REVIEW

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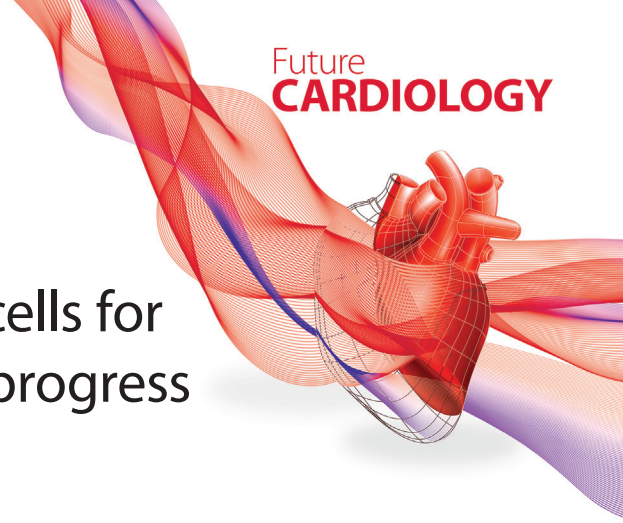

Allogeneic cardiosphere-derived cells for myocardial regeneration: current progress and recent results

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Early-phase clinical testing of autologous cardiosphere-derived cells (CDCs) has yielded intriguing results, consistent with therapeutic myocardial regeneration. However, autologous therapy is associated with significant technical, timing, economic and logistic constraints, prompting researchers to explore the potential of allogeneic CDC therapy. CDCs exhibit a favorable immunologic antigenic profile and are hypoimmunogenic *in vitro*. Preclinical studies in immunologically mismatched animals demonstrate that allogeneic CDC transplantation without immunosuppression is safe and produces sustained functional and structural benefits through stimulation of endogenous regenerative pathways. Currently, allogeneic human CDCs are being tested clinically in the ALLSTAR and DYNAMIC trials. Potential establishment of clinical safety and efficacy of allogeneic CDCs combined with generation of highly standardized, 'off-the-shelf' allogeneic cellular products would facilitate broad clinical adoption of cell therapy.

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• allogeneic cells • cardiosphere-derived cells • cell therapy • heart regeneration • heart repair

Despite significant advances in pharmacological and device-based therapies, ischemic heart disease remains the number one cause of death and years of life lost in the world [1]. In an attempt to address the need for development of novel therapies, cell therapy has emerged over the past 15 years as a potential therapeutic approach for ischemic cardiomyopathy and heart failure [2]. Among the multiple cell types that have been tested (including cells isolated from skeletal muscle [3], the bone marrow [4,5] or the heart itself) [6,7] cardiosphere-derived cells (CDCs) [8] appear particularly attractive for myocardial repair and regeneration, given the intriguing results observed in animal studies and in the first-in-man CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction (CADUCEUS) trial [7,9]. The majority of studies of cell therapy to date have been conducted using autologous (or syngeneic) cells. However, autologous cell therapy is associated with significant limitations (described later), which have prompted researchers to explore the potential of allogeneic cell therapy [10–13]. In this review, we focus on allogeneic CDCs as potential therapeutic agents for heart disease. We describe the *in vitro* immunologic properties of CDCs (from the standpoint of allogeneic cell transplantation), summarize the available *in vivo* preclinical data on allogeneic CDCs, discuss ongoing clinical trials of allogeneic CDCs and touch upon the critical issue of development of potency assays for allogeneic cellular products. The focus of this review lies solely on allogeneic CDCs (or other heart-derived cells that appear similar to CDCs). General discussion of immunologic barriers to allogeneic cell transplantation or discussion of other allogeneic cell types (e.g., allogeneic mesenchymal stromal cells) is intentionally cursory. The reader is referred elsewhere for reviews on these topics [14–16].

