Chapter 14
Stem Cell Therapy to Improve Acute Myocardial Infarction Remodeling

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Abbreviations

3D	Three dimensional
ADSC	Adipose-derived stem cell
AMI	Acute myocardial infarction
Ang-1α	Angiopoietin-1α
BM	Bone marrow
BM-MNC	Bone marrow-derived mononuclear cell
BM-SC	Bone marrow stem cells
CABG	Coronary artery bypass grafting
CDC	Cardiosphere-derived cells
CSC	Cardiac stem cell
CVD	Cardiovascular disease
ECM	Extracellular matrix
EDV	End-diastolic volume
EF	Ejection fraction
EHT	Engineered heart tissue
EPC	Endothelial progenitor cell
ESC	Embryonic stem cell
ESV	End-systolic volume
FGF	Fibroblast growth factor
Flk1	Fetal liver kinase 1
GCP	Glycolytic cardiac progenitor
GCSF	Granulocyte colony-stimulating factor
HF	Heart failure
HGF	Hepatocyte growth factor
IC	Intracoronary
IGF	Insulin-like growth factor

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ILK	Integrin-linked kinase
IM	Intramyocardial
iPSC	Induced pluripotent stem cell
Isl1	Insulin gene enhancer protein-1
IV	Intravenous injection
LV	Left ventricular
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cell
NSTEMI	Non-ST segment elevation myocardial infarction
PCI	Percutaneous coronary intervention
PEU	Polyester urethane
PEUU	Polyester urethane urea
PGS	Polyglycerol sebacate
PU	Polyurethane
SC	Stem cell
Sca1	Stem cell antigen-1
SDF1	Stromal cell-derived factor 1
SMC	Skeletal myoblast cell
SP	Side population
SSEA	Stage-specific embryonic antigen 1
STEMI	ST-segment elevation myocardial infarction
VEGF	Vascular endothelial growth factor

14.1 Introduction

14.1.1 General Considerations for Myocardial Infarction

Ischemic heart disease remains the leading cause of death worldwide [1]. According to the American Heart Association, 720,000 Americans experienced a new coronary artery event in 2018, with the median survival after a first myocardial infarction (MI) being 8.4, 5.6, 7, and 5.5 years, respectively, for white males, white females, Black males, and Black females [2]. The burden of cardiovascular disease (CVD) and MI affects low- and middle-income countries disproportionately, where 80% of CVD-related deaths occur [3]. While a large majority of the risk factors associated with ischemic heart disease such as high serum cholesterol, hypertension, diabetes, obesity, and smoking are modifiable, family history, age, male sex, and female sex associated with postmenopausal status cannot be altered [4].

Myocardial infarction is broadly defined as myocardial death secondary to prolonged ischemia and can result from multiple etiologies including coronary artery occlusion, supply/demand imbalance, MI related to percutaneous coronary intervention (PCI), stent thrombosis, MI associated with coronary artery bypass grafting (CABG), and others [5]. Rupture or erosion of an atherosclerotic coronary plaque, with resultant exposure of highly thrombogenic material, is the most common inciting factor for coronary occlusion [6]. While a completely occlusive thrombus in the coronary circulation results in an ST-segment elevation MI (STEMI), incomplete thrombosis, or occlusion in the presence of well-established collaterals, results in a non-ST elevation MI (NSTEMI) or unstable angina [7, 8].

14.1.2 Current Myocardial Infarction Standard of Care

Patients with a suspected acute coronary syndrome should be immediately evaluated with an electrocardiogram and cardiac troponin testing. These diagnostic tests, along with history of symptoms, group patients into those suffering from STEMI, NSTEMI, or nonischemic chest pain, distinctions that dictate further care [7, 8]. Initial medical care of patients with STEMI and NSTEMI includes oxygen, analgesics, nitrates, beta-blockers, antiplatelet, and anticoagulation therapy [7, 8]. Urgent reperfusion of ischemic myocardium is the primary therapeutic goal in both groups. All patients with a STEMI should undergo percutaneous coronary intervention within 90 minutes of presentation, while those suffering from NSTEMI undergo immediate, early, and elective PCI depending on time of symptom onset [7, 8]. Diagnostic angiography delineates the extent of disease and dictates reperfusion strategies including stenting, fibrinolysis, or CABG.

Despite urgent reperfusion, life-threatening post-MI complications arise depending on the amount and location of lost myocardium. These complications can be grouped into five subtypes including ischemic, mechanical, arrhythmic, embolic, and inflammatory [9]. Coronary artery disease, including MI, is the number one cause for development of heart failure (HF) in the United States [10]. With improved medical and interventional care, patients are living longer post MI, resulting in a projected increase of HF from six to over eight million by 2030 [11].

14.1.3 Post-Myocardial Infarction Cardiac Remodeling

Following an MI, the injured myocardium and surrounding tissue undergo a series of early and late remodeling changes in an attempt to compensate for the ischemia-induced damage [12]. The early remodeling phase occurs hours to days post MI and includes myonecrosis-induced inflammation, matrix metalloproteinase (MMP)-driven collagen matrix breakdown, thinning and dilation of ventricular walls, as well as fibroblast-induced scar formation [13, 14]. Over the

subsequent weeks to months, uninjured myocardium hypertrophies eccentrically overcompensate for increased stress, further contributing to ventricular dilation. As preload increases without resultant change in ventricular contractility, the ejection fraction (EF) decreases, and dilated cardiomyopathy and resultant HF ensue, worsened by the adult myocardium's limited ability to recover after ischemia [15].

Unfortunately, current therapies aimed at decreasing pathologic post-MI remodeling and HF are limited and include pharmacological treatments to decrease scarring and tissue ischemia, devices and implants aimed at restoring heart function, as well as transplantation [16]. Mammalian myocardium has traditionally been viewed as a non-regenerative organ, and although resident cardiac stem cells (CSC) contribute to cardiac regeneration and some evidence of mammalian heart regeneration exists in animal models, resident stem cells lack the capacity to regenerate all of the myocardium lost after an MI [17, 18]. Furthermore, the contribution of CSC to cardiac regeneration remains highly controversial. Reports of cardiomyocyte exchange in humans range from 50 to 100% during a normal life span, with many such reports having been retracted secondary to lack of reproducibility [19, 20]. Delivery of endogenous and exogenous cardiac progenitor cells to increase myocardial regeneration post MI and in ischemic cardiomyopathy has recently been explored in animal and human studies, with promising results [21].

