MAYO CLINIC

Breakthroughs in Cell Therapy for Heart Disease: Focus on Cardiosphere-Derived Cells

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CME Activity

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Abstract

The clinical reality of cell therapy for heart disease dates back to the 1990s, when autologous skeletal myoblasts were first transplanted into failing hearts during open-chest surgery. Since then, the focus has shifted to bone marrow–derived cells and, more recently, cells extracted from the heart itself. Although progress has been nonlinear and often disheartening, the field has nevertheless made remarkable progress. Six major breakthroughs are notable: (1) the establishment of safety with intracoronary delivery; (2) the finding that therapeutic regeneration is possible; (3) the increase in allogeneic cell therapy; (4) the effect of increasing mechanistic insights; (5) glimmers of clinical efficacy; and (6) the progression to phase 2 and 3 studies. This article individually reviews these landmark developments in detail and concludes that the field has reached a new phase of maturity where the prospect of clinical impact is increasingly imminent.

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Each year, approximately 1 million Americans have a myocardial infarction (MI).[1] Although acute mortality has decreased in recent decades because of the universal adoption of reperfusion therapy,[2] up to 36% of MI survivors will develop heart failure (HF) and consequently be at increased risk for premature death.[3] Whether due to MI or another cause, HF affects approximately 5 million Americans.[1] Patients are unable to exercise normally (in the extreme, they become bedbound) and experience shortness of breath. Current therapy relies on drugs that block various maladaptive signaling pathways, such as β-adrenergic blockers and angiotensin inhibitors. Additional benefit can sometimes be gained from pacemakers that attempt to normalize the pattern of cardiac contraction. Although such drugs and devices can attenuate the progression of HF, no treatment modality currently

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available addresses the root cause, which is a loss of functional heart muscle.[4] Cell therapy for heart disease aims to regenerate viable myocardial tissue that has been lost to disease. The main targets to date have been MI and HF. In the case of MI, the goal is to avert the progression to HF; in already established HF, cell therapy seeks to halt further deterioration or even to reverse the disease. Clinical trials have resulted in inconsistent partial restoration of cardiac structure and function,[5] giving cause for optimism but leaving much room for improvement.

In reflecting on the field, I have identified 6 major developments that have the potential to shape future progress. Time will tell just how durable these developments are and whether they will ultimately be hailed as genuine breakthroughs, but this article lists and discusses them one at a time. The perspective is personal, as will be evident from the fact that the work highlighted in 3 of the 6 bullets is my own. Nevertheless, I attempt to temper what may be seen as self-congratulatory enthusiasm with a number of caveats and concerns regarding the vast remaining gaps in our knowledge.

BREAKTHROUGH 1: ESTABLISHMENT OF SAFETY WITH INTRACORONARY DELIVERY

Skeletal myoblasts were the first cells to be applied to heart disease, on the logical premise that autologous satellite cells might develop into mature contractile units when implanted ectopically into the diseased heart.[6] The paradigm involved harvesting skeletal muscle biopsy specimens from patients with HF who were to undergo elective cardiac surgery; myoblasts would be grown ex vivo and then reimplanted by direct intramyocardial injection at the time of surgery. Despite early enthusiasm regarding this therapy, skeletal myoblasts eventually proved to be risky (ventricular arrhythmias were frequent) and without much functional benefit: the 300-patient phase 2 Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial was halted after an interim analysis of the first 97 randomized patients revealed no robust trend to efficacy.[7]

Since then, the focus has shifted to other cell types and to percutaneous catheter–based delivery methods. In 2001,[8] the first acute MI patient was treated with bone marrow–derived mononuclear cells (BMMCs). The paradigm has been oft-repeated and, collectively, forms the basis for the most substantive clinical experience to date with cell therapy for heart disease. After conventional intervention to restore patency of the occluded coronary artery, patients undergo bone marrow aspiration for derivation of BMMCs. The cells are rather finicky: details of manufacturing importantly influence potency, likely contributing to heterogeneous results among trials.[9,10] Typically, 1 to 14 days after MI, BMMCs are reintroduced into the patient via the intracoronary route using a balloon catheter inflated at the site of the initial blockage.

