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Combining stem cells in myocardial infarction: The road to superior repair?

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Abstract

Myocardial infarction irreversibly destroys millions of cardiomyocytes in the ventricle, making it the leading cause of heart failure worldwide. Over the past two decades, many progenitor and stem cell types were proposed as the ideal candidate to regenerate the heart after injury. The potential of stem cell therapy has been investigated thoroughly in animal and human studies, aiming at cardiac repair by true tissue replacement, by immune modulation, or by the secretion of paracrine factors that stimulate endogenous repair processes. Despite some successful results in animal models, the outcome from clinical trials remains overall disappointing, largely due to the limited stem cell survival and retention after transplantation. Extensive interest was developed regarding the combinational use of stem cells and various priming strategies to improve the efficacy of regenerative cell therapy. In this review, we provide a critical discussion of the different stem cell types investigated in preclinical and clinical studies in the field of cardiac repair. Moreover, we give an update on the potential of stem cell combinations as well as preconditioning and explore the future promises of these novel regenerative strategies.

Abbreviations: ALDH, aldehyde dehydrogenase; BM, bone marrow; BM-MNC, bone marrow mononuclear cell; CASC, cardiac atrial appendage stem cell; CDC, cardiosphere-derived cell; CPC, cardiac progenitor cell; CVD, cardiovascular disease; Cx43, connexin43; DMOG, dimethyloxalylglycine; EPC, endothelial progenitor cell; EV, extracellular vesicle; hESC, human embryonic stem cell; HIF, hypoxia-inducible factor; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell; iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; Isl-1, islet-1; LAD, left anterior descending; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MI, myocardial infarction; MSC, mesenchymal stem cell; Sca-1, stem cell antigen-1.

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KEYWORDS
cotransplantation, myocardial infarction, myocardial regeneration, preconditioning, stem cells

1 | INTRODUCTION

Cardiovascular disease (CVD) has a high global impact on society as it is the leading cause of mortality and disability worldwide.[1] Myocardial infarction (MI) is a well-known clinical manifestation of CVD and can rapidly lead to heart failure by destroying approximately 25% of the ventricular tissue.[2] Due to the limited regenerative potential of the heart, cardiac injury such as MI leads to irreversible massive loss of approximately one billion cardiomyocytes in the left ventricle. Despite treatment of MI by revascularization strategies and therapeutic approaches to slow down ventricular remodeling, current therapies are unable to replace lost cardiac tissue, thereby not preventing progression towards heart failure.[3] At end-stage heart failure, heart transplantation is the only option for those patients, but cannot be offered to all patients mainly due to a shortage of organ donors and the risks accompanied with this operation.[4] The latter emphasizes the need for new strategies to regenerate lost cardiac tissue.

For a long time, it was believed that the heart could not regenerate damaged myocardium after injury. At the beginning of this century, this notion was rejected by the discovery of cardiomyocyte proliferation in mammalian hearts.[5] Although cardiomyocyte turnover occurs, the renewal rate of the heart remains less than 1% per year,[6] underlining the fact that endogenous cardiac regeneration is a rare event that is insufficient to repair millions of lost cardiomyocytes in the infarcted tissue. The search for the ideal approach to promote regeneration of the injured heart, with the goal to prevent or treat heart failure has raised a lot of interest for researchers and clinicians. Cell transplantation is one of the most well-studied regenerative strategies for cardiac repair. The potential of cell-based therapies using stem cells or other cell types has been investigated thoroughly in animal studies as well as in clinical trials.[7] Unfortunately, despite some promising resulting in animal models, a successful stem cell therapy to regenerate injured cardiac tissue has not reached the clinic yet.

Generally, there are two mechanisms by which stem cell therapy can induce cardiac repair (Figure 1). A first mechanism involves the generation of new cardiomyocytes after cardiac injury to truly replace the lost cardiac tissue. This requires differentiation of stem or progenitor cells (e.g., embryonic stem cells, induced pluripotent stem cells and cardiac stem cells) into functional cardiomyocytes and electromechanical coupling of those with the host myocardium. Remuscularization of the heart may be the most intuitional strategy to restore cardiac function after MI, but this cannot be achieved by the majority of the stem cell types already investigated because they do not differentiate and integrate into cardiac tissue. A second mechanism involved in tissue repair relies on the specific properties of the stem cells, namely their capacity to secrete many factors. Indeed, it is likely that most stem cells could promote cardiac repair through their paracrine effects. Stem cells are a rich source of soluble factors as well as extracellular vesicles which can modulate endogenous repair processes by stimulating angiogenesis, reducing fibrosis, increasing cardiomyocyte survival rate, and affecting the immune response.

In the following sections, we review the various stem cell types that were investigated in the last two decades in the field of cardiac repair, focusing on their characteristics as well as their potential in both preclinical and clinical studies. We also discuss the combinational use of these stem cell types in cardiac regeneration and explore the promises of stem cell preconditioning. While the amount of studies using one or two stem cell types as treatment for MI is still growing, our review aims to give an update on the current insights as well as an analytical view on the future directions.

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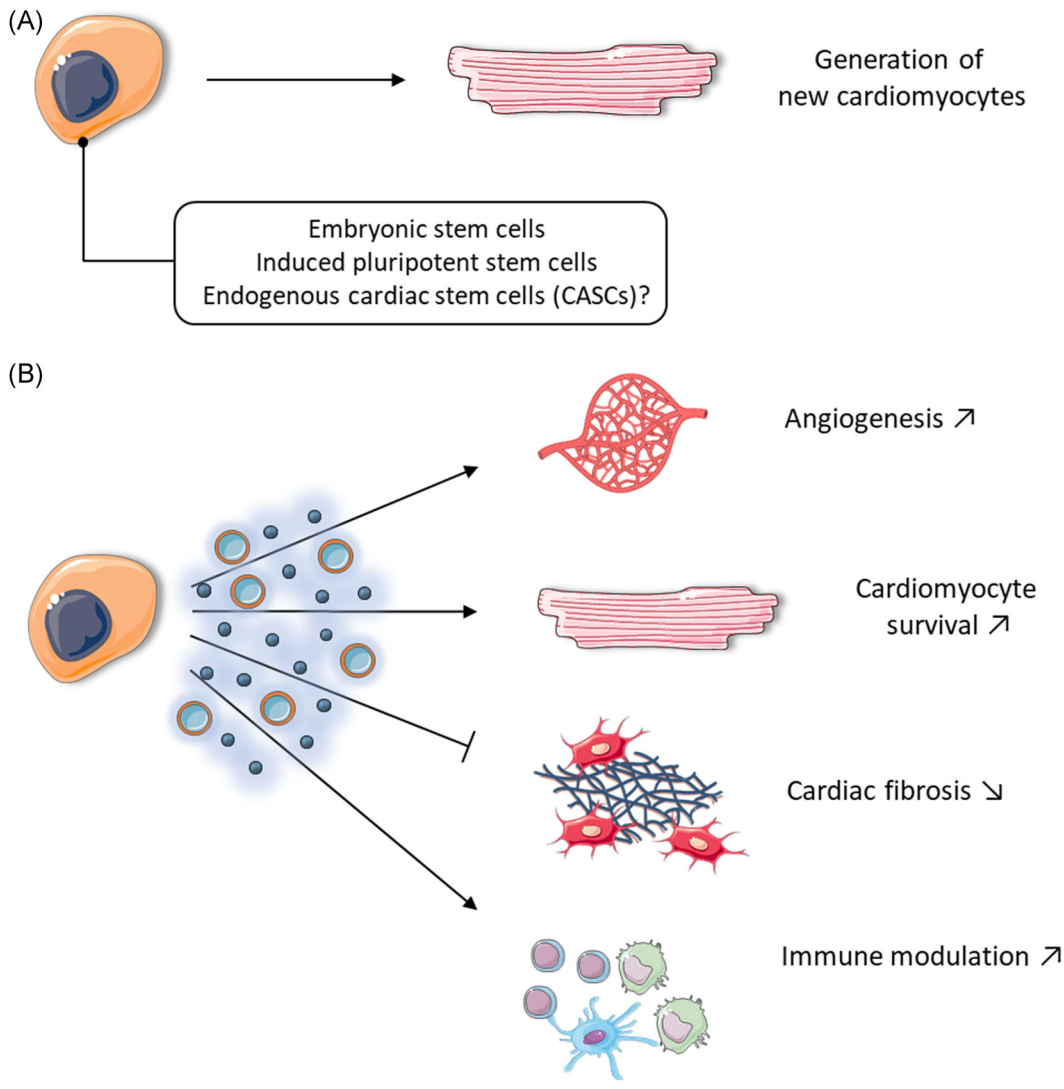