14.2 Stem Cells in Cardiac Regeneration

14.2.1 Exogenous Cellular Sources

Although cellular transfer for treatment of ischemic heart disease is a relatively new field, with the first clinical trial occurring in 2000, a multitude of cellular sources have been trialed to date in preclinical and clinical models of MI and HF, with relatively few cellular types remaining unexplored [22].

Exogenous cardiac progenitor and stem cells of clinical interest include skeletal myoblast cells (SMC), bone marrow-derived mononuclear cells (BM-MNC), bone marrow-derived populations including lin-c-kit+, CD133+, CD133-/CD34+, c-kit+, and Sca1+, mesenchymal stem cells (MSC), adipose-derived stem cells (ADSC), endothelial progenitor cells (EPC), induced pluripotent stem cells (iPSC), as well as embryonic stem cells (ESC) including early cardiovascular (Isl1+/SSEA1+) cells. Although exogenous stem cell therapy demonstrates some improvement of cardiac function post MI, direct cardiomyogenic differentiation from these cells is rare [23].

Non-satellite CD34-/CD45-/Sca1- stem cells isolated from skeletal muscle have demonstrated rhythmic beating similar to cardiomyocytes and when transplanted into adult mice differentiate into cardiac tissue, while C-kit+Sca1- cells improved survival, enhanced cardiac function, reduced regional strain, and attenuated remodeling in mice [24].

In rodent studies, embryonic stem cell-derived cardiomyocytes attenuated progression of HF after acute myocardial infarction (AMI) by reducing ventricular dilation and improving global left ventricular (LV) function. Subsequent studies established that human embryonic cell cardiomyocytes can limit AMI size and preserve LV contractility [25]. Further, xenotransplantation of cardiac-committed mouse embryonic cells into ovine models has shown that ESC are immune privileged and cardiomyocytes from human ESC are capable of repopulating rat hearts [24].

MSC can be derived from adult peripheral blood, adipose tissue, bone marrow, and neonatal umbilical cord, amnion, cord blood, and placenta [26]. They are potent stimulators of angiogenesis and cardiac regeneration and have been shown to be superior than hematopoietic stem cells in rodent post-MI models [27, 28]. Although they improve tissue regeneration predominantly via paracrine mechanisms, some porcine studies have shown that MSC injected intramyocardially can differentiate into vascular smooth muscle or endothelial cells. Furthermore, human umbilical cord blood-derived MSC preconditioned with 5-aza transdifferentiated into cardiomyocytes, when transplanted into mouse models of MI, preventing infarct expansion and improving heart function [24].

The groundbreaking discovery of iPSC generation via in vitro reprogramming of adult cells into a pluripotent state, and subsequent differentiation into any lineage, transformed the field of regenerative medicine [29]. Although their use in humans remains limited, cardiomyocytes, endothelial cells, and smooth muscle cells derived from iPSC have been tested on porcine infarct models with resultant reduction in infarct size, ventricular wall stress, and apoptosis [30].

14.2.2 Endogenous Cellular Sources

The concept of resident cardiac stem cells is not very well established, with reports quoting vastly different cardiomyocyte renewal capacity [19, 20]. At best, CSC account for approximately 1/30,000 cells in the human heart, although this number increases post injury, likely secondary to migration from bone marrow [31]. The hallmark of CSC is their ability to differentiate into every cardiac lineage including myocytes, fibroblasts, smooth muscle, and endothelial cells. Multiple previous studies have shown their contribution to cardiac regeneration [18, 32]. Specific subtypes of CSC implicated in cardiac regeneration include cardiosphere-derived cells, c-Kit+ cells, insulin gene enhancer protein 1 (Isl1+) progenitor cells, fetal liver kinase 1 (Flk1+) progenitor cells, glycolytic cardiac progenitors (GCP), stage-specific embryonic antigen1 (SSEA1+) progenitors, side population (SP) progenitors, as well as stem cell antigen-1 (Sca1+) progenitors [21]. CSC are localized mainly in the atria of the heart, including the right atrial appendage, and are more numerous in the subepicardium compared to the myocardium [33].

CSC have greater potential to differentiate into cardiomyocytes compared to MSC and in animal studies show potential to reduce post-MI scar size and vascular

overload [18, 34]. Other studies have shown their ability to engraft in the myocardium, recruit endogenous stem cells, and attenuate myocyte apoptosis, via release of growth factors and promotion of angiogenesis [25]. Lastly, administration of human W8B2+ CSC into rat hearts 1 week post MI improved cardiac function and reduced scar tissue formation [35].

SP progenitor cells differentiate into cells expressing sarcomeric proteins including troponin and cardiac α-actinin [36]. Flk-1+ cells can give rise to myocardial, endothelial, and smooth muscle lineages, and their concentration in the circulation increases in humans during an MI [37, 38]. Although Isl1+ cells are capable of differentiating into mature cardiomyocytes, they can only be extracted from neonatal tissue [39]. C-kit+ cells migrate through infarcted myocardium, give rise to cardiomyocytes, and reduce oxidative stress and apoptosis in cardiac and noncardiac cell populations [40, 41]. Sca1+ progenitors are found in myocardial stromal tissue, can be differentiated into myocardium, smooth muscle, and endothelial cells, and their absence leads to myocardial contractile dysfunction in rodents [42–44]. Rodent SSEA+ cells express surface markers which signal cardiomyogenic differentiation potential, form beating colonies when co-cultured with primary cardiomyocytes, and induce myocardial regeneration and functional improvement post MI in animal studies [45]. GCP are isolated from the epicardial/subepicardial hypoxic environment; they express all cardiac stem cells markers and differentiate into endothelial, smooth muscle, and cardiac lineages [46].

In the clinics, skeletal myoblasts were the first cell type to be transferred to human hearts. Early clinical trials focused mainly on bone marrow-derived cells including unselected progenitor/stromal/hematopoietic cells, with a gradual transition to more specific cellular populations including hematopoietic stem and progenitor (CD34+, CD133+) cells and later MSC. More recently, the focus of trials has shifted to various cardiac-committed cell types, including C-kit+ and cardiosphere-derived cells, especially given their increased preclinical success. Embryonic-derived early cardiovascular cells are the most recent cellular type to be examined, and the first trial using iPSC-derived cardiomyocytes is in the early planning phase [22, 47]. Treatment and delivery models for post-acute MI myocardial salvage and ischemic cardiomyopathy regeneration overlap, mononuclear cells have seen a larger success in post-MI studies, while CSC and embryonic-derived early myocardial progenitors have been more extensively studied in ischemic cardiomyopathy trials. This chapter will focus on the specific results of clinical trials in early post-MI patients.