The salient finding has been the superior safety record of intracoronary BMMCs. Figure 1 shows the results of a meta-analysis of 7 trials that involved 660 patients.[11] Compared with baseline, BMMC transfer performed 4 to 7 days after MI decreased revascularization, cumulative clinical events of death or recurrent MI, culprit artery restenosis, and ventricular arrhythmia. The lack of excess arrhythmias in BMMC-treated patients is particularly notable. Although BMMCs are the only cell type for which results for large numbers of patients are available, the general pattern of safety with intracoronary delivery has held up so far with cardiac-derived cells as well.[12-14] One feature that BMMCs and cardiac-derived cells share is a predominantly indirect mechanism of action: long-term engraftment is not required for durable benefit.[15-17] The problem of arrhythmia is related to conduction block and inhomogeneity of repolarization; these factors are likely to be much more severe with skeletal myoblasts (that do not integrate electrically in the myocardium) or pluripotent cell–derived products. Indeed, Cingolani and I[18] have speculated that indirectly acting cells will be less arrhythmogenic than those that engraft, differentiate, and proliferate in vivo. The idea is that endogenous regeneration is likely to cause less electrical instability than transplantation of highly proliferative cells; the latter may colonize the heart, producing barriers to conduction and/or aberrant repolarization. The finding that intracoronary delivery of nonengrafting cells is safe, particularly with regard to arrhythmia, represents a major breakthrough for the field of cell therapy.

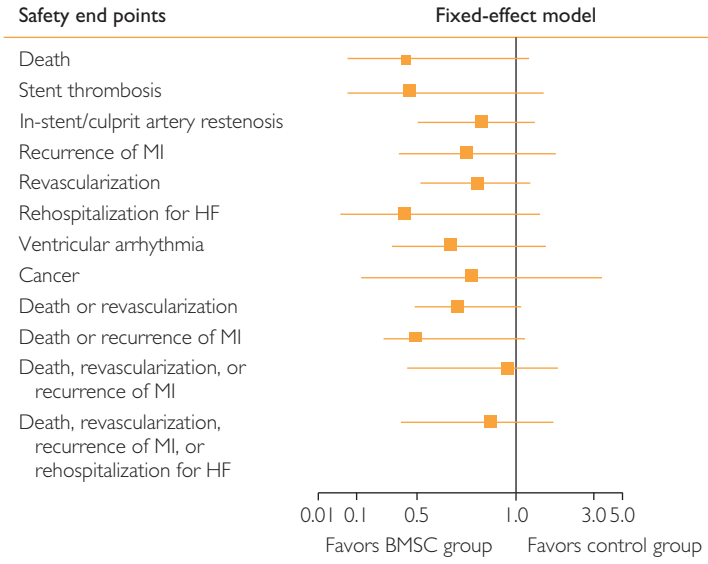

FIGURE 1. Meta-analysis of safety data from 5 major bone marrow mononuclear cell (BMMC) trials, as performed by Zhang et al.[11] Odds ratios of BMMC infusion therapy with respect to individual and cumulative clinical end points are listed. Adapted from Zhang et al[11] with permission. BMSC = bone marrow stem cell; HF = heart failure; MI = myocardial infarction; OR = odds ratio.