FIGURE 1 Mechanisms by which stem cells promote cardiac repair. Two mechanisms are involved by which stem cell therapy can repair the infarcted cardiac tissue. (A) Tissue replacement. Stem cells differentiate into functional cardiomyocytes that replace the lost myocardial tissue and electromechanically couple with the host myocardium. Examples include embryonic stem cells, induced pluripotent stem cells and cardiac stem cells such as CASCs. (B) Paracrine stimulation. Most stem cell types secrete paracrine factors and extracellular vesicles that promote host repair mechanisms such as increasing tissue perfusion as well as cardiomyocyte survival, reducing interstitial fibrosis and interfering with the host immune system in both the acute and chronic phase, leading to improved cardiac function. CASC, cardiac atrial appendage stem cell. *Source*: This image was created using Servier Medical Art [Color figure can be viewed at wileyonlinelibrary.com]

2 | STEM CELL TYPES FOR MYOCARDIAL REPAIR

Many stem cell types, such as skeletal myoblasts, bone marrow-derived stem cells, embryonic stem cells, induced pluripotent stem cells, and endogenous cardiac stem cells, have been investigated as candidates to improve cardiac function after MI. Despite inconsistent results in animal models, most of the studies showed an overall beneficial

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effect of these stem or progenitor cells on cardiac regeneration. Because of the strong urge to find a therapy for MI patients that can significantly improve quality of life, these cell types knew a rapid transition towards human trials. Although some minimal improvements in cardiac function were observed, the overall outcome from clinical trials remains disappointing. This indicates that major obstacles still need to be solved and that the mechanisms underlying the repair as well as current limitations need to be fully understood before a cell therapy can be successfully translated to the clinic. Since few studies directly compared different stem cell types, this review will discuss in-depth the most important progenitor and stem cell types for cardiac repair used in animal studies (Table 1) and human clinical trials (Table 2).

2.1 | Skeletal myoblasts

In an attempt to repair injured myocardium, the first preclinical and clinical studies focused on undifferentiated skeletal muscle-derived myoblasts, also referred to as satellite cells. Myoblasts reside in the skeletal muscle and fuse with injured muscle fibers to induce regeneration.[77] Because of their autologous origin, their ability to rapidly expand, their spontaneous differentiation into muscle cells and their high resistance to ischemia, myoblasts were early candidates for cardiac repair. In one of the first animal experiments, Chiu et al. harvested skeletal myoblasts from muscle biopsies for autologous transplantation in a canine model of myocardial cryoinjury.[8] Fourteen weeks after transplantation, skeletal myoblasts showed differentiation into cardiomyocyte-like cells. Furthermore, Taylor et al. showed that skeletal myoblasts improved myocardial contractility upon autologous transplantation in a rabbit model of cryoinfarct.[9] However, it should be noted that results were mitigated as these successful results were only observed in 9 of 12 rabbits while five rabbits showed a deterioration in myocardial performance (i.e., stroke work and strain) after transplantation. In addition, no differentiation of skeletal myoblasts into functional cardiomyocytes was observed. It seems that the modest beneficial effects of skeletal myoblasts on ventricular remodeling does not rely on the generation of new cardiomyocytes. Rather, it might be explained by their paracrine and cardioprotective effects[78] as well as their ability to revascularize ischemic tissue and to reduce cardiac fibrosis.[79,80] Additional experiments in small and large animal models using skeletal myoblasts showed inconsistent results. McConnell et al. demonstrated that skeletal myoblast transplantation in sheep with ischemic heart failure did not improve left ventricular ejection fraction (LVEF) and ventricular pressures.[10] However, the study of van den Bos et al. showed that ventricular wall thickening was improved in the infarct area and remote regions 4 weeks after cryoinjury in rabbits.[11] This discrepancy between studies is probably due to the use of different species and the variety in experimental procedure to induce myocardial injury.[81]

Despite inconsistent results in animal studies, the positive findings opened the doors towards clinical trials. Although the first clinical studies using autologous skeletal myoblasts in small groups of patients reported little adverse events and an improvement in cardiac function,[48,49] the MAGIC phase II trial in a larger number of patients did not detect a significant improvement in left ventricular function and revealed ventricular arrhythmias after myoblast transplantation.[50] These negative results are due to the low engraftment potential of skeletal myoblasts in cardiac tissue and the lack of electromechanical coupling with cardiomyocytes through connexin43 (Cx43),[82] indicating that skeletal myoblasts are unable to transdifferentiate into functional cardiomyocytes.[83] Since skeletal myoblasts cannot functionally repair the injured heart and can only minimally reverse ventricular remodeling by paracrine effects, it can be concluded that skeletal myoblasts have a limited potential for cardiac repair.

2.2 | Bone marrow (BM)–derived stem cells

BM is widely used for regenerative purposes both in animal and clinical studies. It is usually isolated from the femur or tibia in an experimental setting or from the posterior iliac crest in human trials. BM contains a mixed pool of cells from hematopoietic and nonhematopoietic lineages that can differentiate into a variety of cell types.

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TABLE 1 Animals studies using stem cells for cardiac repair

Reference	Animal model	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Skeletal myoblast						
Chiu et al.[8]	Cryoinjury in canine	$5–7.5 \times 10^6$	i.m.	20–25 min after MI	Differentiation into cardiomyocyte-like cells	1 d to 14 w
Taylor et al.[9]	Cryoinjury in rabbits	1×10^7	i.m.	1 w after MI	No cardiomyogenic differentiation	3–6 w
McConnell et al.[10]	Ischemic heart failure in sheep by microembolization	3×10^8	i.m.	ND	LVEF ↔ LVEDP ↔	6 w
van den Bos et al.[11]	Cryoinjury in rabbits	$189–240 \times 10^6$	i.m.	3 w after MI	Regional wall thickness ↗	4 w
Bone marrow mononuclear cells						
Orlic et al.[12]	LAD ligation in mice	$3–20 \times 10^4$	i.m.	3–5 h after MI	No differentiation into functional cardiomyocytes	9 d
Ciulla et al.[13]	Cryoinjury in rats	20 × 10^6	i.v.	7 d after MI	Homing to injured cardiac tissue	14 d
Kamihata et al.[14]	LAD ligation in minipigs	1 × 10^8	i.m.	60 min after MI	LVEF ↗ Regional blood flow ↗	12 w
Tomita et al.[15]	Cryoinjury in rats	1×10^6	i.m.	3 w after MI	Capillary density ↗ Differentiation into cardiac-like cells	5 w
Kobayashi et al.[16]	LAD ligation in rats	5×10^6	i.m.	Directly after MI	Capillary density ↗	4 w
Endothelial progenitor cells						
Kawamoto et al.[17]	LAD ligation in nude rats	1×10^6	i.v.	3 h after MI	Capillary density ↗ Fractional shortening ↗	28 d
Kupatt et al.[18]	I/R in pigs	5×10^6	i.v.	60 min after I/R	Infarct size ↘	7 d
Kawamoto et al.[19]	LAD ligation in nude rats	1×10^5	i.m.	10 min after MI	Capillary density ↗ Fibrotic area ↘	4 w
Kawamoto et al.[19]	Chronic MI in Yorkshire porcine	1×10^7	i.m.	4 w after MI	LVEF ↗ Capillary density ↗	4 w

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TABLE 1 (Continued)

Reference	Animal model	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Aicher et al.[20]	LAD ligation in nude rats	1×10^6	i.v.	24 h after MI	Homing to infarct border zone	4 d
Hematopoietic stem cells						
Jackson et al.[21]	I/R in irradiated mice	2×10^3	BM transplant	10 w before I/R	Stem cell engraftment	2–4 w
Murry et al.[22]	LAD ligation in mice	$5–10 \times 10^4$	i.m.	5 h after MI	No differentiation into cardiomyocytes	1–2 w
Nygren et al.[23]	LAD ligation or cryoinjury in mice	1×10^6	i.m.	ND	Engraftment No differentiation into cardiomyocytes	9–28 d
Scherschel et al.[24]	LAD ligation in mice	1×10^5	i.m.	3-5 h after MI	No differentiation into functional cardiomyocytes	9–10 d
Limbourg et al.[25]	LAD ligation in mice	7.5×10^3	i.v.	30 min after MI	Infarct size ↔ No engraftment	4 w
Deten et al.[26]	LAD ligation in mice	1×10^7	i.v.	6 h after MI	Infarct size ↔	6 w
Templin et al.[27]	I/R in mice	5×10^5 and 1×10^7	i.c.	24 h after I/R	LVEF ↗ only in high-dose group	5 w
Gardiwal et al.[28]	I/R in mice	1×10^7	i.c.	24 h after I/R	Infarct size ↘ Electrophysiology ↔	6 w
Mesenchymal stem cells						
Fukuda et al.[29]	LAD ligation in irradiated mice	ND	BM transplant	8 w before MI	Cardiomyogenic differentiation	8 w
Armiñán et al.[30]	LAD ligation in nude rats	1.2×10^6	i.m.	7 d after MI	Fractional shortening ↗ Infarct size ↘ Capillary density ↗	4 w
Shake et al.[31]	I/R in domestic porcine	6×10^7	i.m.	2 w after I/R	Engraftment	6 m
Silva et al.[32]	LAD ligation in canine	100×10^6	i.m.	30 d after MI	LVEF ↗	30 d