14.2.3 Paracrine Factors, Exosomes, and Direct Cellular Reprogramming

Despite showing some clinical efficacy, stem cell therapy is associated with several important limitations including immune rejection, tumorigenicity, and arrhythmogenicity. In addition, few cells survive after transplantation despite improved

myocardial function, suggesting that the mechanism of their action is predominantly paracrine in nature [48]. A recent study of ischemia/reperfusion injury in rodents showed that intracardiac injection of two separate adult-derived stem cells improved cardiac function without altering the number of new cardiomyocytes. The proposed mechanism for this improvement was selective induction of CCR2+ and CX3CR1+ macrophages, resulting in altered fibroblast activity, extracellular matrix (ECM) content, and enhanced mechanical properties [49]. In order to circumvent these obstacles, researchers have begun to study stem cell-derived paracrine factors, exosomes, and cells directly reprogrammed into cardiac progenitors, although no such studies have yet entered clinical trials, despite animal studies showing promising results.

MSC-derived growth factors and cytokines derived from cell culture supernatants have been shown to decrease inflammation, decrease myocyte apoptosis, recruit endogenous stem cells, and decrease infarct size [25]. In further animal and in vitro studies, MSC-conditioned medium increased neovascularization and fibrosis while improving cardiomyocyte contractility [50].

Exosomes are 40–100-micron vesicles released from cells by fusion with cellular membranes and carry mRNA, miRNA, as well as antiapoptotic and proangiogenic proteins. Exosomes are involved in cell signaling, mediate stem cell paracrine effects, and improve resident cardiac stem cell function without the downsides associated with direct cellular use [48]. In murine models, ESC-derived exosomes increased cardiomyocyte proliferation, upregulated the number of cardiac progenitor cells, and increased cardiac repair following ischemic injury. MSC-derived exosomes also reduced the size of postischemic infarcts, in animal models, via increased cardiac progenitor cell proliferation and decreased fibroblast proliferation [51, 52]. In addition, MSC-derived exosomal miRNA upregulated angiogenesis in post-infarct ischemia [48].

One of the major challenges associated with iPSC use in clinical trials include their tumorigenic potential in an undifferentiated state. Accordingly, new protocols have been designed to directly reprogram cells via induction of lineage-specific factors, without passage through a pluripotent and tumorigenic state [53]. For example, three factors including Gata4, Mef2c, and Tbx5 reprogram cardiac fibroblasts into induced cardiomyocytes. Further addition or modification of reprogramming factors microRNAs has been shown to promote reprogramming efficiency and maturation [54]. In vivo reprogramming of cells following acute MI in animal models has been reported and resulted in improved cardiac function and reduced fibrosis [55]. Most recently, direct in vivo reprogramming has been achieved without genomic integration of viral DNA with the use of a *Sendai virus* vector, which remains outside of the nucleus [56].

14.2.4 Lineage-Specific Considerations

The advantages and drawbacks of specific cellular subtypes in post-MI regenerative therapy are summarized in Table 14.1. Skeletal muscle cells are easier to obtain although likely only provide structural benefits, as opposed to forming new cardiac

Table 14.1 Advantages and drawbacks of lineage-specific cell therapy in myocardial regeneration

Cellular Source	Advantages	Disadvantages
Skeletal muscle cells	1. Less invasive harvest 2. Large source pool 3. Provide structural support	1. No transdifferentiation into cardiomyocytes 2. Low survival 3. Arrhythmogenic in large quantities
MSC	1. Minimally invasive harvest 2. Multiple source pools 3. Differentiation into osteoblasts, chondrocytes, myocytes, and adipocytes 4. High self-renewal, proliferative, and differentiation capacity 5. Beneficial paracrine signaling 6. Immunomodulatory	1. Relatively low yield in peripheral blood and bone marrow 2. Source-dependent variation in quality and yield
Hematopoietic stem cells	1. Minimally invasive harvest 2. Multiple source pools 3. Differentiation into cardiomyocytes and endothelial cells 4. Simultaneously capable of myogenesis and angiogenesis	1. Relatively low yield 2. Difficult in vitro maintenance 3. Unknown signaling pathways
Embryonic stem cells	1. Pluripotent 2. Genomic stability 3. Large differentiation and proliferation potential	1. Derived from human blastocysts, ethical dilemmas 2. Tumorigenic when undifferentiated 3. Immunogenic
iPSC	1. Pluripotent 2. In vitro reprogramming from adult cells 3. Minimally invasive harvest 4. No ethical dilemmas	1. Inefficient differentiation 2. Tumorigenic when undifferentiated 3. Often require viral transfection resulting in genomic instability
Adult-derived stem cells	1. No ethical dilemmas 2. Low risk of immune rejection	1. Limited source 2. Invasive harvesting technique 3. Unclear regeneration potential

Summary of advantages and drawbacks of specific cells used in post-MI regenerative therapy *MSC* mesenchymal stem cells, *iPSC* induced pluripotent stem cells

tissue, secondary to lack of transdifferentiation. In addition, 90% of injected cells die within a few days, and higher cellular counts are arrhythmogenic [24].

Stem cell sources can be divided into three main groups: embryonic, induced, and adult. Embryonic stem cells are pluripotent and can differentiate into all three germ layers and have genomic stability and good differentiation and proliferative capacity [57]. They are, however, derived from human blastocysts and require the destruction of embryos to attain, raising ethical dilemmas, in addition to having tumorigenic and immunogenic potential [58, 59]. In human and rodent studies, it was noted that transplanted embryonic stem cells generated small numbers of cardiomyocytes [60]. As they are reprogrammed in vitro from adult cells, iPSC avoid the ethical dilemmas associated with embryonic stem cells. Differentiation of iPSC

into adult cells is at times inefficient, and they are teratogenic in their undifferentiated states. Furthermore, cells that are derived via viral transfection suffer from genomic instability [61]. Adult-derived cardiac stem cells also avoid ethical dilemmas associated with embryonic stem cells, and they carry a lower risk of immune rejection. However, they are obtained via invasive techniques and have a limited regeneration potential [62].

