 Koh GY, Klug MG, Soonpaa MH, Field LJ. Differentiation and long-term survival of C2C12 myoblast grafts in heart. J Clin Invest 1993; 92(03):1548–1554
2 Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med 1998;4(08):929–933
3 Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7(04):430–436
4 Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410(6829):701–705
5 Strauer BE, Brehm M, Zeus T, et al. Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction [in German]. Dtsch Med Wochenschr 2001;126(34-35):932–938
6 Kalil RA, Ott D, Sant'Anna R, et al. Autologous transplantation of bone marrow mononuclear stem cells by mini-thoracotomy in

dilated cardiomyopathy: technique and early results. Sao Paulo Med J 2008;126(02):75–81
7 Fisher SA, Doree C, Mathur A, Martin-Rendon E. Meta-analysis of cell therapy trials for patients with heart failure. Circ Res 2015; 116(08):1361–1377
8 Nowbar AN, Mielewczik M, Karavassilis M, et al; DAMASCENE writing group. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. BMJ 2014;348(7109): g2688
9 Moyé L. DAMASCENE and meta-ecological research: a bridge too far. Circ Res 2014;115(05):484–487
10 Veltman CE, Soliman OII, Geleijnse ML, et al. Four-year follow-up of treatment with intramyocardial skeletal myoblasts injection in patients with ischaemic cardiomyopathy. Eur Heart J 2008;29 (11):1386–1396
11 Menasché P, Alfieri O, Janssens S, et al. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 2008;117(09):1189–1200
12 Leobon B, Garcin I, Menasche P, Vilquin J-T, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci U S A 2003;100 (13):7808–7811
13 Roell W, Lewalter T, Sasse P, et al. Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. Nature 2007; 450(7171):819–824
14 Uchinaka A, Tasaka K, Mizuno Y, et al. Laminin α2-secreting fibroblasts enhance the therapeutic effect of skeletal myoblast sheets. Eur J Cardiothorac Surg 2017;51(03):457–464
15 Miyagawa S, Domae K, Yoshikawa Y, et al. Phase I clinical trial of autologous stem cell-sheet transplantation therapy for treating cardiomyopathy. J Am Heart Assoc 2017;6(04):e003918
16 Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004; 428:664–8. doi:10.1038/nature02446.
17 Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 2004;428:668–73. doi:10.1038/nature02460.
18 Schächinger V, Erbs S, Elsässer A, et al; REPAIR-AMI Investigators. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J 2006; 27(23):2775–2783
19 Dill T, Schächinger V, Rolf A, et al. Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. Am Heart J 2009;157 (03):541–547
20 Assmus B, Rolf A, Erbs S, et al; REPAIR-AMI Investigators. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circ Heart Fail 2010;3(01):89–96
21 Traverse JH, Henry TD, Pepine CJ, et al; Cardiovascular Cell Therapy Research Network (CCTRN). Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA 2012;308(22):2380–2389
22 Traverse JH, Henry TD, Pepine CJ, et al; Cardiovascular Cell Therapy Research Network (CCTRN). Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA 2012;308(22):2380–2389
23 Assmus B, Dimmeler S, Zeiher AM. Cardiac cell therapy: lost in meta-analyses. Circ Res 2015;116(08):1291–1292
24 Mathur A, Fernández-Avilés F, Dimmeler S, et al; BAMI Investigators. The consensus of the Task Force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for the treatment of acute myocardial infarction and heart failure: update 2016. Eur Heart J 2017;38 (39):2930–2935
25 Pokushalov E, Romanov A, Chernyavsky A, et al. Efficiency of intramyocardial injections of autologous bone marrow mononuclear cells in patients with ischemic heart failure: a randomized study. J Cardiovasc Transl Res 2010;3(02):160–168
26 Bartunek J, Behfar A, Dolatabadi D, et al. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. J Am Coll Cardiol 2013;61(23): 2329–2338
27 Bartunek J, Davison B, Sherman W, et al. Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial design. Eur J Heart Fail 2016;18(02):160–168
28 Bartunek J, Davison B, Sherman W, et al. Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial design. Eur J Heart Fail 2016;18(02):160–168
29 Henry TD, Traverse JH, Hammon BL, et al. Safety and efficacy of ixmyelocel-T: an expanded, autologous multi-cellular therapy, in dilated cardiomyopathy. Circ Res 2014;115(08):730–737