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TABLE 1 (Continued)

Reference	Animal model	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Embryonic stem cells						
Chong et al.[33]	I/R in monkeys	ND	i.m.	2 w after I/R	Engraftment Arrhythmias	84 d
Zhu et al.[34]	LAD ligation in monkeys	1×10^7	i.m.	30 min after MI	No engraftment LVEF ↔	28–140 d
Liu et al.[35]	I/R in monkeys	7.5×10^8	i.m.	2 w after I/R	LVEF ↗ No teratoma, 1 arrhythmia	28 d to 12 w
Induced pluripotent stem cells						
Mauritz et al.[36]	LAD ligation in mice	5×10^5	i.m.	Directly after MI	Infarct size ↘ LVEF ↗	14 d
Ye et al.[37]	I/R in pigs	6×10^6	i.m.	15 min after I/R	Engraftment No arrhythmias	4 w
Shiba et al.[38]	I/R in monkeys	4×10^8	i.m.	Directly after MI	LVEF ↗ Engraftment	12 w
Gao et al.[39]	I/R in pigs	8×10^6	2 cellular patches	Directly after I/R	LVEF ↗ Engraftment	4 w
Kashiyama et al.[40]	LAD ligation in monkeys	3.6×10^6	4 cellular patches	14 d after MI	LVEF ↗	6 m
Endogenous cardiac stem cells						
Oh et al.[41]	I/R following permanent LAD ligation in mice	1×10^6 (Sca-1 cells)	i.v.	7 h after MI	Cardiomyogenic differentiation Fusion in myocardium	2 w
Dergilev et al.[42]	LAD ligation in rats	ND (c-kit$^+$ cells)	1 cell sheet	Directly after MI	Infarct size ↘ Differentation into cardiomyocytes and endothelial cells	14–60 d
Bearzi et al.[43]	LAD ligation in mice and rats	4×10^4 (c-kit$^+$ cells)	i.m.	0-5 d after MI	Engraftment Cardiomyogenic differentiation	5–21 d

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TABLE 1 (Continued)

Reference	Animal model	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Zaruba et al.[44]	LAD ligation in mice	ND (c-kit$^+$ cells)	i.m.	ND	No cardiomyogenic differentiation	7–13 d
Cheng et al.[45]	LAD ligation in mice	1×10^5 (CDC cells)	i.m.	Directly after MI	Infarct wall thickness ↗ LVEF ↗	3 w
Kasai-Brunswick et al.[46]	LAD ligation in rats	5×10^5 (CDC cells)	i.m.	63 d after MI	LVEF ↔	60 d
Fanton et al.[47]	I/R in minipigs	$83 \pm 126 \times 10^6$ (CASCs)	i.m.	2 m after I/R	LVEF ↗ Infarct size ↘ Cardiomyogenic differentiation and engraftment	2 m

Abbreviations: BM, bone marrow; CASCs, cardiac atrial appendage stem cells; CDC, cardiosphere-derived cells; d, days; h, hours; i.c., intracoronary; i.m., intramyocardially; I/R, ischemia-reperfusion; i.v., intravenously; LAD, left anterior descending coronary artery; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; m, months; MI, myocardial infarction; min, minutes; ND, not determined; Sca-1, stem cell antigen-1; w, weeks; ↗, increase; ↘, decrease; ↔, no significant difference.

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TABLE 2 Clinical studies using stem cells for cardiac repair

Reference	Number of patients	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Skeletal myoblast						
Smits et al.[48]	5	$196 \pm 105 \times 10^6$	i.m.	>4 w after MI	Ventricular tachycardia in 1/5 patients LVEF ↗	6 m
Menasché et al.[49]	10	$530–1215 \times 10^6$	i.m.	2–3 w after MI	Ventricular tachycardia in 4/9 patients 1 early postoperative death	5–17.5 m
Menasché et al. (MAGIC) [50]	97	$400 \text{ or } 800 \times 10^6$	i.m.	>4 w after MI	LVEF ↔ Arrhythmic events	6 m
Bone marrow mononuclear cells						
Strauer et al.[51]	20	6–7 infusions of $1.5–4 \times 10^6$	i.c.	5–9 d after MI	No adverse events	3 m
Assmus et al. (TOPCARE-AMI)[52]	40	245×10^6	i.c.	4.3 ± 1.5 d after MI	No adverse events LVEF ↗	4 m
Meyer et al. (BOOST)[53]	60	$24.6 \pm 9.4 \times 10^8$	i.c.	4.8 ± 1.3 d after PCI	LVEF ↔	18 ± 6 m
Lunde et al. (ASTAMI)[54]	100	$54–130 \times 10^6$	i.c.	6 ± 1.5 d after MI	LVEF, LVEDV and infarct size ↔	6 m
Lunde et al. (ASTAMI)[55]	100	$54–130 \times 10^6$	i.c.	6 ± 1.5 d after MI	Exercise capacity and quality of life ↔	6 m
Schächinger et al. (REPAIR-AMI)[56]	204	$236 \pm 174 \times 10^6$	i.c.	3-7 d after PCI	LVEF ↗	4 m
Janssens et al. [57]	67	$304 \pm 128 \times 10^6$	i.c.	1 d after PCI	LVEF ↔	4 m
Hendrikx et al.[58]	20	$60 \pm 31 \times 10^6$	i.m.	217 ± 162 d after MI	LVEF, LVEDV and LVESV ↔	4 m

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TABLE 2 (Continued)

Reference	Number of patients	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Perin et al. (FOCUS-CCTRN)[59]	92	100×10^6	i.m.	<12 h after BM aspiration	LVESV ↔ Maximal oxygen consumption ↔	6 m
Choudry et al. (REGENERATE-AMI) [60]	92	$59.8 \pm 59.9 \times 10^6$	i.c.	403-1312 min after PCI	LVEF ↔	1 yr
Endothelial progenitor cells						
Noiseux et al. (IMPACT-CABG)[61]	40	$0.5–10 \times 10^6$ ($CD34^+$/$CD133^+$/$CD45^+$ cells)	i.m.	>2 w after MI	No adverse events LVEF ↔	6 m
Mansour et al. (COMPARE-AMI)[62]	37	ND ($CD34^+$/$CD133^+$ cells)	i.c.	<12 h after BM aspiration	No adverse events	12 m
Manginas et al.[63]	24	$16.9 \pm 4.9 \times 10^6$ ($CD133^+$ cells) and $8 \pm 4 \times 10^6$ ($CD34^+$ cells)	i.c.	ND	LVEF ↗	11.3 ± 3 m
Bartunek et al.[64]	35	$12.6 \pm 2.2 \times 10^6$ ($CD133^+$ cells)	i.c.	11.6 ± 1.4 d after MI	Adverse coronary and arrhythmic events LVEF ↗	4 m
Mansour et al.[65]	38	ND	i.c.	ND	Luminal loss of infarct-related coronary artery ↗	8 m
Hematopoietic stem cells						
Vanderheyden et al.[66]	24	ND	i.c.	7 d and 4 m after MI	LVEF ↔	8 m
Losordo et al.[67]	24	5×10^4, 1×10^5 and 5×10^5	i.m.	<60 d after MI	Severe adverse events	6 m

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TABLE 2 (Continued)

Reference	Number of patients	Dose (cells)	Delivery route	Timing of delivery	Primary outcome	Follow-up time
Mesenchymal stem cells						
Chen et al.[68]	69	48–60 × 10^9	i.c.	18.4 ± 0.5 d after PCI	LVEF ↗	6 m
Guijarro et al. (MESAMI)[69]	10	61.5 × 10^6	i.m.	ND	No severe adverse events related to cell therapy	2 yr
Suncion et al. (POSEIDON) [70]	30	20, 100 or 200 × 10^6	i.m.	ND	LVEF ↔	13 m
Katritsis et al.[71]	11	1–2 × 10^6	i.c.	242 ± 464 d after MI	LVEF ↔	4 m
Embryonic stem cells						
Menasché et al.[72]	6	ND	Cellular patch	ND	No arrhythmias No teratomas	12 m
Induced pluripotent stem cells						
Yla-Herttuala et al.[73]	3	ND	Cellular patch	ND	ND	ND
Endogenous cardiac stem cells						
Bolli et al. (SCIPIO) [74]	81	0.5–1 × 10^6 (c-kit$^+$ cells)	i.c.	4 ± 1 m after CABG	No adverse events	12 m
Makkar et al.[75] Malliaras et al. (CADUCEUS)[76]	31	12.5–25 × 10^6 (CDC cells)	i.c.	1.5–3 m after MI	LVEF ↔ No adverse events	12 m

Abbreviations: BM, bone marrow; CABG, coronary artery bypass grafting; CDC, cardiosphere-derived cells; d, days; h, hours; i.c., intracoronary; i.m., intramyocardially; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; m, months; MI, myocardial infarction; ND, not determined; PCI, percutaneous coronary intervention; w, weeks; yr, year; ↗, increase; ↔, no significant difference.