Mesenchymal stem cells are adult fibroblast-like cells and can differentiate into osteoblasts, adipocytes, and cardiomyocytes, among others [63]. Mesenchymal stem cells can be extracted from peripheral blood, bone marrow, dental pulp, placenta, umbilical cord, or adipose tissue with minimally invasive biopsy. They can self-renew, proliferate, and differentiate, as well as promote growth of adjacent tissue via strong paracrine signaling pathways [64]. MSC have immunosuppressive properties; they decrease the immune response by inhibiting T-cell proliferation and cytotoxicity while increasing the production of regulatory T cells. Drawbacks of MSC include small number in bone marrow and blood, as well as source-dependent variation [25].

Hematopoietic stem cells are multipotent cells, with capacity to differentiate into multiple lineages including cardiomyocytes and endothelial cells [65]. Although they can be harvested from peripheral blood and bone marrow, bone marrow yields are higher [66]. Hematopoietic stem cells are perfect regenerative candidates as they can achieve myogenesis and angiogenesis concomitantly, although their low numbers, difficult in vitro maintenance, and unknown signaling pathways need to be improved [66]. Endothelial progenitor cells are also found in the bone marrow and peripheral blood although in very low concentrations. They can differentiate into endothelial cells and participate in angiogenesis [67]. The number of circulating EPC increases with myocardial ischemia and cytokine release, infiltrating the injured myocardium and possibly differentiating into myocytes [68, 69].

14.3 Stem Cell Delivery Methods

14.3.1 Delivery Methods in Humans

The ideal delivery platform for stem cell therapy in cardiac regeneration should use a noninvasive technique that directly delivers cells to the site of infarct, to prevent cellular loss via aberrant homing. The carrier vehicle for cells should promote survival in the ischemic environment, facilitate retention and promote stem cell differentiation, augment paracrine effects, and protect native myocardium from scarring and arrhythmias [70]. Despite continued studies predominantly in animal studies, no such vehicle exists for use in clinical trials. At present, stem cell delivery can be accomplished via intravenous injection, intracoronary infusion, direct epicardial and endocardial injection, as well as topical application at the time of surgery [24]. Peripheral intravenous (IV) injection is by far the least invasive, though

studies examining homing of radioactively labeled bone marrow cells into infarcted myocardium did not reveal any signal in the heart [71]. Intracoronary and intramyocardial delivery is by far the most commonly used methods reported in clinical trials [72]. Intracoronary (IC) infusion is less invasive than intramyocardial injection and can be achieved in an antegrade or retrograde fashion. IC delivery is also less arrhythmogenic and has been associated with a modest improvement in EF and infarcted area size [72]. Despite these benefits, IC delivery results in delivery of only 1.3–2.6% of cells into the infarcted myocardium, with the majority of cells circulating to the liver or spleen [71]. In addition, IC injection depends on patency of coronary arteries and is associated with a small risk of embolization [73, 74]. Intramyocardial delivery (IM) of cells facilitates their delivery to target tissues and can be accomplished via transepicardial, transendocardial, or transcoronary routes [57]. Transepicardial injection requires direct exposure of the heart, and all intramuscular injections are associated with ventricular arrhythmias [75].

14.3.2 Implantable and Injectable Systems

Cellular scaffolds and hydrogels enhance stem cell survival, and while hydrogels can retain cells at desired locations, scaffolds provide mechanical support to adjacent structures; unfortunately, both require invasive topical application [76, 77]. In rodent studies, human bone marrow CD133+ cells delivered in collagen patches increased local angiogenesis, though the cells themselves failed to differentiate into cardiomyocytes [78]. In addition to collagen, multiple other substrates mimic the ECM of the heart, including polyurethane (PU), poly(ester urethane) (PEU), polyester urethane urea (PEUU), and poly(glycerol sebacate) (PGS) [24]. Animal studies of biodegradable PU patches promoted the contractile phenotype of smooth muscle cells and improved cardiac remodeling [79].

Hydrogels composed of materials such as fibrin, Matrigel, alginate, and polyethylene glycol can all be modified to resemble the physical properties of cardiac tissue [24]. ECM and collagen containing hydrogels allowed for differentiation of human ESC into functional cardiomyocytes in vitro, and cell-impregnated alginate hydrogels delivered to murine hearts reduced left ventricular remodeling [80, 81]. Engineered heart tissue (EHT) has been developed from type I collagen and neonatal heart tissue. When sutured onto rat hearts in vivo, this tissue becomes electrically integrated and perfused [82, 83]. Finally, engineered heart muscle has been developed by ESC-derived cardiomyocytes onto EHT [84].

To overcome the invasive methods required for scaffold and hydrogel delivery, gelling systems based on materials including fibrin glue, collagen, Matrigel, hyaluronic acid, and alginate have been developed that undergo a fluid-to-solid transition when in vivo, allowing catheter-based delivery [24]. Self-assembling RAD16-II scaffolds induced angiogenesis, retained myocytes, and promoted ESC differentiation into MHC-positive cells [85]. Catheter-delivered, collagen-encapsulated bone marrow cells showed improved LV function, and vascularization and

self-assembling peptides loaded with insulin-like growth factor (IGF) allowed for sustained release of paracrine factors [86, 87]. Acellular alginate is undergoing clinical trials to prevent ventricular remodeling [24].

14.4 Clinical Trials of Post-MI Regeneration

14.4.1 Trial Design

A query of completed clinical trials in post-acute MI stem cell therapy shows that approximately 28 studies have been completed thus far (Table 14.2) [88–119]. The number of patients randomized varied from 20 to 250 in each trial. The intracoronary route of cell delivery after initial diagnostic and therapeutic PCI, used in 25 out of 29 trials, was the most widely used method of cell delivery. One study delivered cells both via the intracoronary and intramyocardial routes concomitantly, one study injected cells intramyocardially at the time of CABG, and two studies delivered cells peripherally via intravenous injection.