BREAKTHROUGH 2: FINDING OF THERAPEUTIC REGENERATION

Regeneration is defined as "regrowth of lost or destroyed parts or organs."[19] Although human BMMC studies have reported reductions in scar size,[20] the effect is solely on scar mass with no reciprocal increase in viable myocardium. Thus, such changes report a decrease in the extent of injury but not regrowth of destroyed parts. During the past 10 years, my laboratory has developed cardiosphere-derived cells (CDCs) as a candidate cell type for regenerative therapy after MI.[21] These heart-derived cells are stem cells in that they exhibit multilineage potential and clonogenicity,[22] but they work primarily through indirect mechanisms.[15] At least 6 independent laboratories worldwide have reproduced the published methods and verified CDCs' identity and utility.[17,23-27] The CDCs were first used clinically in the Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) trial.[13,14] The CADUCEUS trial tested the safety and efficacy of intracoronary autologous CDCs in 17 patients with left ventricular dysfunction and a recent MI (1.5-3 months earlier) compared with 8 routine care controls. In more than 12 months of follow-up, safety end points, including arrhythmia, were not significantly different in control and CDC-treated groups. Contrast-enhanced magnetic resonance imaging (MRI) revealed reductions of scar mass at 6 and 12 months in CDC-treated patients (but not in controls; Figure 2, A, left). Scar reduction is notable, but tissue regeneration was manifested by an unprecedented increase in viable tissue (Figure 2, A, right). The reductions in scar mass correlated with the increases in viable mass (Figure 2, B), consistent with (but not proving) the idea that scar is being converted to viable tissue as a consequence of treatment with CDCs. Histologic analysis in animal models reveals that CDCs do not induce myocyte hypertrophy[13,28]; in contrast, cell size tends to be smaller, consistent with an increase in cell number. Moreover, porcine studies that compare contrast-enhanced MRIs with histologic sections confirm that MRI accurately reports scarred and viable myocardium after CDC therapy.[28]

Neither scar mass nor viable heart mass changed over time in the CADUCEUS controls,[13,14] in line with the traditional belief that chronic MI injury is irreversible: once formed, scar does not resolve on its own, and, once

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lost, living heart muscle does not spontaneously regrow. The CADUCEUS trial was the first controlled clinical trial to report an increase in viable tissue as a result of cell therapy.[13,14] Limited MRI data from the Stem Cell Infusion in Patients with Ischemic Cardiomyopathy trial of c-kit+ cells also indicated increases in viable myocardium in cell-treated patients, but no controls underwent imaging.[29] The finding that iatrogenic cardiac regeneration is indeed possible represents a major breakthrough for the field of cell therapy.

BREAKTHROUGH 3: THE RISE OF ALLOGENEIC CELL THERAPY

During the first decade of cell therapy for heart disease, most clinical trials were conducted using autologous cells. This approach has the advantage that it avoids immunologic rejection, but autologous therapy requires patient-specific tissue harvesting, cell processing, and quality control, imposing significant risk, expense, and inflexibility with regard to the timing of treatment. In addition, cell efficacy may vary with donor age and comorbidities. The use of allogeneic cells, if safe and effective, would obviate such limitations, enabling the generation of highly standardized "off-the-shelf" cell products. The obvious disadvantage is the risk of immune rejection, which may limit effectiveness whether or not it poses safety hazards. Nevertheless, because most of the observed functional benefit is attributable to indirect pathways even with heart-derived cells,[15-17] rejection of allogeneic cells may not be an issue if it occurs after the cells have exerted their beneficial paracrine effects and if the resulting benefits are durable.

For some time, mesenchymal stem cells (MSCs) have been developed for potential therapeutic application. Allogeneic MSCs or their precursors have been used in various early-phase human trials of MI and HF, with no safety concerns reported to date.[30] The Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration trial of allogeneic CDCs after MI, currently in progress (http://clinicaltrials.gov/show/NCT01458405), is based on my laboratory's discoveries that allogeneic CDC transplantation without immunosuppression is safe, promotes cardiac regeneration, and improves heart function in rats[17] and pigs[28] with MI. Figure 3 shows results of a porcine study of allogeneic CDCs after MI,[28] with reduction of scar, increase of viable tissue, and preserved global function in cell-treated animals relative to vehicle-only controls. The indirect mechanism of action rationalizes the persistence of benefit despite the evanescent survival of transplanted cells. Once activated, endogenous regenerative pathways have their own momentum, not requiring the continued presence of CDCs. The increasing recognition of the safety and efficacy of allogeneic cells, specifically MSCs and CDCs, represents a major breakthrough for the field of cell therapy.