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Combined with their ability to produce various growth factors and cytokines, BM-derived stem cells are considered as a promising therapy for ischemic diseases.

2.2.1 | Mononuclear cells

Unfractionated bone marrow-derived mononuclear cells (BM-MNCs) represent a heterogeneous population containing stem cells and progenitor cells, including endothelial progenitor cells, hematopoietic stem cells, and mesenchymal stem cells. Studies in mice have shown that after BM transplantation, these BM-derived cells appeared in various tissues such as liver, brain, and heart, and that they possibly contributed to tissue repair in response to injury.[84] Cardiovascular researchers theorized that BM-MNCs were able to colonize the infarcted myocardium, proliferate, and transdifferentiate into cardiovascular endothelial cells and cardiomyocytes. In this context, Makino et al. investigated whether BM-MNC-derived cells could transdifferentiate in vitro into cardiomyocytes.[85] They used immortalized cells obtained by subculturing BM-MNCs of 4 months and showed that 5-azacytidine treatment induced differentiation into cardiomyocyte-like cells with spontaneous contractions. However, because the spontaneous action potentials were not compared to action potentials of host cardiomyocytes, it is unknown whether these cultured BM-MNCs really differentiated into functional cardiomyocytes. Orlic et al. also demonstrated improved ventricular pressures after transplanting BM-derived cells in the infarct border zone.[12] Engrafted cells showed expression of cardiac myosin, but no electrophysiological properties were measured. Consequently, their transdifferentiation into functional cardiomyocytes remains to be confirmed. In addition, studies have shown that intravenously injected BM-MNCs can home towards injured skeletal and cardiac muscle tissue in mice and rats.[13,86] It was suggested that this trafficking process of BM-MNCs through the circulation to the site of tissue damage was induced by chemotactic factors produced by the injured cells.

Subsequent research in various animal models of MI demonstrated that BM-MNCs improved cardiac function and enhanced myocardial perfusion.[14–16] However, only few animal studies indicate that these are related to the transdifferentiation capacity of BM-derived cells into cardiomyocytes. Rather, it appears that BM-derived cells, in particular mesenchymal stem cells, act through the paracrine release of soluble factors to limit myocardial damage and to improve cardiac function.[87,88] As such, these promising results in animal studies paved the way for clinical trials in patients with acute MI. Strauer et al. demonstrated the safety and efficacy of intracoronary infusion of autologous BM-MNCs in a clinical setting.[51] The TOPCARE-AMI trial showed similar results regarding safety and efficacy of intracoronary BM-MNC transplantation after MI, with remarkable improvements in global LVEF, wall motion in the infarct region and left ventricular end-systolic volumes (LVESV) at 4 month follow-up.[52] However, a nonrandomized control group was used, questioning the beneficial results observed. Additional human studies were performed showing negative results concerning the efficacy of intracoronary BM-MNC transplantation after MI. In the BOOST randomized, controlled trial, a significant effect with regard to the primary outcome LVEF was only observed at 6-month follow-up (52.0% ± 12.4% in control group vs. 56.7% ± 12.5% in BM-MNC group), but not after 18 months.[53] Furthermore, confirming these outcomes, the ASTAMI study found no effects on global left ventricular function[54] nor on quality of life.[55] The REPAIR-AMI randomized trial showed a small improvement in LVEF of 3.9% on average between placebo and transplant groups.[56] It is worth mentioning that such a small increase in LVEF may not result in an improved quality of life, thereby questioning the clinical relevance of this cell therapy. In comparison, intravenous administration of the β-blocker metoprolol increased LVEF with 2.67% in patients with acute MI compared to placebo treatment.[89] In addition, sacubitril/valsartan treatment in patients with LVEF < 35% increased LVEF from 29.6% ± 5.9% to 34.8% ± 6.2%.[90] Altogether and especially compared to current standard therapy, BM-MNC transplantation remains overall a disappointing and inconsistent therapy for patients with acute MI.[57–60]

Interestingly, a very recent paper by the Molkentin group indicates that intracardiac injection of either MNC or cardiac MSC induced regional $CCR2^{+}$ and $CX3CR1^{+}$ macrophage accumulation and provided functional

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rejuvenation in a mouse temporally heart ischemia model.[91] This selective macrophage response altered cardiac fibroblast activity and ameliorated the mechanical properties of injured area. As freeze/thaw-killed cells as well as a chemical inducer of the innate immune response had the same effect on macrophage accumulation and associate myocardial repair, this unique identified mechanism warrants further examination.

2.2.2 | Endothelial progenitor cells

A possible explanation for the controversial results of BM-MNC transplantation could be the heterogenicity of this cell population, suggesting that purification of the appropriate cell type could improve clinical outcome. Some studies focused on endothelial progenitor cells (EPCs), which are mostly isolated from BM or peripheral blood based on surface markers CD34, CD31, and CD133. EPCs are similar to stem cells in their self-renewability and clonogenicity; yet EPCs are unipotent cells that can only differentiate into endothelial cells.[92] A 6-day coculture of EPCs with rat cardiomyocytes induced cardiomyocyte-like calcium transients in these EPCs.[93] However, since no further functional experiments regarding electrophysiology and cardiomyocyte contractility were performed and no in vivo studies confirmed these data, it remains unknown whether these cells really differentiated into mature functional cardiomyocytes. Therefore, it is assumed that the main potential of EPCs lies in their role to revascularize damaged tissue. In addition to directly contributing to the formation of new blood vessels through vasculogenesis, EPCs promote migration of mature endothelial cells by the paracrine release of proangiogenic factors, such as vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1).[94,95] Animal studies in nude mice and rats have demonstrated that infusion of ex vivo expanded EPCs promoted neovascularization[96] and improved left ventricular function after MI.[17] Furthermore, embryonic EPC transplantation in pigs subjected to ischemia-reperfusion injury improved cardiac function by decreasing infarct size.[18] Following studies in ischemic rat and swine models demonstrated an increase in capillary density after EPC transplantation, associated with a decrease in fibrotic area and an improved LVEF.[19] Aicher et al. even showed that myocardial injury stimulates mobilization of transplanted EPCs.[20]

In the first clinical trial, homing of EPCs to the acute ischemic event was confirmed in humans.[97] The same findings were observed in patients with congestive heart failure.[98] Since studies have shown that EPCs are generally enriched in cell populations expressing the surface markers CD34 and CD133, clinical trials often used $CD34^+$ or $CD133^+$ EPCs for transplantation after MI.[99] The IMPACT-CABG clinical trial transplanted $CD133^+/CD34^+/CD45^+$ EPCs intramyocardially in patients with chronic ischemic cardiomyopathy.[61] No adverse events were observed but also no significant improvement in LVEF was seen at 6-month follow-up. In a subsequent phase 2 trial (COMPARE-AMI), LVEF was significantly increased after 12 months compared to baseline.[62] However, no comparison was made with a control group. In accordance with these results, intracoronary infusion of $CD34^+$[63] and $CD133^+$[63,64] EPCs in other studies also improved LVEF compared to baseline. It should be noted that these significant improvements are only small increases in LVEF of ~3%–7%, indicating a poor clinical relevance. Unfortunately, intracoronary administration of $CD133^+$ EPCs resulted in higher restenosis rates and increased risk for atherosclerosis.[64,65] Nonetheless, comparison of results between clinical trials is difficult because of the variability of the used EPC subtype.[100]

2.2.3 | Hematopoietic stem cells (HSCs)

HSCs are multipotent stem cells that reside in the BM and give rise to all blood cells (i.e., hematopoiesis).[101] Since de novo hematopoiesis occurs in the early heart tube during embryonic development, it was suggested that HSCs could play an important role in myocardial regeneration.[102] The interest in HSCs became even larger when Jackson et al. identified incorporated donor-derived $CD34^-$ HSCs (so-called side population cells) into both cardiac muscle tissue and vascular structures, where they showed cardiomyocyte-like and endothelial cell-like characteristics.[21] In contrast, subsequent studies have demonstrated that HSCs do not transdifferentiate into cardiomyocytes after MI,

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but rather fuse with resident cardiomyocytes.[22,23] Scherschel et al. have shown that these engrafted BM-derived cells lack specific characteristics of functional cardiomyocytes, such as intracellular calcium transients.[24] Additionally, HSCs did not improve infarct size and showed no permanent engraftment after permanent MI in mice.[25] Furthermore, Lin$^-$ c-kit$^+$ HSCs did not repair the infarcted mouse heart.[26]

Researchers tried to improve HSC therapeutic efficiency by genetic transduction. In this context, it was demonstrated that β-catenin modification of HSCs can significantly improve cardiac function in mice models of ischemia-reperfusion without changing electrophysiological properties of cardiomyocytes.[27,28] Unfortunately, subsequent clinical trials using HSCs had the same negative outcome. Both intracoronary[66] and intramyocardial[67] autologous transplantation of HSCs did not improve cardiac performance and the latter even showed an increased frequency and severity in adverse events. However, Qazilbash et al. demonstrated in a retrospective study that allogeneic transplantation of HSCs was a safe treatment in patients with low LVEF.[103] It can be concluded that HSC transplantation is inconsistent regarding safety and that HSCs cannot restore cardiac function, thereby emphasizing the limited potential of these cells as a therapy for MI.