Autologous, rather than allogenic, cells were most commonly used, with just five studies employing allogenic sources (Table 14.2). Autologous bone marrow (BM)-derived mononuclear cells were the most commonly studied, followed by autologous bone marrow-derived unselected progenitor cells. Several studies further sorted out autologous bone marrow-derived hematopoietic, endothelial, endothelial/cardiac, and early progenitor cells based on differential expression of various combinations of cell surface markers including CD34, CD45, CD133, CXCR4, among others. Less common cell sources included autologous bone marrow-derived MSC from commercially available products, allogenic Wharton's jelly-derived MSC, and autologous peripheral blood stem cells. Although more extensively studied in the context of heart failure, as compared with acute MI, autologous cardiosphere-derived stem cells and allogenic cardiac stem cells have also been examined.

The timing of cell delivery and number of cells varied widely across studies. Despite all being acute post-MI models, therapy was delivered anywhere from less than 24 hours to several months post-initial therapeutic PCI. Although the ideal timing of cell delivery has not yet been standardized, the majority of studies implemented the therapeutic intervention within 10 days of PCI. Comparison of early (3–6 weeks) versus late (3–4 months) delivery did not change the primary outcome, increased left ventricular ejection fraction (LVEF), and decreased infarct size [94]. Final cell count delivered differed significantly between and often within studies; all studies used a magnitude of cells on the order of millions, in the range of 1.9–1300 million cells. Although the majority of studies used a fixed number across participants, several studies used weight-based dosing of 0.5–five million cells/kg. In preparations containing mixed cell subtypes, such as nucleated and mononuclear cells, the percentage of cells between subjects varied to a small degree.

Table 14.2 Clinical trials in post-myocardial infarction stem cell therapy

Study	Cell type	Cell number	Route	*N*	Time	Outcome
TIME (2012) [89]	Autologous BM-MNC	100 million	IC	40	3–10 days post MI	No change in LVEF Decreased LVEDV
Late TIME (2010) [119]	Autologous BM-MNC	150 million	IC	120	3–7 days post MI	No change in LVEF or LV function
SWISS-AMI (2013) [91]	Autologous BM-MNC	50–500 million	IC	200	5–7 days or 3–4 weeks post MI	No change in LVEF
MYSTAR (2009) [94]	Autologous BM-MNC	Intramyocardial (E/L): 200.3/194.8 million Intracoronary (E/L): 1300/1290 million	IC + IM	60	3–6 weeks or 3–4 months post MI	Increased LVEF and decreased infarct size in E/L groups
SCAMI (2013) [100]	Autologous BM-MNC	381 million	IC	42	5–7 days post MI	No change in EF, infarct size, EDV, and ESV
MI3 (2015) [103]	Autologous BM-MNC	558 million	IC	250	Median 15 days post MI	No change in LVEF
BONAMI (2011) [114]	Autologous BM-MNC	98.3 million	IC	100	9.3 days post MI	No change in LVEF or myocardial viability
ASTAMI (2006) [109]	Autologous BM-MNC	Median 68 million	IC	100	Median 6 days post MI	No change in LVEF
FINCELL (2008) [106]	Autologous BM-MNC	Mononuclear: Mean 402 million	IC	80	2–6 days post MI	Improved LVEF
TECAM (2015) [104]	Autologous BM-MNC ± GCSF	BM-MNC: 83 million BM-MNC + GCSF: 560 million	IC	120	3–5 days post MI	No change in LVEF/ LVESV Reduced infarct size
COMPARE CPM-RMI (2018) [102]	Autologous BM-MNC v CD133+ cells	BM-MNC: 564.63 million CD133+: 8.19 million	IM	77	26.5–30 days post MI	Improved LVEF and decreased wall thickening in both groups Decreased nonviable segments in CD133+ group

REGENT (2009) [118]	Autologous BM-MNC v CD34+/CXCR4+ cells	BM-MNC: Median 178 million CD34+/CXCR4+: Median 1.9 million	IC	200	Median 7 days post MI	Improved LVEF in both cellular groups only in baseline EF < 37%
NCT00264316 (2006) [93]	Autologous BM-SC	304 million nucleated and 172 million mononuclear	IC	67	24 hours post MI	Reduced infarct size Improved regional systolic function No change in LVEF
REPAIR-AMI (2006) [97]	Autologous BM-SC	CD34+/CD45+: Mean 3.6 million CD34+/CD133+/CD45+: Mean 2.5 million	IC	204	3–7 days post MI	Improved LVEF Reduced death, MI, revascularization
REGENERATE-AMI (2016) [101]	Autologous BM-SC	Mononuclear: 59.8 million CD34+: 1.9 million	IC	100	<24 hours post MI	Improved myocardial salvage index No change in LVEF
BOOST (2004) [108]	Autologous BM-SC	Nucleated: 24.6 million CD34+: 9.5 million Hematopoietic colony forming: 3.6 million	IC	60	Mean 4.8 days post MI	Improved LVEF
NCT00363324 (2010) [113]	Autologous BM-SC	402 million	IC	78	2–6 days post MI	Improved LVEF in patients with baseline below average EF
TOPCARE-AMI (2004) [95]	Autologous BM-CD34+/CD45+ cells ± circulating progenitor cells	BM-CD34+/CD45+: 213 million CPC: 16 million	IC	59	3–7 days post MI	Improved LVEF in both groups Decreased ESV in both groups

(continued)

Table 14.2 (continued)

Study	Cell type	Cell number	Route	*N*	Time	Outcome
AMR1 (2011) [105]	Autologous BM-CD34+ cells	5, 10, 15 million	IC	31	Median 8.3 days post MI	Reduced infarct size and perfusion with increasing cellular doses
PreSERVE AMI (2017) [111]	Autologous BM-CD34+ cells	Mean 14.9 million	IC	161	Mean 9.3 days post MI	No change in resting myocardial perfusion
SEED-MSC (2014) [98]	Autologous BM-MSC	72 million	IC	60	1 month post MI	Improved LVEF
IRB SCH2011–006 (2018) [116]	Autologous BM-MSC	7.2x107 cells	IC	26	1 month post MI	Improved LVEF
NCT00114452 (2009) [92]	Allogenic BM-MSC	0.5, 1.6, 5 million cells/kg	IV	53	1–10 days post MI	Improved LVEF Improved global symptoms score
NCT00883727 (2015) [107]	Allogenic BM-MSC	2 million cells/kg	IV	20	2 days post MI	No change in EF No change in perfusion
NCT01291329 (2015) [99]	Allogenic Wharton's jelly MSC	6 million	IC	116	5–7 days post MI	Improved LVEF Decreased ESV/EDV Increased myocardial viability and perfusion
MAGIC Cell (2004) [110]	Autologous peripheral blood SC mobilized with GCSF	100 million	IC	27	2–270 days post MI	Improved exercise capacity, perfusion, systolic function Increased stent stenosis with GCSF