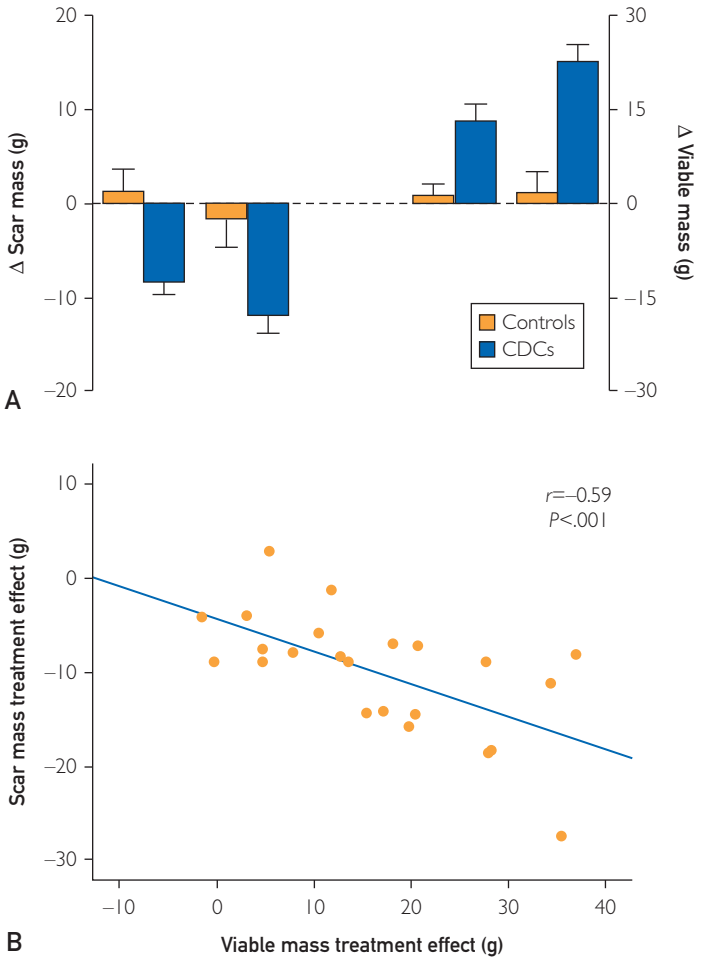

FIGURE 2. Changes in scar mass and viable mass in Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction trial patients. A, Differences in scar mass from baseline to 6 or 12 months (left) and differences in viable left ventricular mass from baseline to 6 or 12 months (right). B, Correlation between the changes in scar mass and the changes in viable mass in cardiosphere-derived cell (CDC)—treated patients at 6 and 12 months. Adapted from Makkar et al[13] and Malliaras et al[14] with permission.