2.2.4 | Mesenchymal stem cells (MSCs)

In 2005, the theory that HSCs could differentiate into cardiomyocytes was rejected and replaced by the idea that the nonhematopoietic, mesenchymal cell population of the BM was responsible for this event.[29] MSCs are defined as plastic-adherent, multipotent cells that express the surface markers CD73, CD90, and CD105. These fibroblast-like cells derived from multiple tissues including BM, adipose tissue, dental pulp and the umbilical cord can differentiate into adipocytes, osteoblasts, and chondrocytes.[104] Since MSCs can differentiate into cells of mesodermal lineages, an enormous interest was developed in using MSCs for therapeutic purposes in myocardial regeneration. Numerous attempts using growth factors such as BMP4, Wnt and fibroblast growth factor (FGF2) as well as coculture with rat cardiomyocytes appeared to induce the generation of cardiomyocyte-like cells from MSCs in vitro. These cells express some marker genes indicative of myocardial differentiation like troponin T, myosin heavy chain and the heart transcription factor Nkx2.5. Nevertheless, other important features of mature cardiomyocytes such as the generation of adult ventricular action potentials, specific ionic currents and the presence of gap junction proteins are not present in these cells.[105,106] The only convincing strategy for clinically relevant cardiomyocyte differentiation requires substantial and direct epigenetic manipulation of BM-MSCs.[107–109] Therefore, it is assumed that in a normal physiological environment this type of stem cells can only rarely differentiate into functional cardiomyocytes.

Nevertheless, there is abundant clinical and experimental evidence that MSCs can contribute to cardiac regeneration. Studies in both small[30] and large animal models[31,32] have demonstrated the therapeutic potential of MSCs by engraftment of injected cells in the myocardium and by improving cardiac outcome. These encouraging results in animal models led to several clinical trials. In an early clinical trial performing intracoronary transplantation of autologous BM-derived MSCs in patients with acute MI, left ventricular function was significantly improved at 6-month follow-up (LVEF of 67% ± 3% in MSC group compared to 54% ± 5% in control group).[68] The more recent MESAMI phase 1 trial showed feasibility and safety of MSC transplantation after chronic ischemia.[69] However, the POSEIDON clinical trial could not demonstrate an increase in segmental ejection fraction of autologous and allogenic transplantation compared to control.[70] Although some beneficial effects were observed, the magnitude of cardiac improvement varies substantially between clinical studies. Therefore, the general consensus is that MSCs contribute to cardiac repair through paracrine effects rather than cardiomyogenic differentiation. Indeed, Kinnaird et al. have demonstrated that VEGF and FGF release by MSCs promotes in vitro and in vivo arteriogenesis, thereby improving myocardial perfusion and reducing tissue ischemia.[88] Furthermore, it is suggested that Akt plays a role in cytoprotecting cardiomyocytes exposed to hypoxia, thereby further supporting the "paracrine hypothesis" of myocardial repair by stem cells.[87] MSCs not only act by producing proregenerative paracrine factors, but also have immunomodulatory properties by interacting directly and indirectly with cells of

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the immune system.[107] Since sustained chronic inflammation is a typical aspect of ischemic cardiomyopathy leading to adverse ventricular remodeling, suppression of the inflammatory response may be indeed an effective strategy to promote cardiac repair.[110] Luger et al. showed that intravenous administration of MSCs in mice with acute MI attenuates neutrophil and natural killer cell responses. Although no significant improvement in cardiac outcome was observed in the acute MI model, MSC treatment in ischemic cardiomyopathy mice (4 weeks post-MI) showed a significant increase in LVEF, thereby emphasizing the crucial role of MSC immune modulation in cardiac repair.[111]

MSC do not only produce soluble factors but extracellular vesicles (EVs). Based on their size and origin, three different subtypes have been identified, namely exosomes (30–150 nm), microvesicles (100 nm–1 μm) and apoptotic bodies (50–5000 nm). Exosomes originate from the intraluminal budding of early endosomes, forming intraluminal vesicles that accumulate in multivesicular bodies. When these bodies fuse with the plasma membrane, their intraluminal vesicles are released as exosomes by the process of exocytosis. Microvesicles are formed by direct pinching of the plasma membrane. Apoptotic bodies are only secreted by cells undergoing apoptotic cell death. While these vesicles, which are produced by virtually all cell types, were first considered to be waste material, later studies have demonstrated that they play an essential role in cell–cell communication by encapsulating and protecting diverse bioactive molecules, including cytokines, growth factors, transcription factors and micoRNA's (miRNA's).[112] After release of their intraluminal content, many signaling cascades are induced in the recipient cell. Recent studies indicate that MSC-derived exosomes have also cardiac therapeutic benefits. This is mediated by the gene products and miRNA in their cargo which induce angiogenesis, alleviate inflammation and decrease cell apoptosis. For example, MSC-derived EVs reduced infarct size, decreased oxidative stress and activated PI3K/Akt pathway enhancing myocardial viability in a mouse model of ischemia-reperfusion.[113] Also ESC and CSC have been shown to produce EVs enhancing cardiac repair.[114] A recent review by Huang et al. and summarizes the exact molecular mechanisms elicited by these remarkable vesicles.[115]

2.3 | Embryonic stem cells

In 1981, Evans et al. were the first to discover the pluripotency of mouse embryonic cells derived from the blastocyst inner mass.[116] These pluripotent embryonic cells are capable to differentiate in cells of all three germ layers. Seventeen years later, Thomson et al. were the first to generate a human embryonic stem cell (hESC) line.[117] By this discovery, multiple researchers focused on using hESCs for the treatment of several diseases including MI. In 2014, Chong et al. showed that transplanted cardiomyocytes derived from hESCs can engraft and electrically couple to host myocardium.[33] Extensive remuscularization of infarcted hearts was observed even 12 weeks after treatment. However, all monkeys suffered from arrhythmias. It is also worth mentioning that sample size was limited. Four years later, Zhu et al. conducted a large study where cardiovascular progenitor cells derived from hESCs were transplanted in a monkey model of MI.[34] In this study, the transplanted cells could not be detected 140 days after transplantation and functional parameters (such as LVEF) minimally increased after treatment. Therefore, it was stated that hESC-derived cardiomyocytes were not suitable for human trials.[118] In contrast, Liu et al. found that hESC-derived cardiomyocytes restored cardiac function after MI in a macaque monkey model.[35] hESC transplantation was considered to be safe in this study since only one animal suffered from minimal arrythmias and no teratomas were detected. These contradictory results are most likely due to differences in study protocol, that is, transplanting a higher dose of stem cells, using completely differentiated cardiomyocytes instead of progenitor cells and delivering the cells at a different time point. Therefore, Menasché et al. were the first to transplant cellular patches containing differentiated hESCs in six patients suffering from left ventricular systolic dysfunction.[72] After 1 year of follow-up, no significant arrhythmias nor cardiac teratomas or off-target proliferation of hESCs were detected, thereby supporting the safety of ESC transplantation. However, sample size needs to be increased to draw meaningful conclusions regarding efficacy of using hESCs-derived patches as treatment for MI. Moreover, the concentration of hESCs, long-term safety, the optimal differentiation stage and

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the structural composition of the scaffold need to be determined before a possible clinical translation can be realized in patients with CVDs.