CADUCEUS (2012) [88]	Autologous CDSC *	12.5, 17.3, 25 million	IC	31	1.5–3 months post MI	Reduced scar mass Increased viable heart mass and regional contractility Increased regional systolic wall thickening No change in LVEF/ESV/EDS
CAREMI (2018) [115]	Allogenic CSC *	35 million	IC	49	5–7 days post MI	No change in infarct size or LV remodeling

Summary table of design and outcomes of current post-MI stem cell therapy clinical trials
N number of participants randomized, *BM-MNC* bone marrow mononuclear cells, *IC* intracoronary, *MI* myocardial infarction, *LVEF* left ventricular ejection fraction, *LVEDV* left ventricular end-diastolic volume, *LV* left ventricular, *E/L* early/late, *IM* intramyocardial, *EF* ejection fraction, *EDV* end-diastolic volume, *ESV* end-systolic volume, *GCSF* granulocyte colony-stimulating factor, *LVESV* left ventricular end-systolic volume, *BM-SC* bone marrow stem cells, *BM-MSC* bone marrow mesenchymal stem cells, *IV* intravascular, *MSC* mesenchymal stem cells, *SC* stem cells, *CDSC* cardiosphere-derived stem cells, *CSC* cardiac stem cell, *endogenous stem cell source

14.4.2 Trial Outcomes

Comparison of outcomes across studies is difficult due to the lack of standardization of timing, inclusion criteria, cell number, and type. Despite these limitations, a generalization can be made that stem cell treatment is associated with only a modest improvement in outcome, as only 64% of the studies examined showed efficacy. Autologous bone marrow-derived mononuclear cells, although most studied, were associated with the least favorable outcomes. Six out of 12 patient cohorts treated with mononuclear cells did not have any significant improvement in any outcome. Three studies showed improvement in LVEF. One study showed decreased left ventricular end-diastolic volume (LVEDV), and one study showed reduced infarct size. Mononuclear cell treatment was also associated with decreased infarct size regardless of treatment timing (3–6 weeks versus 3–4 months) in one study and decreased systolic wall thickening in another. LVEF improved in one study only in a subset of patients with initial EF < 37%.

Autologous BM-derived stem cells, including hematopoietic, endothelial, cardiac/hematopoietic, and early progenitor cells, showed by far the highest efficacy rates with 90% of studies showing a significant increase in various outcome parameters. Six studies reported increased LVEF, up to as much as 18 months after treatment. Perhaps most notably, treatment with a combination of CD34+/CD45+ and CD34+/CD133+/CD45+ cells reduced combined death, recurrent MI, and any revascularization procedures at 1 year. Other outcomes associated with BM-derived stem cell treatment included reduced myocardial infarct size, recovery of regional systolic function and myocardial deformation, improved perfusion, decrease in end-systolic volume (ESV), improved myocardial salvage index, decreased systolic wall thickening and nonviable segments, as well as increased LVEF in patients with baseline EF < 37%.

Autologous BM-MSC improved LVEF in two studies, while allogenic BM-MSC were only efficacious 50% of the time, though they were only used in two studies. They increased LVEF and global symptom score in patients at 6 months in one cohort, although no change in LVEF or perfusion was observed in another study at the same time point. Wharton's jelly-derived MSC were associated with a higher absolute increase in myocardial viability and perfusion at 4 months, as well as increased LVEF and decreased end-systolic and diastolic volumes at 18 months.

Autologous cardiosphere-derived stem cells reduced scar mass and increased viable heart mass, regional contractility, and regional systolic wall thickening, though there was no appreciable change in LVEF at 12 months. Interestingly, allogenic cardiac stem cells were not associated with a change in infarct size or LV remodeling. Peripheral blood stem cells mobilized with granulocyte colony-stimulating factor (GCSF) increased exercise capacity, myocardial perfusion, and systolic function, although the use of GCSF was associated with a higher rate of in-stent stenosis at 6 months. Beneficial effects based on cellular type are summarized in Table 14.3 and Fig. 14.1.

Table 14.3 Benefits after acute MI-based on cell type

Cellular subtype	Improvements seen
Autologous BM-MNC	Increased LVEF Decreased LVEDV Decreased infarct size Decreased systolic wall thickening
Autologous CD133+ cells	Increased LVEF Decreased nonviable segments Decreased systolic wall thickening
Autologous CD34+/CXCR4+ cells	Increased LVEF in patients with EF < 37%
Autologous BM-SC	Increased LVEF Reduced myocardial infarct size Improved recovery of regional function Reduced combined death, recurrence of MI, and need for revascularization Improved myocardial salvage index Improved regional myocardial deformation
Autologous BM-CD34+ cells	Reduced infarct size Improved perfusion
Autologous BM-CD34+/CD45+ cells	Increased LVEF Decreased ESV
Autologous circulating peripheral blood stem cells	Increased LVEF Decreased ESV Increased exercise capacity Improved myocardial perfusion Improved systolic function
Autologous BM-MSC	Increased LVEF
Allogenic BM-MSC	Improved LVEF Improved global symptoms score
Allogenic Wharton's jelly MSC	Increased LVEF Improved infarct perfusion Increased myocardial viability Decreased ESV/EDV
Autologous CDSC	Reduced scar mass Increased viable heart mass Increased regional contractility Increased regional systolic wall thickening

Summary of advantageous post-acute MI outcomes based on cell source
BM-MNC bone marrow-derived mononuclear cell, *LVEF* left ventricular ejection fraction, *LVEDV* left ventricular end-diastolic volume, *EF* ejection fraction, *BM-SC* bone marrow-derived stem cells, *MI* myocardial infarction, *ESV* end-systolic volume, *BM-MSC* bone marrow mesenchymal stem cells, *MSC* mesenchymal stem cell, *EDV* end-diastolic volume, *CDSC* cardiosphere-derived stem cell

14.4.3 Stem Cell Therapy in Ischemic Cardiomyopathy

Although the primary focus of stem cell therapy remains to prevent myocardial loss and allow for regeneration of tissue immediately after an MI, clinical trials are also underway to evaluate the ability of stem cells to remuscularize and reactivate innate