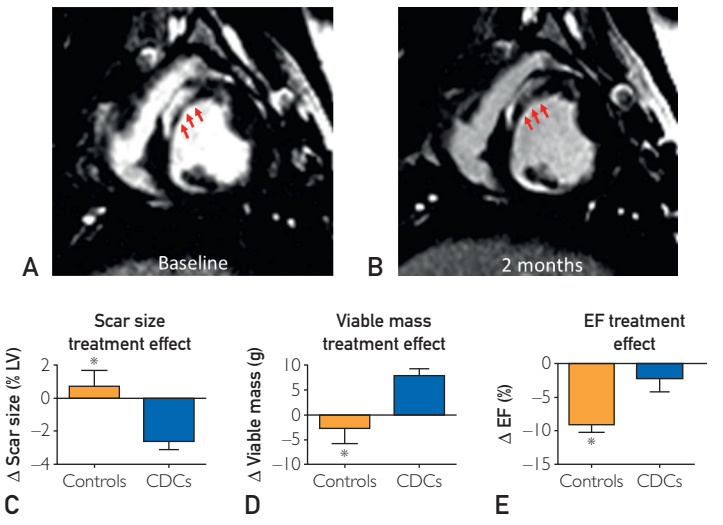

FIGURE 3. Allogeneic cardiosphere-derived cells (CDCs) attenuate adverse remodeling and improve global function in a porcine preclinical post–myocardial infarction (MI) model. Matched cine short-axis images at baseline (3 weeks after MI) (A) and 2 months after CDC treatment (B). Changes in scar mass (C), viable mass (D), and ejection fraction (EF) (E), respectively, between placebo-treated controls and CDC-treated animals 2 months after therapy vs baseline values in each animal. Adapted from Malliaras et al[28] with permission.

BREAKTHROUGH 4: INCREASING MECHANISTIC INSIGHTS

The guiding principle underlying the use of stem cells to achieve regeneration is the idea that injected cells will engraft, proliferate, and differentiate, thereby repopulating the injured heart. However, in many published studies, cell transplantation produces beneficial effects despite poor retention and minimal long-term survival of transplanted cells.[31] How can transient, paltry short-term cell survival suffice to produce lasting benefits? Multiple lines of evidence now indicate that most of the beneficial effects of transplanted CDCs are indirect (Figure 4 shows that, after human CDCs are injected in severe combined immunodeficiency mice, most of the "new" heart and vascular cells are of mouse origin[15]); in the extreme, allogeneic CDCs are cleared completely within several weeks, but their functional and structural benefits persist at least 6 months.[17] Thus, long-term transplanted cell survival is not required for sustained benefit. This appears to be true for many other nonpluripotent cells.[5,16]

The CDCs are rich biological factories, secreting diffusible factors that promote angiogenesis, recruit endogenous progenitor cells, and coax surviving heart cells to proliferate[15,32,33]; on the other hand, injected CDCs suppress maladaptive left ventricular remodeling,[32] apoptosis,[34,35] tissue fibrosis,[36] and inflammation after MI. Although it is possible that CDCs secrete a complex medley of individual growth factors that collectively produce distinctive benefits, the involvement of master regulators, such as microRNAs, would help tie together the various effects without postulating complex mixtures of many secreted factors. MicroRNAs are short noncoding RNAs that regulate gene expression by targeting families of transcripts for degradation and thereby play pivotal roles in development, homeostasis, and disease.[37] The roles of several microRNAs in the heart have been elucidated by recent studies; cardiovascular effects include modulation of susceptibility to oxidative stress and induction of cardiomyocyte proliferation.[38,39] Moreover, microRNAs are known to confer long-lasting benefits and fundamental alterations of the injured microenvironment.[37]

How might microRNAs be transferred from CDCs to surrounding myocardium? Exosomes are lipid-bilayer vesicles secreted by a variety of cells that play important roles in paracrine and autocrine signaling[40]; they have, for example, been implicated as mediators of the angiogenic effects of $CD34^+$ endothelial progenitor cells.[41] Exosomes are particularly rich in nocoding RNA, including microRNAs.[42] Exosomes can cross biological membranes, and their lipid-bilayer structure protects the cargo from degradation, enabling the natural delivery of microRNAs to targets. We are currently testing the hypothesis that CDC-exosomes mimic and

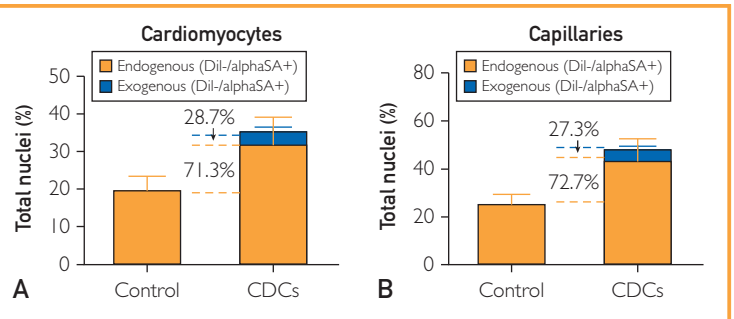

FIGURE 4. Indirect regeneration from cardiosphere-derived cells (CDCs). Three weeks after myocardial infarction, human CDC-treated mice had more cardiomyocytes (A) and capillaries (B) in the infarct area compared with controls (injected only with vehicle). More than 70% of the additional cardiomyocytes and capillaries are of endogenous (orange) origin (blue). Adapted from Chimenti et al[15] with permission.