30 Bartel RL, Cramer C, Ledford K, et al. The Aastrom experience. Stem Cell Res Ther 2012;3(04):26
31 Patel AN, Henry TD, Quyyumi AA, et al; ixCELL-DCM Investigators. Ixmyelocel-T for patients with ischaemic heart failure: a prospective randomised double-blind trial. Lancet 2016;387(10036): 2412–2421
32 Patel AN, Geffner L, Vina RF, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. J Thorac Cardiovasc Surg 2005;130(06):1631–1638
33 Losordo DW, Henry TD, Davidson C, et al; ACT34-CMI Investigators. Intramyocardial, autologous CD34+ cell therapy for refractory angina. Circ Res 2011;109(04):428–436
34 Stamm C, Nasseri B, Hetzer R. Cardiac stem cells in patients with ischaemic cardiomyopathy. Lancet 2012;379(9819):891–892, author reply 891–892
35 Noiseux N, Mansour S, Weisel R, et al. The IMPACT-CABG trial: a multicenter, randomized clinical trial of CD133(+) stem cell therapy during coronary artery bypass grafting for ischemic cardiomyopathy. J Thorac Cardiovasc Surg 2016;152(06):1582–1588.e2
36 Nasseri BA, Ebell W, Dandel M, et al. Autologous CD133+ bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: the Cardio133 trial. Eur Heart J 2014;35(19): 1263–1274
37 Donndorf P, Kaminski A, Tiedemann G, Kundt G, Steinhoff G. Validating intramyocardial bone marrow stem cell therapy in combination with coronary artery bypass grafting, the PERFECT Phase III randomized multicenter trial: study protocol for a randomized controlled trial. Trials 2012;13(01):99
38 Clifford DM, Fisher SA, Brunskill SJ, et al. Long-term effects of autologous bone marrow stem cell treatment in acute myocardial infarction: factors that may influence outcomes. PLoS One 2012;7 (05):e37373
39 Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(04):315–317
40 Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003;18(04):696–704
41 Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic

cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004;94(05):678–685
42 Hoogduijn MJ. Are mesenchymal stromal cells immune cells? Arthritis Res Ther 2015;17(01):88
43 Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006;107 (04):1484–1490
44 Glennie S, Soeiro I, Dyson PJ, Lam EW-F, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 2005;105(07):2821–2827
45 Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 2013;15(06):641–648
46 Iwase T, Nagaya N, Fujii T, et al. Comparison of angiogenic potency between mesenchymal stem cells and mononuclear cells in a rat model of hindlimb ischemia. Cardiovasc Res 2005;66(03): 543–551
47 Guillot PV, Gotherstrom C, Chan J, Kurata H, Fisk NM. Human first-trimester fetal MSC express pluripotency markers and grow faster and have longer telomeres than adult MSC. Stem Cells 2007;25 (03):646–654
48 Nekanti U, Dastidar S, Venugopal P, Totey S, Ta M. Increased proliferation and analysis of differential gene expression in human Wharton's jelly-derived mesenchymal stromal cells under hypoxia. Int J Biol Sci 2010;6(05):499–512
49 Bieback K, Kern S, Klüter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 2004;22(04):625–634
50 Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res 2010;107(07):913–922
51 Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg 2002;73(06): 1919–1925, discussion 1926
52 Vrijsen KR, Maring JA, Chamuleau SA, et al. Exosomes from cardiomyocyte progenitor cells and mesenchymal stem cells stimulate angiogenesis via EMMPRIN. Adv Healthc Mater 2016; 5(19):2555–2565
53 Arslan F, Lai RC, Smeets MB, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res (Amst) 2013;10(03):301–312
54 Glenn JD, Whartenby KA. Mesenchymal stem cells: emerging mechanisms of immunomodulation and therapy. World J Stem Cells 2014;6(05):526–539
55 Fukuda K, Fujita J. Mesenchymal, but not hematopoietic, stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction in mice. Kidney Int 2005;68(05): 1940–1943
56 Ohtani K, Dimmeler S. Epigenetic regulation of cardiovascular differentiation. Cardiovasc Res 2011;90(03):404–412
57 Heldman AW, DiFede DL, Fishman JE, et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. JAMA 2014;311(01):62–73
58 Boquest AC, Shahdadfar A, Brinchmann JE, Collas P. Isolation of stromal stem cells from human adipose tissue. Methods Mol Biol 2006;325:35–46
59 Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004;109(10):1292–1298