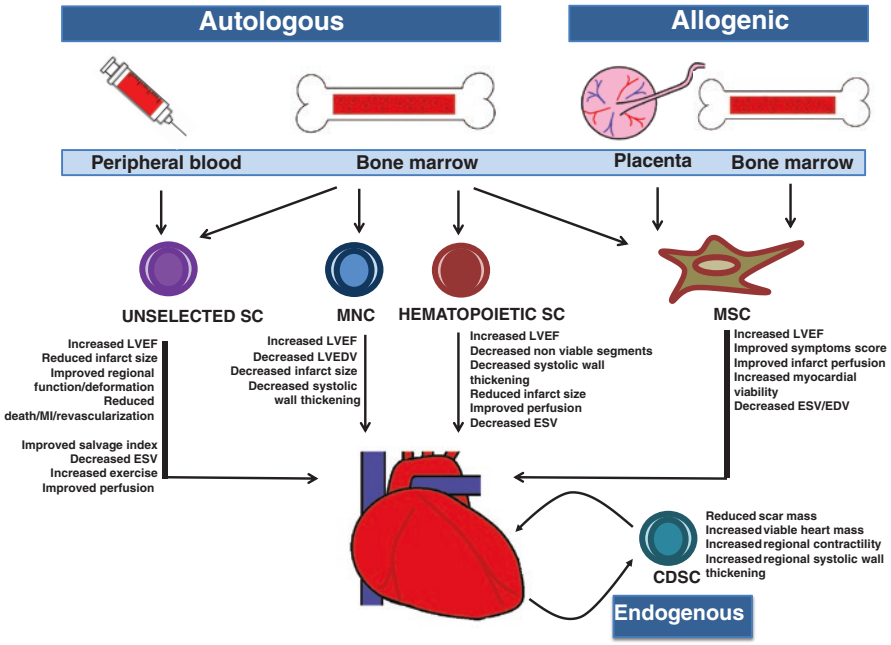

Fig. 14.1 Stem cell types and benefits in treatment after MI**.** SC, stem cell; MNC, mononuclear cell; MSC, mesenchymal stem cell; LVEF, left ventricular ejection fraction; MI, myocardial infarction; ESV, end-systolic volume; LVEDV, left ventricular end-diastolic volume; EDV, end diastolic volume; CDSC, cardiosphere-derived stem cell

cardiac regeneration pathways in models of heart failure secondary to chronic cardiomyopathy [22]. Similar to studies targeting treatment of acute MI, cells evaluated in ischemic cardiomyopathy include skeletal myoblasts, bone marrow-derived unselected and selected stem cells, MSC, embryonic stem cells, and cardiac-committed progenitor cells [22].

Skeletal myoblasts do not appear to improve LVEF [120]. Unselected bone marrow stem cells are less commonly used in HF models although appear to have as little efficacy as when used in acute MI trials [121–125]. Bone marrow-derived hematopoietic stem and progenitor cells appear to improve unstable angina, but their efficacy in HF is less established [126–129]. Similar to post-MI studies, MSC appear to be among the most efficacious in HF models [130–133]. Cardiac stem cells including KIT+ and cardiosphere-derived cells (CDC) both appear to show some efficacy in clinical trials [134, 135]. Transplantation of embryonic stem cell-derived cardiac progenitor cells appears to confer a symptomatic benefit, although a very small number of patients have been evaluated thus far, necessitating further trials [136]. Analysis of completed clinical trials in chronic HF suggests MSC and CSC as the most promising cell types, and although some efficacy has been established, many more clinical trials and optimal delivery vehicles are needed before stem cell therapy becomes standard of care.

14.5 Limitations of Stem Cells Therapy

A substantial limitation of stem cell therapy post-MI is the low homing, retention, and differentiation rate of cells in the ischemic microenvironment of the infarcted heart [137]. Human studies have shown a 39% cellular retention rate just 1 hour following transplantation that is attributable to high rates of apoptosis [71]. The high rate of cell death after transplantation can be attributed to inflammation, mechanical injury, hypoxia, and reperfusion injury [138]. Furthermore, loss of matrix attachment during cell preparation and following injection contributes to programmed cell death [139]. Ischemia is a major hurdle for stem cell populations to differentiate into cardiomyocytes, particularly ones that become electromechanically integrated [24].

Although most clinical trials demonstrate safety following stem cell transfer, with only a few complications reported, animal studies have shown increased risk of ventricular arrhythmias following human cardiomyocyte transfer into guinea pigs and nonhuman primates [140, 141]. In addition, isolation of adequate quantity of stem cells, expansion, and optimal delivery methods that allow for cell retention and differentiation are lacking [28]. Although peripheral and bone marrow stem cells are easier to harvest, attaining adequate number of organ-derived cells, such as cardiac stem cells, is invasive and often low yield [28]. Several clinical trials have also shown that transplanted cells may not be capable of integration and electrochemical coupling, suggesting that their effects are predominantly paracrine in nature and may not add directly to myocyte mass [142].

Meaningful decisions and meta-analyses of clinical trial data are difficult to interpret and synthesize in light of heterogeneity of trial design and reporting. Clinical trials completed thus far have varying, though usually low, number of participants. Primary outcomes measured vary from study to study, and some lack diverse clinical assessment tools. Inclusion criteria, stem cell type and number, delivery methods, and timing vary greatly across trials. Some studies lack placebo groups, making them prone to observation bias, while others evaluate safety only without efficacy. Variable outcomes across studies can easily be attributed to the heterogeneous number and quality of cells used [143].

14.5.1 Modifications to Enhance Cell Function

Multiple strategies including in vitro cellular preconditioning or reprogramming via environmental, pharmacological, and genetic means have been explored in order to increase in vivo cell survival [137]. These strategies include culturing cells under ischemic conditions, supplementing culture medium with growth factors, as well as transfecting cells with proangiogenic and anti-apoptotic factors [57]. Culturing MSC in low oxygen conditions prior to transplant activates survival pathways, upregulates pro-survival genes, increases anti-apoptotic genes including Akt and

eNOS, and upregulates pro-angiogenic cytokines including vascular endothelial growth factor (VEGF) [144]. Additionally, hypoxia allows cells to preserve stemness and promote differentiation and proliferation in vivo [145]. In vitro burst exposure of cells to low levels of oxidative stress and thermal shock treatment also improves cell viability and functional outcomes [146, 147].