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mediate the beneficial effects of CDCs and that these exosomes are replete with microRNAs.[43]

Regardless of the precise mediators that may turn out to be operative, there has been a major conceptual shift from canonical stem cell–based mechanisms to the notion that most clinically-applied cells work indirectly. The practical implications are multifarious: we have already alluded to the implications for arrhythmogenicity and allogeneic therapy. The recognition of dominant indirect mechanisms also stimulates the search for next-generation therapeutic candidates that may be able to harness the benefits of cell therapy without the vagaries of cell harvesting, processing, and delivery. By rationalizing allogeneic therapy and opening up new prospects for cell-free products, our increasing mechanistic understanding represents a major breakthrough for the field of cell therapy.

BREAKTHROUGH 5: GLIMMERS OF CLINICAL EFFICACY

The BMMC experience has been notable for little evidence of benefit in surrogate end points, namely, an inconsistency of improvements in ejection fraction and scar size and the absence of rigorous evidence of genuine myocardial regeneration. Nevertheless, it is intriguing that significant benefits on clinical end points have been reported. The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction study reported favorable clinical outcomes associated with cell therapy, sustained at 5 years of follow up,[44] despite being underpowered to detect such differences. At 5 years, the composite end point of death, MI, or revascularization exhibited an odds ratio of 0.62 in favor of the BMMC-treated group relative to placebo (P=.03). In addition, trends in favor of BMMC therapy with regard to hard clinical end points have also emerged from meta-analyses.[11,45]

In an attempt to reconcile equivocal functional benefits with extraordinary clinical outcomes, the following considerations are relevant. First, ejection fraction (which is load dependent) may not be the best-suited surrogate marker for assessing the effects of cell therapy. In patients with MI, scar size measured by MRI is a better predictor of mortality compared with ejection fraction.[46] Second, patients enrolled in the first generation of BMMC clinical trials had well-preserved ventricular function on average, leaving little room for improvement. The principal patient population has been a first-MI population, who received prompt reperfusion and state-of-the-art medical therapy so that the extent of injury is limited. Meanwhile, the greatest benefits of stem cell therapy occur in patients with the greatest MI-induced myocardial damage (eg, in the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction study,[47] the Finnish Stem Cell Study,[48] and the Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction study,[49] the major determinant of functional recovery after cell therapy was low baseline ejection fraction). This finding has major implications for the design of future clinical studies: cell therapy may maximize its potential for successful myocardial repair and regeneration by targeting a sicker patient population. Third, the effects of BMMC therapy on surrogate end points, although seemingly modest, are comparable to what is achieved by established therapeutic strategies, including primary percutaneous coronary intervention, thrombolysis, angiotensin-converting enzyme inhibition, or β-blocker therapy, which are used routinely in clinical practice and confer a survival benefit.[50] However, this conclusion is largely on the basis of meta-analyses and should be taken with a grain of salt.

The increasing recognition that BMMCs have clinical benefits, despite little signal in terms of surrogate end points, represents a major breakthrough for the field of cell therapy. The results give reason to hope that emerging cell types, with greater effects on scar size or ejection fraction, will have superior clinical benefits.