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Mesenchymal stromal cells: the prototypical 'universal donor' cells for heart repair

Allogeneic mesenchymal stromal cells (MSCs) are considered the prototypical 'universal donor' cells for cardiac applications. MSCs exhibit an immunologic profile that renders them attractive for allogeneic transplantation without concurrent immunosuppression: they express major histocompatibility complex (MHC) class I, but lack expression of MHC class II surface antigens or costimulatory molecules. In addition, MSCs exert multiple immunosuppressive and immunomodulatory effects *in vitro*, as they have been shown to interact with virtually all immune cells involved in adaptive and innate immunity. More specifically, MSCs (through both paracrine and contact-dependent mechanisms) inhibit T-cell and B-cell proliferation, inhibit the maturation and cytotoxicity of natural killer (NK) cells, dampen the respiratory burst of neutrophils, inhibit the maturation of monocytes into dendritic cells and impair the antigen-presenting function of dendritic cells (see [15] for an excellent review). Preclinical studies in large animal models of ischemic cardiomyopathy have demonstrated that allogeneic MSC transplantation without immunosuppression is safe and produces benefits that appear equivalent to those produced by autologous cells [17]. With regard to clinical translation, early-phase clinical testing of allogeneic MSCs in patients with heart disease has yielded promising results; allogeneic human MSCs have demonstrated a favorable safety profile (without eliciting a significant immune memory response in transplanted recipients) and have produced encouraging hints of efficacy [10–12]. It should be noted that even immunoprivileged cells like MSCs are eventually rejected after *in vivo* transplantation without concurrent immunosuppression [18–21]; however, since their mechanism of action is indirect [22], rejection of MSCs may not be of therapeutic significance if it is delayed long enough to allow them to exert their reparative paracrine effects.

Properties of CDCs & clinical translation of autologous CDC therapy

Over the past decade, CDC therapy has navigated the entire trajectory from discovery through early-stage clinical trials [23]. Unlike many other cell types that have reached the clinic, CDCs followed a systematic preclinical developmental program, comprising *in vitro* studies (to optimize cell culture methods), small animal studies (proof-of-concept studies) and large animal studies (to optimize dosage, formulation and delivery), before moving on to human subjects in the first-in-man CADUCEUS trial. After reviewing the accumulated preclinical data in their totality, we herein attempt to summarize the most important properties of CDCs.

CDCs are cultured as follows: in brief, myocardial specimens (obtained from percutaneous biopsies, surgical biopsies or explanted donor hearts) are minced and plated in primary culture to yield cardiac outgrowth cells. Outgrowth cells are then harvested and plated into suspension culture, in order to generate spherical multicellular clusters termed cardiospheres (CSps) [24]. CSps provide a microenvironment that favors upregulation of stemness and cell–matrix interactions [24,25]. CSps are collected, replated and further expanded in monolayer culture to yield CDCs [8]. CDCs are cells of intrinsic-cardiac origin [26]. Surface markers are uniformly positive for endoglin (CD105, the TGF-β receptor subunit) and uniformly negative for the pan-hematopoietic marker CD45 [8]. A fraction of CDCs (25–60%) expresses CD90 [27–29] (a marker of MSCs), and a small minority (~3%) expresses c-Kit (a putative marker of endogenous cardiac progenitors) [27]. Importantly, it has been demonstrated that the $CD105^+$/$CD90^-$/$c\text{-}Kit^-$ population of CDCs constitutes the active fraction, in terms of therapeutic efficacy; the $c\text{-}Kit^+$ fraction and the $CD90^+$ fraction are neither necessary for, nor contributory to, the regenerative efficacy of CDCs [27]. CDCs do not express markers typically associated with fibroblasts or myofibroblasts (<4% of CDCs express discoidin domain-containing receptor 2 or smooth muscle actin) [30] and, at least in their naive state, CDCs are generally negative for sarcomeric proteins [27]. Autologous CDCs can be isolated from percutaneous endomyocardial biopsy specimens and can be readily expanded in culture [8], yielding clinically relevant numbers in a timely manner [7]. Several animal studies from multiple independent laboratories have demonstrated that CDCs improve cardiac function, decrease scar size and increase viable myocardium postmyocardial infarction (MI) in mice [8,31–32], rats [30,33–36] and pigs [37–41]. While the therapeutic superiority of CDCs over other adult progenitor cells has not been established, preliminary comparative studies suggest that CDCs appear to be more potent than [32,37]

(or at least equally effective as [22]) other clinically applicable cell types. Importantly, from a translational perspective, dose-ranging studies in clinically relevant large animal models of ischemic cardiomyopathy have demonstrated that intracoronary infusion of CDCs (up to 300,000 CDCs/kg of body weight) does not cause microvascular obstruction and cardiac injury [38].