Preconditioning of cells with several therapeutic drugs increased secretion of growth factors, including vascular endothelial growth factor (VEGF), angiopoietin-1α (Ang-1α), stromal cell-derived factor-1 (SDF-1), hepatocyte growth factor (HGF), and IGF [148]. Several mitochondrial potassium channel opening drugs, including pinacidil and diazoxide, suppress apoptosis and increase cell survival in ischemic conditions [149, 150]. In one study, treatment of cardiac stem cells with hydrogen peroxide increased endothelial and vascular smooth muscle gene expression and angiogenesis [25]. In vivo treatment with statins increased cell survival and differentiation, while in vitro treatment improved function of endothelial progenitor cells [151, 152]. Pre-treatment of several cell lines with oxytocin improves their response to oxidative stress and differentiation into cardiomyocytes and vascular cells [153, 154]. Multiple other drug classes including trimetazine, β-mercaptoethanol, caspase inhibitors, 5-Azacytidine, and the kinase inhibitor Imatinib have been used in vitro to increase cell viability, confer resistance to oxidative injury, increase cellular engraftment, and prime cellular differentiation toward a cardiac fate, respectively [155–157].

Genetic manipulation of stem cells prior to transfer is another strategy used to improve efficacy, as transgenes can be targeted to release pro-angiogenic and chemoattractant factors, as well as anti-apoptotic proteins. For example, insertion of the pro-survival gene Pim-1 kinase into cardiac stem cells decreased infarct scar mass in a pig model [25]. Transformation of stem cells with IGF-1, which induces expression of survival genes, enhanced survival, engraftment, and differentiation [158]. IGF-1-transformed MSC showed efficacy in improving ejection fraction in animal studies. Overexpression of Ang-1, HGF, VEGF, and MyoD in post-MI studies have consistently shown improved cellular retention, likely secondary to increased angiogenic potential of pre-treated cells [159–161]. Akt-modified bone marrow-derived MSC survival is upregulated via secretion of numerous growth factors, including bFGF, HGF, IFG-1, and VEGF [162].

Because adhesion to an extracellular matrix is important for the survival of several stem cells, notably MSC, injection of cells and lack of healthy ECM in infarcted hearts potentiate apoptosis. To address this, overexpression of tissue transglutaminase in MSC increased survival leading to improved restoration of cardiac function [163]. Transfection of integrin-linked kinase (ILK), which contributed to cell adhesion and ECM assembly, improves cellular survival in hypoxic conditions and reduces infarct size in animal studies [164].

Resident stem cells become senescent and lose their regenerative capacity with age, resulting in reduced proliferation, differentiation, and metabolic activity [165]. These changes are driven by telomere shortening and upregulation of p53 genes [166]. For example, MSC derived from older patients are not as efficacious in post-MI models as those derived from younger patients [167]. Strategies to combat

senescence have been examined and include modification of human cardiac progenitor cells with Pim-1 and upregulation of the WNT/β-catenin signaling pathway, both of which result in improved cellular function [168, 169].

14.6 Future Directions

Although stem cell therapy after MI is gaining momentum with promising initial results, multiple limitations must be overcome to realize the full potential that cellular therapy has to offer. The optimal cell source for use in clinical trials must be determined. Although embryonic stem cells confer immune privilege, they are associated with ethical dilemmas and are teratogenic in undifferentiated forms. While embryonic stem cell-derived cardiac precursors eliminate teratogenic potential, their differentiation protocols currently produce low yields and must be improved. Resident cardiac stem cells are difficult to harvest and are low in number. Bioreactors and devices to standardize and improve differentiation yields are on the horizon, although further research needs to be accomplished [170].

iPSC are an ideal cell candidate for clinical translation since they are derived from adult somatic cells via noninvasive techniques and can repopulate any cardiac lineage. Although the first iPSC clinical trial is currently being planned, nonviral transfection protocols to derive iPSC cells must be optimized to prevent genomic instability. Furthermore, differentiation protocols and elimination of undifferentiated cells via induced cell apoptosis must ensure patient safety. Paracrine effects of cell therapy must be defined more clearly, and the potential of exosomes must be studied, as use of exosomes alone without cellular transfer could realize the full potential of iPSC cells.

Cell survival and homing, particularly with intravenous and intracoronary routes, are extremely low, with cell loss being exacerbated by the ischemic post-infarct environment. Preconditioning of cells prior to transfer via genetic modifications and drug treatments, as well as improved homing mechanisms, must be developed to improve the number of cells participating in repair. In addition, methods of preventing resident stem cell senescence and improve mobilization must be elucidated.

Optimal delivery methods for stem cell treatment must be redesigned. Although intramyocardial injections deliver cells directly to infarcted areas, they are invasive and associated with generating pro-arrhythmogenic foci. Intracoronary and intravenous injections suffer from poor cellular homing. While patches and scaffolds afford the added benefit of maintaining an optimal scaffold, they can only be delivered at the time of surgery. Gelling systems loaded with cytokines and pro-survival proteins must be refined to allow for noninvasive delivery. In addition, three-dimensional (3D) and bioprinted cellularized vascular constructs are currently being developed.

Currently, protocols for clinical trials of stem cell therapy vary greatly and lack standardization. In order to make meaningful comparisons and interpretations across trials cell type, delivery methods and timing, as well as measured outcomes, must be standardized.

14.7 Conclusion

Myocardial infarction and ischemic cardiomyopathy confer significant morbidity and mortality, yet despite best medical care, many patients who suffer from an MI go on to develop heart failure, secondary to myocardial necrosis and pathologic myocardial remodeling. The population of resident cardiac stem cells available to replenish lost cells is low and easily overwhelmed by ischemia. Although the design of clinical trials is not uniform, and comparisons cannot be easily made, delivery of both endogenous and exogenous stem cells to ischemic myocardium has shown some efficacy at reducing infarct size and improving long-term function.

Several issues are currently being addressed in order to optimize stem cell efficacy. In addition to standardizing cellular type, delivery method, and timing, clinical trials must focus on similar outcomes. The optimal cell type and differentiation methods are being determined, with iPSC and exosomes holding great promise. The most direct, least invasive delivery method and improvement of cell homing and survival are yet to be overcome. Despite all of these obstacles, stem cell therapy holds great promise in post-MI regeneration.

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