2.4 | Induced pluripotent stem cells

With the discovery of hESCs, the field of regenerative medicine was revolutionized and researchers started to investigate human pluripotent stem cells. Yamanaka et al. achieved to directly generate pluripotent stem cells from somatic cells when introduced to the embryonic factors Oct3/4, Sox2, c-Myc, and Klf4.[119] By this Nobel-prize winning technology, the ethical controversy of hESCs was bypassed. A new era of generating induced pluripotent stem cells (iPSCs) was initiated. In vitro studies have demonstrated that iPSCs differentiated into ventricular, atrial and nodal cell-like phenotypes, based on their electrophysiological characteristics.[120,121] The first-generation iPSC-derived cardiomyocytes (iPSC-CMs) remained immature cells resembling a more embryonic or fetal phenotype. Structurally these iPSC-CMs did not exhibit organized sarcomere striations and they had a low expression of mature cardiomyocyte-related genes, e.g. for the ryanodine receptor RyR2.[122] More recently, the application of physical and mechanical stimuli, culturing in 3D settings or fatty acids, has been shown to provide a powerful strategy to render more developmentally mature iPSC-CMs.[123,124]

Animal studies were performed to further explore the potential of these differentiated iPSCs. Mauritz et al. transplanted iPSC-derived cardiac progenitor cells in a mouse model of MI.[36] After 2 weeks of follow-up, these cell-derivatives showed a high proliferation capacity and formed large grafts. However, whether functional cardiomyocytes were generated remains uncertain since a sarcomeric pattern, specific for muscle cells, was not observed. Instead, transplanted cells showed vascular-like properties. In 2014, Ye et al. differentiated human iPSCs not only into cardiomyocytes, but also into smooth muscle cells and endothelial cells.[37] This mixture of cells was intramyocardially injected in a porcine model of acute MI. Four weeks after transplantation, cells integrated into host myocardium leading to an improved cardiac function. No ventricular arrhythmias were reported. This study highlighted the potential of human iPSC therapy for cardiac repair. In a subsequent study, allogeneic transplantation of differentiated iPSCs was performed in primate hearts after MI.[38] LVEF and cardiac contractility were significantly increased in the treated group at 12-week follow-up. Furthermore, the iPSCs-derived cardiomyocytes integrated into the host myocardium. Even if no teratomas were reported, nonlethal ventricular tachycardias were observed. Therefore, Gao et al. started to use iPSCs-loaded cellular patches containing differentiated cardiomyocytes, smooth muscle cells and endothelial cells.[39] These patches improved cardiac performance and limited adverse remodeling without inducing arrhythmias. However, teratoma formation was not investigated. In line with these results, Kashiyama et al. improved LVEF in monkeys with chronic MI by transplanting iPSC-loaded cellular patches.[40]

A first clinical trial was initiated in which iPSCs-derived cardiac cell sheets were transplanted into three patients suffering from heart failure.[73] Unfortunately, to date, no results are reported yet and the therapeutic potential of iPSCs for ischemic heart diseases remains controversial.[125,126] Some studies report that iPSCs retain their epigenetic memory from the somatic cell source: iPSCs derived from cardiomyocytes differentiate more efficiently into cardiomyocytes, redering more mature cells compared to iPSCs derived from other tissues.[127] Furthermore, undifferentiated iPSCs can have a potential risk to form teratomas or induce arrhythmias, indicating that heterogeneity of transplanted iPSCs must be limited. Long-term culture of these iPSCs can induce genetic instability and karyotypic abnormalities. Additionally, strategies to evade possible immune rejections are needed after transplantation.[128] While generally it is considered that immune response using iPSC-derived cells is low, it cannot ruled out that reprogramming itself can induce genetic or epigenetic defects in iPSCs which possibly directly or indirectly contribute to the possible immunogenicity of these cells.[129] To solve the issues of possible immune reactions as well as genetic instability, research efforts are dedicated to establish genetically stable iPSC cell banks (to use HLA-matched iPSCs for cell therapy) as well as the development of several strategies for immune tolerance (e.g., overexpression of both CTLA4-Ig and PD-L1).[130] Altogether, data indicate

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that a large patient cohort and long-term follow-up studies are mandatory before a possible translation to the clinic can be achieved.

2.5 | Endogenous cardiac stem cells

Because of the limited cardiomyogenic differentiation potential of adult stem cells, the search for a cell-based regenerative therapy continued by exploring resident cardiac progenitor cells (CPCs). Since these cells originate from the heart and may be "preprogrammed" to become cardiomyocytes, they are considered to be more suitable for cardiac regeneration. Whether cardiac regeneration emanates from CPCs or is derived from proliferation of dedifferentiated adult cardiomyocytes, remains elusive.[131] Nevertheless, several endogenous stem cell populations were reported in the adult heart based on the expression of specific surface markers as well as functional stem cell characteristics. CPCs expressing markers such as islet-1 (isl1),[132] stem cell antigen-1 (Sca-1),[41] MDR-1/ABCG2 (cardiac side population cells),[133] and the c-kit receptor[42] have been well-studied and show cardiomyogenic differentiation in vitro and in vivo. Extensive interest was developed regarding the c-kit$^+$ CPCs as they showed functional differentiation and integration into the host myocardium, leading to improvement of cardiac function and a decrease in infarct size.[42,43] The SCIPIO clinical trial, using intracoronary infusion of c-kit$^+$ cells, showed an increase in left ventricular systolic function and a decrease in infarct size.[134] However and remarkably, reported data failed to be repeated by others. Concerns were raised regarding the reliability of the SCIPIO trial, leading to the retraction of this paper together with other papers of the research group because of fraudulent data.[74] Zaruba et al. found that c-kit$^+$ CPCs are unable to undergo cardiomyogenic differentiation when cocultured with fetal cardiomyocytes or when transplanted into normal and infarcted adult mouse hearts.[44] Furthermore, Cheng et al. demonstrated that c-kit expression is neither necessary for, nor contributory to the regenerative efficacy of CPCs in humans.[45] Moreover, c-kit$^+$ cells in the heart were specifically identified as mast cells and are, as such, not able to regenerate myocardium.[135]

Since isolation of cells based on surface markers is limited by the antibody-antigen interaction, other research groups focused on the functional stem cell characteristic of sphere formation. Cardiospheres are formed from atrial or ventricular biopsies and result in the outgrowth of the so-called cardiosphere-derived cells (CDCs).[136] Transplantation of CDCs improved both infarct wall thickness and LVEF in a mouse model of chronic MI.[45] In contrast, a recent study intramyocardially injecting CDCs in rats with MI did not increase LVEF compared to control.[46] Despite contradictory results in preclinical studies, the CADUCEUS clinical trial was initiated performing intracoronary infusion of CDCs in patients with MI.[75,76] No improvement in LVEF was observed at 12-month follow-up. Although not significant, several severe adverse events, hospitalizations and non-sustained ventricular tachycardia were reported in the CDC-treated group. Moreover, it is worth mentioning that sphere formation is not a unique stem cell marker for cardiac progenitor cells, but also for MSCs and myofibroblasts, and that cardiospheres are formed by aggregation of cells rather than cell cloning. Therefore, using sphere formation as a single stem cell marker may be an inaccurate isolation method.[137]

Another promising method to isolate stem cells from cardiac tissue is based on a high aldehyde dehydrogenase (ALDH) enzyme activity, which is a general stem cell marker and may be an important enzyme for stem cell protection by metabolizing toxic aldehydes.[138,139] A new ALDH$^+$CD34$^+$CD45$^-$ cardiac stem cell type was identified in human atrial appendages and was therefore called 'cardiac atrial appendage stem cell' (CASC).[140] A first in vitro study of CASCs cocultured with neonatal rat cardiomyocytes showed differentiation of CASCs towards a cardiac phenotype, expressing specific cardiomyocyte markers such as cardiac troponin T and cardiac troponin I. Moreover, Fanton et al. demonstrated that transplantation of CASCs in a minipig model resulted in cardiomyogenic differentiation, with specific expression of ventricular proteins such as ventricular myosin light chain 2.[47] Data indicate that CASCs, which originate from the atria, may differentiate towards a ventricular phenotype. CASCs transplantation also improved in vivo cardiac function in minipigs by increasing LVEF and reducing scar size at 2-month follow-up. Additionally, it was shown that CASCs promote angiogenesis in vitro and in ovo via paracrine mechanisms.[141] Although no clinical trials using CASCs transplantation have

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been performed yet, preclinical studies already demonstrate that CASCs are considered as a safe treatment since no arrhythmias were observed and no tumors were formed upon in vivo transplantation.[47,140]

3 | COMBINATIONAL USE OF STEM CELLS IN MYOCARDIAL INJURY

Differentiation capacity is an important, but not the only factor determining the efficacy of stem cell approaches. Any cell-based therapy is limited in its regenerative potential by the low cell engraftment, resulting in a minimal, although sometimes significant, improvement in functional outcome. Finding the most appropriate stem cell type for cardiac regeneration is usually considered as the end of the road. However, to obtain true tissue repair, the restricted engraftment and retention of stem cells should also be tackled. Research has been performed combining stem cells with biomaterials, such as collagen and fibrin scaffolds, polysaccharide-based biomaterials, and polymer-based materials. Biomaterials have been shown to slightly improve cell retention after transplantation.[142] However at the moment, the results remain disappointing for the patient. Therefore, cotransplantation and priming of stem cells became of large interest as novel strategies to improve cardiac repair (Figure 2).