As far as the mechanism of action underlying CDC-induced myocardial repair goes, it is now evident that indirect mechanisms trump stemness; even though CDCs are clonogenic, self-renewing and multipotent [42], the salutary effects of CDC therapy should be attributed to indirect mechanisms of action, rather than direct cardiomyogenic differentiation of administered cells [30,31]. Accordingly, it has been shown that CDCs, both through paracrine and contact-dependent mechanisms, promote endogenous repair and regeneration by stimulating angiogenesis, recruitment of endogenous progenitors and proliferation of resident cardiomyocytes in the peri-infarct area [30–31,43–44]. While the mediators of these regenerative effects have not been fully elucidated yet, fate-mapping studies have demonstrated that CDCs stimulate recruitment of endogenous progenitors partially through secretion of SDF-1. In addition, CDCs are potent secretors of exosomes (rich in miRNAs), which appear to be critical agents of CDC-induced regeneration, angiogenesis and cardioprotection [45–47]. Among the several miRNAs enriched in CDC-derived exosomes, miR-146a, miR-210 and miR-132 appear particularly intriguing; preliminary mechanistic studies have demonstrated a cardioprotective and regenerative effect of miR-146a [46,47], an antiapoptotic effect of miR-210 [46] and a proangiogenic effect of miR-132 [46].

With regard to clinical translation, two clinical trials of autologous CDCs (the CADUCEUS trial and the TICAP trial) have been completed [7,9,29]. The Phase I CADUCEUS trial (performed in the Cedars-Sinai Heart Institute, CA, USA and the Johns Hopkins Hospital, MD, USA) investigated the feasibility, safety and preliminary efficacy of intracoronary infusion of autologous CDCs in patients with convalescent MI [7,9]. Thirty-one patients with subacute MI and left ventricular dysfunction were randomized in a 2:1 manner to receive either intracoronary infusion of 12.5–25 million autologous CDCs (grown from endomyocardial biopsies) or standard care and were followed over a 1-year period. Twenty-five patients (17 treated, 8 controls) were included in the per-protocol analysis. Autologous CDCs were successfully harvested from endomyocardial biopsies and grown in sufficient numbers to achieve the required CDC dose in 17/20 patients; three technical manufacturing failures occurred. Intracoronary infusion of autologous CDCs in the infarct-related artery did not raise significant safety concerns. Analysis of exploratory efficacy endpoints, measured by cardiac MRI, revealed a decrease in scar size, an increase in viable myocardium and improved regional contractility of infarcted myocardium (but not ejection fraction) in CDC-treated patients compared to controls. While the lack of increased benefit in ejection fraction after CDC therapy may appear paradoxical at first (ejection fraction improved similarly – by ~6% – in CDC-treated and control patients), it needs to be highlighted that ejection fraction is dependent on several parameters (including volume load, ventricular geometry and electrical activation pattern) and therefore may not be the best-suited surrogate marker for evaluating efficacy of cell therapy [23]. Importantly, the CDC-induced decrease in scar size was consistent with the increase in ejection fraction observed in cell-treated patients, as predicted by the natural relationship between scar size and ejection fraction in convalescent MI [9]. In addition, scar shrinkage correlated with an increase in viable myocardium [7,9] and with improvement in regional function [9], suggesting genuine therapeutic myocardial regeneration. While the fidelity of contrast-enhanced cardiac MRI in assessing tissue viability after cell therapy has recently been called into question [48,49], studies in large animal models of convalescent MI have demonstrated that cardiac MRI can accurately measure scarred and viable myocardium after cell administration and thus can be reliably used for assessing regenerative efficacy of cell therapy [40,50].