BREAKTHROUGH 6: PROGRESSION TO PHASE 2 AND 3 STUDIES

All too often in cell therapy, as in many other fields, promising early-phase trial results fail to be confirmed in larger studies.[51] Well-powered and rigorously designed (randomized, placebo-controlled, double-blind) large-scale clinical trials with long-term follow-up, focusing on hard clinical end points, are mandatory to determine whether the changes in surrogate end points (eg, scar size, ventricular volumes, and ejection fraction) are consistent and whether

TABLE. Selected Ongoing Phase 2 and 3 Clinical Trials Targeted at Heart Disease[a,b]

Indication and cell type	Sponsor	Phase
Heart failure		
Autologous adipose derived cells	Cytori	2
Autologous expanded BM fractions	Aastrom	2
Autologous BM cardiopoietic MSCs	Cardio3Biosciences	2
SDF-1 plasmid	Juventas	2
Allogeneic MPCs	Teva	3
Post-MI		
Autologous CD34	Amorcyte	2
Allogeneic CDCs	Capricor	2
Autologous BMMCs	European Union	3
Autologous BM CD133	Asklepios Proresearch	2

[a]BM = bone marrow; MSCs = mesenchymal stem cells; MPCs = mesenchymal precursor cells; MI = myocardial infarction; CDCs = cardiosphere-derived cells; BMMCs = bone marrow mononuclear cells.
[b]See www.clinicaltrials.gov for details of each study.

they translate into increased survival and reduced morbidity.[52] Fortunately, several such trials are in progress. The Table lists the major ongoing trials, their phase (2 or 3), corporate sponsors (Effect of Intracoronary Reinfusion of Bone Marrow—Derived Mononuclear Cells on All-Cause Mortality in Acute Myocardial Infarction trial, exceptionally, is paid by public funds from the European Union), and the cell type under study. Notable among these are the phase 2 Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration trial of allogeneic CDCs (http://clinicaltrials.gov/show/NCT01458405) and the phase 3 Effect of Intracoronary Reinfusion of Bone Marrow—Derived Mononuclear Cells on All-Cause Mortality in Acute Myocardial Infarction trial of BMMCs (http://clinicaltrials.gov/show/NCT01569178). The progress from small-scale observational studies to larger studies focusing on clinical end points reflects the increasing interest in specific therapeutic candidates by commercial entities. Without such commercial sponsorship, the potential of the field will never fully materialize, and the wide dissemination of reliable products will be impossible. The progression of selected cell products into advanced-phase clinical trials represents a major advance for the field of cell therapy.

CONCLUSION

In the past several years, we have progressed from a profusion of hype to the point of having a solid basis for moving forward. With the good fortune of prevalent safety to date, we have managed to avoid the sort of debacle that derailed gene therapy for more than a decade.[53] The evidence that therapeutic regeneration is possible, in a setting where conventional wisdom teaches that scar is irreversible, catapults the field onto a new plane yet to be achieved by any other treatment approach. The increasing evidence that allogeneic cells can be safe and effective takes cell preparation and manufacturing into the commercial mainstream and away from the cottage industry paradigm where it has been stalled for so long. Our increasing insights into the mechanism of action of transplanted cells helps us to decide what is and is not rational as we set priorities for future work. The glimmers of clinical efficacy in trials to date, coupled with the increasing number of advanced-phase clinical studies currently in progress, give new reasons for excitement. Other laudable developments not reviewed here include efforts to increase efficacy by conditioning the myocardial environment (eg, CELLWAVE trial[54]) or enhancing cardiogenesis of nonresident stem cells (eg, Cardiopoietic Stem Cell Therapy in Heart Failure trial[55]); such efforts can only enhance progress. In summary, the field has reached an unprecedented phase of maturity in which the prospect of clinical effect is increasingly plausible, if not likely. Exciting times lie ahead.

Abbreviations and Acronyms: **BMMCs** = bone marrow mononuclear cells; **CADUCEUS** = Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction; **CDCs** = cardiosphere-derived cells; **HF** = heart failure; **MI** = myocardial infarction; **MRI** = magnetic resonance imaging; **MSCs** = mesenchymal stem cells

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The Symposium on Regenerative Medicine will continue in an upcoming issue.

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