3.1 | Preclinical studies combining two stem cell types

Taking advantage of the knowledge gathered on stem cells, combining the use of different stem cell types displaying different specific properties in cardiac regeneration might be the clue and deserves further attention. Over the past 15 years, numerous studies focused on cotransplantation of MSCs and EPCs after myocardial injury

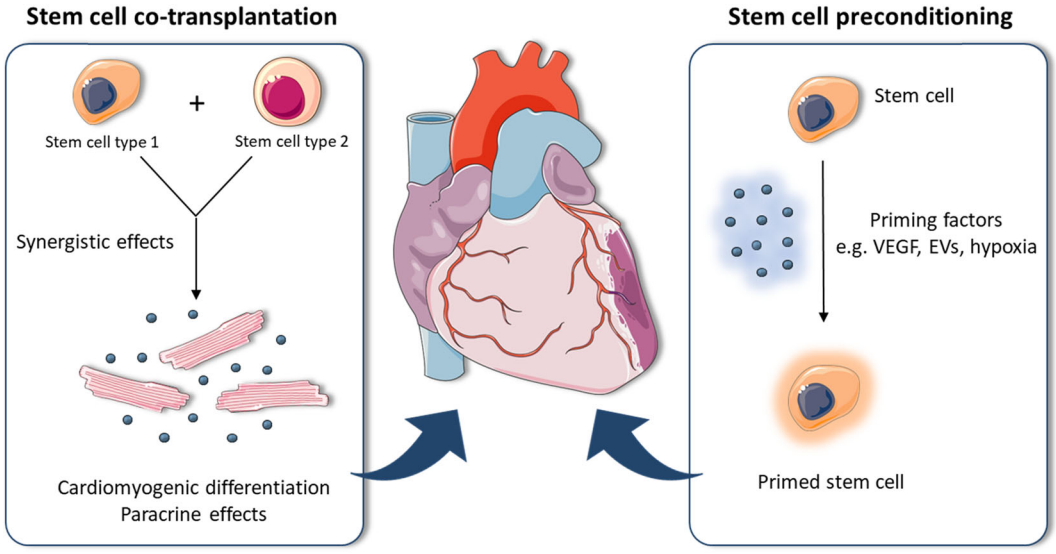

FIGURE 2 Strategies to enhance cardiac repair after injury. Two cell-based approaches are currently under investigation to improve cardiac regeneration by promoting stem cell retention and survival upon transplantation: (left) cotransplantation of different stem cell types can improve cardiac function through synergistic paracrine effects on the host myocardium and through differentiation into functional cardiomyocytes; (right) several priming strategies enhance stem cell properties, making them more resistant to the detrimental microenvironment in which they are transplanted. EVs, extracellular vesicles; VEGF, vascular endothelial growth factor. *Source*: This image was created using Servier Medical Art [Color figure can be viewed at wileyonlinelibrary.com]

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(Table 3). Zhang et al. transplanted 2×10^6 EPCs, 2×10^6 MSCs, or a combination of 1×10^6 EPCs and 1×10^6 MSCs (EPC/MSC group) into a rat model of isoproterenol-induced myocardial injury.[143] All groups improved cardiac performance significantly compared to control, with the greatest improvement in the EPC/MSC group. Combining EPCs and MSCs also showed a significant improvement in cardiac function, for example, ejection fraction and fractional shortening, compared to EPC or MSC treatment alone. Although capillary density was not different in the EPC/MSC group compared to EPC transplantation, fibrosis in the EPC/MSC group was significantly lower than in the stem cell groups used alone. In addition, transplanting a combination of umbilical cord MSCs and $CD34^+$ cells from umbilical cord blood in an MI rabbit model increased left ventricular fractional shortening, but did not improve other cardiac parameters compared to MSC transplantation.[144] Cotransplantation also increased capillary density 4 weeks after MI compared to the MSC group. In contrast, Suuronen et al. demonstrated that combining MSCs and blood-derived mononuclear cells resulted in a significantly lower LVEF in comparison with mononuclear cell treatment.[145]

Additionally, researchers focused on combining MSCs and c-kit^+ cells to further improve cardiac function after MI (Table 3). Bao et al. transplanted 1×10^6 BM-MSCs, 1×10^6 c-kit^+ cells or a combination of 1×10^5 BM-MSCs and 1×10^5 c-kit^+ cells in a rat model of chronic MI.[146] Cotransplantation of BM-MSCs and c-kit^+ cells was superior to transplantation of BM-MSCs or c-kit^+ cells alone in improving LVEF and fractional shortening. Infarct size was significantly decreased compared to BM-MSC therapy, but not to c-kit^+ cell transplantation. However, Williams et al. showed no significant difference in cardiac outcome between cotransplantation of MSCs and c-kit^+ cells, and transplantation of either MSCs or c-kit^+ cells alone in a porcine model of ischemia–reperfusion.[147] These results were confirmed by two other studies showing no significant difference in scar mass and only a minimal improvement in cardiac function between the MSC/c-kit^+ cell group and MSCs alone 3 months after ischemia–reperfusion in Göttingen minipigs.[148,149] Altogether and despite inconsistent results, one could speculate that combining stem cells for transplantation could further improve cardiac function through synergistic paracrine effects on cardiomyocyte survival, proliferation and angiogenesis.[146] Windmolders et al. also demonstrated that MSCs can enhance migration of endogenous cardiac stem cells by secreting specific factors.[151] They showed that MSC conditioned medium improved CASC migration in vitro, suggesting that the platelet-derived growth factor pathway was responsible for this induced CASC migration. In addition, Hatzistergos *et al*. showed that MSCs enhanced the proliferation and chemotaxis of c-kit^+ cells in vitro via the SDF-1/CXCR4 and the stem cell factor/c-kit^+ pathway.[152]

Two studies also investigated whether coadministration with stem cells improved stem cell survival after MI. Indeed, Derval et al. demonstrated that a combination with unfractionated BM cells significantly improved the engraftment of MSCs but not of EPCs compared to monotherapy of these stem cells in a murine model of MI. Monotherapy of EPCs and MSCs as well as cotransplantation of these cells with unfractionated BM cells resulted in an increased capillary density.[153] In addition, Bao et al. showed that combining c-kit^+ cells and BM-MSCs enhanced engraftment of both cell types compared to transplantation of c-kit^+ cells and MSCs alone.[146] A recent study by Park et al. combined the use of human iPSC-CMs with human MSCs in a rat model of MI (Table 3). Epicardial patch implantation of MSCs improved the engraftment and maturation of intramyocardially injected human iPSC-CMs. This also resulted in a significant higher fractional shortening and LVEF, and less fibrosis in comparison with treatment of human iPSC-CMs alone.[150]

3.2 | Clinical studies combining two stem cell types

Clinical trials were already initiated using a combination of stem cells in patients with ischemic cardiomyopathy (Table 4). Already in 2005, a clinical study was published in which the combination of autologous EPC and MSC transplantation in patients with an anteroseptal MI was reported. However, they did not use two differently

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TABLE 3 Animal studies combining stem cell types for cardiac repair

Reference	Animal model	Dose (cells)	Delivery route	Timing of delivery	Primary outcome (compared to monotherapy)	Follow-up time
Mesenchymal stem cell + Endothelial progenitor cell						
Zhang et al.[143]	Isoproterenol-induced myocardial injury	1×10^6 (MSC) + 1×10^6 (EPC)	i.m.	4 w after myocardial injury	LVEF ↗ Capillary density ↔ Fibrosis ↘	12 w
Li et al.[144]	LAD ligation in rabbits	5×10^6 (MSC) + 5×10^5/kg ($CD34^+$ cells)	i.m.	4 w after MI	LV fractional shortening ↗ Capillary density ↗	4 w
Suuronen et al.[145]	LAD ligation in rats	5×10^5 (MSC) + 5×10^5 (MNC)	i.m.	3 w after MI	LVEF ↘ LV fractional shortening ↘	4 w
$C\text{-}kit^+$cells + Mesenchymal stem cells						
Bao et al.[146]	LAD ligation in rats	5×10^5 ($c\text{-}kit^+$ cells) + 5×10^5 (MSC)	i.m.	4 w after MI	LVEF ↗ LV fractional shortening ↗ Infarct size ↘	4 w
Williams et al.[147]	I/R in porcine	1×10^6 ($c\text{-}kit^+$ cells) + 200×10^6 (MSC)	i.m.	14 d after I/R	LVEF ↔	4 w
Karantalis et al.[148]	I/R in Göttingen minipigs	1×10^6 ($c\text{-}kit^+$ cells) + 200×10^6 (MSC)	i.m.	3 m after I/R	LVEF ↗ Scar mass ↔	3 m
Natsumeda et al.[149]	I/R in Göttingen minipigs	1×10^6 ($c\text{-}kit^+$ cells) + 2×10^8 (MSC)	i.m.	3 m after I/R	LVEF ↔ Scar mass ↔	3 m
Mesenchymal stem cells + induced pluripotent stem cells						
Park et al.[150]	LAD ligation in rats	1×10^6/ml (MSC) + 1×10^6 (iPSC-CM)	i.m. (iPSC-CM) + patch (MSC)	<1 d after MI	LVEF ↗ Fractional shortening ↗ Fibrosis ↘ Engraftment	8 w

Abbreviations: d, days; EPC, endothelial progenitor cell; i.m., intramyocardially; iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; I/R, ischemia-reperfusion; LAD, left anterior descending coronary artery; LV, left ventricular; LVEF, left ventricular ejection fraction; m, months; MI, myocardial infarction; MNC, mononuclear cell; MSC, mesenchymal stem cell; w, weeks; ↗, increase; ↘, decrease; ↔, no significant difference.