The Phase I TICAP trial (Transcoronary Infusion of Cardiac Progenitor Cells in Patients with Single Ventricle Physiology, performed in Okayama University Hospital, Japan) investigated the feasibility, safety and preliminary efficacy of global intracoronary infusion (i.e., infusion in all three major coronary arteries) of autologous CDCs in pediatric patients with hypoplastic left heart syndrome [29]. Eighteen patients (~2 years old) with hypoplastic left heart

syndrome were prospectively assigned to receive global intracoronary infusion of autologous CDCs (300,000/kg of body weight) after staged palliation (n = 10), followed by eight controls with staged palliation alone. Fourteen patients (seven treated, seven controls), followed over an 18-month period, were included in the per-protocol analysis. Autologous CDCs were successfully grown in 9/10 patients; one technical manufacturing failure occurred. Intracoronary infusion of CDCs into all three major coronary arteries did not raise significant safety concerns. In addition, hints of efficacy were observed; pediatric patients treated with autologous CDCs exhibited improved ventricular function (measured by cardiac MRI), reduced heart failure symptoms and improved somatic growth compared with controls. The preliminary efficacy signals observed in these early-Phase trials of CDC therapy need to be tested in larger clinical trials.

Limitations of autologous cell therapy

Most clinical trials of cell therapy to-date have been conducted using autologous cells. While autologous cell therapy carries no risk of immune rejection, it requires patient-specific tissue harvesting, cell manufacturing and quality control and is, therefore, associated with important limitations. First, autologous tissue harvesting (via endomyocardial biopsies in the case of autologous CDCs) is invasive and not risk-free [51]. Second, culture of autologous cells may be complicated by technical manufacturing failures; in CADUCEUS, three such failures occurred: one bacterial contamination, one cytogenetic abnormality and one failure to achieve the minimal CDC dose for infusion [7]. Third, expansion of autologous heart-derived cells to clinically relevant numbers poses significant timing constraints (4–6 weeks for CDCs) [7], which prohibit administration of autologous heart-derived cellular products in acute MI or early post-MI. Fourth, donor age and comorbidities may [52] (or may not) [53] negatively impact upon cell quality, resulting in decreased cell potency. Finally, the technical, economic and logistic constraints associated with autologous cell therapy preclude broad adoption of cell therapy and limit its clinical application to few specialized centers.

The aforementioned limitations of autologous therapy could be overcome by the use of allogeneic cells. The obvious disadvantage of allogeneic therapy is the risk of immune rejection of transplanted cells, which could limit effectiveness of cell therapy (due to rapid rejection of transplanted cells), raise safety concerns (as it could theoretically result in immune-related myocardial injury), and induce allosensitization of the recipient, which (if robust and persistent) could complicate future organ transplant. However, if allogeneic cell therapy were proven to be safe and effective, it would open up a new paradigm in cellular therapeutics. Large-scale expansion of cells derived from allogeneic tissues could be performed in specialized manufacturing labs under strict quality control. In the case of heart-derived cells, attractive sources of allogeneic tissue include hearts explanted from organ donors but not used for transplantation and surgical myocardial discards. Highly standardized, 'off-the-shelf' allogeneic cellular products would subsequently be shipped to hospitals throughout the world and banked for future use, thus enabling broad adoption and timely application of cell therapy in a cost-efficient manner. This attractive novel paradigm has motivated researchers to investigate the safety and efficacy of allogeneic therapy for myocardial repair.

Figure 1 depicts schematically the preclinical developmental program and the clinical translation of allogeneic CDCs. The major findings of each step along the pathway to clinical translation are presented.

In vitro immunologic properties of heart-derived cells

Malliaras *et al.* investigated the *in vitro* immunologic properties of allogeneic rat and human CDCs [30]. CDCs were shown to express MHC class I, but not MHC class II surface antigens or CD80/CD86 costimulatory molecules. This antigenic profile appears favorable for allogeneic applications, since expression of MHC class I antigens would protect donor CDCs from recipient NK cells [54]; lack of expression of MHC class II antigens would allow donor CDCs to escape direct recognition by recipient CD4 T-helper cells; and the lack of costimulatory molecules on the surface of donor CDCs would prevent activation of recipient effector T cells by MHC class I surface antigens [55]. Mixed lymphocyte reactions demonstrated that allogeneic CDCs exhibit minimal *in vitro* immunogenicity, manifested as negligible lymphocyte proliferation and pro-inflammatory cytokine secretion.