60 Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 2004;110(03):349–355
61 Sengenès C, Miranville A, Maumus M, de Barros S, Busse R, Bouloumié A. Chemotaxis and differentiation of human adipose tissue CD34+/CD31- progenitor cells: role of stromal derived factor-1 released by adipose tissue capillary endothelial cells. Stem Cells 2007;25(09):2269–2276
62 Rodríguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc Natl Acad Sci U S A 2006;103(32):12167–12172
63 Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res 2007;100(09):1249–1260
64 Valina C, Pinkernell K, Song Y-H, et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J 2007;28(21):2667–2677
65 Perin EC, Sanz-Ruiz R, Sánchez PL, et al. Adipose-derived regenerative cells in patients with ischemic cardiomyopathy: the PRECISE Trial. Am Heart J 2014;168(01):88–95.e2
66 Comella K, Parcero J, Bansal H, et al. Effects of the intramyocardial implantation of stromal vascular fraction in patients with chronic ischemic cardiomyopathy. J Transl Med 2016;14(01):158
67 Alt EU, Senst C, Murthy SN, et al. Aging alters tissue resident mesenchymal stem cell properties. Stem Cell Res (Amst) 2012; 8(02):215–225
68 Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24(05):1294–1301
69 Rebelatto CK, Aguiar AM, Moretão MP, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med (Maywood) 2008; 233(07):901–913
70 Nekanti U, Mohanty L, Venugopal P, Balasubramanian S, Totey S, Ta M. Optimization and scale-up of Wharton's jelly-derived mesenchymal stem cells for clinical applications. Stem Cell Res (Amst) 2010;5(03):244–254
71 Can A, Ulus AT, Cinar O, et al. Human Umbilical Cord Mesenchymal Stromal Cell Transplantation in Myocardial Ischemia (HUC-HEART Trial). A study protocol of a phase 1/2, controlled and randomized trial in combination with coronary artery bypass grafting. Stem Cell Rev 2015;11(05):752–760
72 Ma N, Ladilov Y, Moebius JM, et al. Intramyocardial delivery of human CD133+ cells in a SCID mouse cryoinjury model: bone marrow vs. cord blood-derived cells. Cardiovasc Res 2006;71(01): 158–169
73 Cargnoni A, Di Marcello M, Campagnol M, Nassuato C, Albertini A, Parolini O. Amniotic membrane patching promotes ischemic rat heart repair. Cell Transplant 2009;18(10):1147–1159
74 Yuan W, Zong C, Huang Y, et al. Biological, immunological and regenerative characteristics of placenta-derived mesenchymal stem cell isolated using a time-gradient attachment method. Stem Cell Res (Amst) 2012;9(02):110–123
75 Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 2004;95(09):911–921
76 Lepilina A, Coon AN, Kikuchi K, et al. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. Cell 2006;127(03):607–619
77 Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. Proc Natl Acad Sci U S A 2007;104(35):14068–14073
78 Goumans M-J, de Boer TP, Smits AM, et al. TGF-beta1 induces efficient differentiation of human cardiomyocyte progenitor cells into functional cardiomyocytes in vitro. Stem Cell Res (Amst) 2007;1(02):138–149
79 Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. Proc Natl Acad Sci U S A 2007;104(35):14068–14073

80 Matsuura K, Honda A, Nagai T, et al. Transplantation of cardiac progenitor cells ameliorates cardiac dysfunction after myocardial infarction in mice. J Clin Invest 2009;119(08):2204–2217
81 Zwetsloot PP, Végh AM, Jansen of Lorkeers SJ, et al. Cardiac stem cell treatment in myocardial infarction: a systematic review and meta-analysis of preclinical studies. Circ Res 2016;118(08): 1223–1232
82 Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 2011;378(9806):1847–1857
83 Li T-S, Cheng K, Malliaras K, et al. Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. J Am Coll Cardiol 2012;59(10):942–953
84 Dehne T, Adam X, Materne E-M, et al. A P19 and P19CL6 cell-based complementary approach to determine paracrine effects in cardiac tissue engineering. Cells Tissues Organs 2014;199(01):24–36
85 Haag M, Stolk M, Ringe J, et al. Immune attributes of cardiac-derived adherent proliferating (CAP) cells in cardiac therapy. J Tissue Eng Regen Med 2013;7(05):362–370
86 Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H. Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. J Mol Med (Berl) 2014;92(04):387–397
87 Gallet R, Dawkins J, Valle J, et al. Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. Eur Heart J 2017;38(03):201–211