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TABLE 4 Clinical studies combining stem cell types for cardiac repair

Reference	Number of patients	Dose (cells)	Delivery route	Timing of delivery	Primary outcome (no results reported)	Follow-up time
Mesenchymal stem cells + Endothelial progenitor cells						
MESENDO (NCT00548613)[154]	20	ND	i.c. or i.m.	2 w after BM aspiration	Safety	3 m
C-kit$^+$cells + Mesenchymal stem cells						
CONCERT-HF (NCT02501811)[155]	144	5×10^6 (c-kit$^+$ cells) + 150×10^6 (MSC)	i.m.	ND	Scar size LV function and structure	12 m
TAC-HFT-II (NCT02503280)[156]	55	199×10^6 (c-kit$^+$ cells) + 200×10^6 (MSC)	i.m.	ND	Incidence of serious adverse events	12 m

Abbreviations: BM, bone marrow; i.c., intracoronary; i.m., intramyocardially; LV, left ventricular; m, months; MSC, mesenchymal stem cell; ND, not determined; w, weeks.

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isolated and characterized stem cells, but a single BM aspirate in which mostly BM-MSCs were present. Therefore, one could consider this study as a monotherapeutic study.[71]

The Combination Stem Cell (MESENDO) Therapy for Utilization and Rescue of Infarcted Myocardium (NCT00548613) phase 1 clinical trial investigated the safety and feasibility in patients with MI receiving either intracoronary or intracardiac cotransplantation of MSCs and EPCs.[154] Furthermore, two ongoing phase 1/2 and phase 2 clinical trials, CONCERT-HF (NCT02501811)[155] and TAC-HFT-II (NCT02503280),[156] respectively, are studying a combination of MSCs and c-kit$^+$ cells in patients with ischemic heart failure. However, no results of clinical studies were published yet. It should be noted that cotransplantation of stem cells with high cardiomyogenic differentiation capacity combined with other cell types might influence action potential propagation between myocytes. Nonmyocardial cells may interfere with gap junction formation and intercellular coupling between differentiated stem cells and host cardiomyocytes, leading to an imperfectly coupled tissue that might induce arrhythmias.[157] In addition, clinicians may be reluctant to combine different stem cell types because of the high labor-intensity and costs related to the cell culturing under GMP-grade conditions. In this respect, alternative strategies should be considered to improve stem cell retention and cardiac regeneration.

3.3 | Priming strategies

In addition to combining two stem cell types, increasing attention has turned towards various priming approaches to improve stem cell properties upon transplantation (Figure 2). Results of regenerative therapies with any stem cell type remain currently limited due to the low long-term survival rate of cells after tissue injection. Up to 99% of the cells dies within a few hours after implantation because of the harsh environment that they encounter associated with death stimuli, nutrient and oxygen deprivation, and inflammatory responses.[158] Several priming strategies have been developed to prepare stem cells for the detrimental microenvironment in which they are transplanted, thereby making them more resistant to death signals and improving the efficacy of the regenerative cell therapy.[159]

Paracrine preconditioning using cell-secreted molecules is a widely used approach to improve stem cell properties. For example, conditioned medium of MSCs can be combined with stem cells since the main effector of MSCs is their secretome. This strategy has the advantage that only one stem cell type needs to be injected and that this does not interfere with the electromechanical coupling of cardiomyocytes. In addition, there is growing interest in the use of extracellular vesicles (EVs) to modulate stem cell function because of their key roles in intercellular communication. Indeed, it has been shown that EVs secreted by right atrial appendage-derived cardiac progenitor cells increased LVEF seven days after acute MI in rats, possibly by their antiapoptotic and proangiogenic actions.[160] Importantly, a recent study pretreated c-kit$^+$ cells with MSC-derived exosomes before injection in a rat model of MI. LVEF was significantly increased while fibrosis was reduced in animals treated with primed c-kit$^+$ cells compared with animals injected with unstimulated cells.[161]

Various studies have reported the beneficial effects of other priming strategies on cardiac repair. Hu et al. demonstrated that hypoxia-preconditioning of MSCs improves stem cell survival and decreases infarct size after transplantation in a rat model of chronic MI.[162] It is suggested that hypoxia-preconditioning can increase expression of prosurvival and proangiogenic factors including hypoxia-inducible factor (HIF)-1α, VEGF, and angiopoietin-1. Similar effects were observed when BM-MSCs were preconditioned with the prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) 24 h before transplantation.[163] DMOG-preconditioning decreased infarct size and improved cardiac function compared to non-preconditioned MSCs. Furthermore, pretreatment of MSCs with angiotensin II was also superior to nonpretreated MSCs in improving cardiac outcome and reducing fibrosis levels.[164] Additionally, growth factor pretreatment before experimental MI has been investigated in animal studies. Vogt et al. demonstrated that direct intramyocardial infusion of human recombinant insulin-like growth factor-2 for 60 min before left anterior descending coronary artery occlusion decreased infarct area in domestic pigs.[165] It was also shown that FGF-2 is a potent angiogenic and cardioprotective protein crucial for stem cell proliferation and survival.[166]

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Even if understudied, priming stem cells for cardiac repair has a high potential in cardiac tissue regeneration and requires further studies to fully understand underlying mechanisms and to evaluate the safety and efficacy of this therapy towards a possible translation in the clinic.

4 | CONCLUSION AND FUTURE PERSPECTIVES

Over the past two decades, various stem and progenitor cells were proposed as ideal candidates to repair injured myocardium through tissue replacement or paracrine stimulation, resulting in numerous animal and human studies. Although the majority of these cells lack the ability to differentiate into functional cardiomyocytes, some stem cells can stimulate endogenous repair processes by secreting a rich source of soluble factors and by modulating the immune system. Until now, results from clinical trials remain overall disappointing, generally due to the restricted cell retention and engraftment. In that prospect, repeated injections of stem cells (e.g. one dose during the acute and another during the late phase), especially of those with therapeutic paracrine actions, should be further explored. In addition, recent findings such as the acute stem cell-induced activation of a selective type of macrophages which enhances cardiac repair, warrants further examination and can shed a new approach on clinical trials.

It has been postulated that one of the reasons of the failure of the translation of preclinical success towards clinical therapies is caused by the lack of sufficient rigor of many preclinical studies: absence of animal randomization and blindation of the investigators, exclusion criteria and sample sizes have not been established a priori, and details of protocols (which may impact outcome) have not been thoroughly standardized.[167] In the future, reproducibility should be increased by demanding in all published studies to incorporate the "Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines" and similarly detailed "standard operating procedures." In the most optimal scenario, the therapeutic potential of stem cells should be proven in two or more independent research centers, as proposed by the CAESAR consortium, using detailed protocols ("CAESAR protocols") for measuring infarct size with a level of rigor analogous to multicentre randomized clinical trials.[168] Also more rigorous report of stem cell source, isolation and culture methods, dose, time of application and delivery mode should be demanded. Standardization of some of these variables (e.g., time of application after myocardial infarction, dose of stem cells) is highly needed and would allow better comparison between different stem cell types and results obtained from different research groups.

The choice to use autologous or allogenic cell transplantation depends on the stem cell type and its main mode of action. For example, MSCs have beneficial effects in the acute phase after MI. As it is impossible to isolate and culture large quantities of autologous MSC fast enough to ensure treatment in the acute phase, allogenic use of MSCs should be the preferred option. As these cells are immunomodulatory, their interaction with the host immune cells is suggested to prevent long-term side-effects and thus rejection. Hence, the allogenic possibility allows the administration of MSC as an "off-the-shelf" product. When tissue replacement is the main aim of the stem cell treatment, which is the case of iPSCs and cardiac stem cell types, autologous administration and thus personalized medicine should be pursued. This would avoid long-term use of immune suppressive agents to avoid rejection of the transplant. As this type of therapy demands delivery of the stem cells in the chronic phase, when the acute ischemic reactions and associated oxidative stress which hamper stem cell survival are diminished, this time can be used to produce autologous patient-derived cells. Studies that compare different timings of stem cell delivery are very scarce, while these are needed to further improve the success of stem cell therapy.

Instead of using only a single stem cell type, the focus has shifted towards using a combination of stem cells for cardiac transplantation. Not only cotransplantation of different cell types, but also several priming approaches are currently under investigation. Although stem cell research in myocardial regeneration has made tremendous progress and the combinational use of stem cells and priming strategies may be promising in improving stem cell retention and in vivo regeneration, the road to use stem cell combinations in the clinic has still some hurdles. Among others, the type and amount of stem cell types as well as route and time of application need to be carefully optimized to maximize their

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synergism. The high costs associated with the isolation and cultivation of two different stem cell types under GMP-grade conditions in large amounts remain a limiting factor and technical advances to decrease related costs are highly needed. In addition, long-term effects of these strategies in patients should be examined. Other concerns include the large patient-to-patient variability and the effect of the age on the stem cell potential. Nevertheless, in this review we show that the therapeutic strategy to use stem cell combinations as well as preconditioning holds great promises and warrants further exploration.

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