Lauden *et al.* performed an in-depth investigation of the *in vitro* immunologic properties of a different (at first glance) type of heart-derived

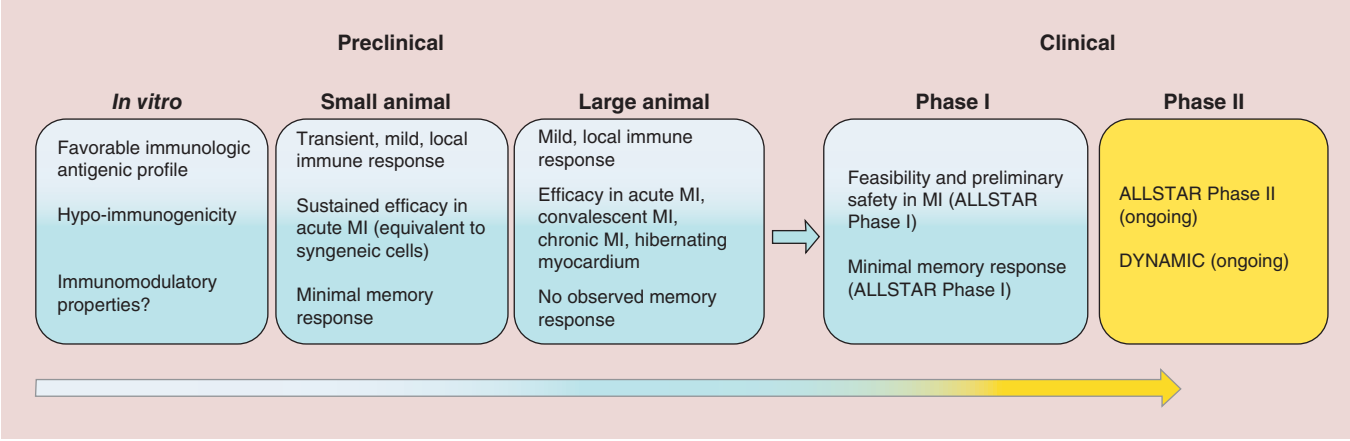

Figure 1. Preclinical developmental program and clinical translation of allogeneic cardiosphere-derived cells. Allogeneic cardiosphere-derived cells followed a systematic preclinical developmental program, comprising *in vitro* studies (to investigate immunological properties), small animal studies (proof-of-concept studies) and large animal studies (to investigate safety and efficacy in clinically relevant models), before moving on to human subjects in the first-in-man ALLSTAR and DYNAMIC clinical trials. The major findings of each step along the pathway to clinical translation are presented. Light blue denotes completed steps, while yellow denotes ongoing investigations.
MI: Myocardial infarction.

cells (termed 'human cardiac progenitor cells' [hCPCs] by the authors) [56]. hCPCs were grown as follows: c-Kit$^+$/CD45$^-$ cells were isolated from human surgical discards and were subsequently expanded in culture. Of note, the isolated heart-derived cells lost expression of c-Kit antigen during *ex vivo* expansion, resulting in generation of a cell population (hCPCs) that exhibited an antigenic profile strikingly similar to that of CDCs (hCPCs were uniformly CD105$^+$/CD45$^-$ and only approximately 4% expressed c-Kit). The loss of c-Kit expression during *ex vivo* expansion is in contrast to previous reports suggesting that c-Kit$^+$ cardiac cells demonstrate a relatively stable phenotype during long-term cell culture [57], and may be attributable to differences in cell culture methods. hCPCs, similar to CDCs, expressed MHC class I, but not MHC class II surface antigens. In addition, hCPCs were negative for costimulatory molecules CD40, CD80, CD86 and CD275, but expressed programmed death ligand 1 (PD-L1) (CD274), a costimulatory molecule of the B7 family that plays a critical role in regulation of T cell-mediated immune responses [58,59]. Mixed lymphocyte reactions demonstrated that allogeneic hCPCs were hypoimmunogenic and possessed immunosuppressive properties (i.e., they suppressed proliferation of preactivated lymphocytes). With regard to the latter, the authors demonstrated that the immunomodulatory capacity of hCPCs was mediated by hCPC-induced expansion of suppressive CD4$^+$/CD25^{high}/CD127^{low}/FoxP3^{high} effector regulatory T cells, via cell–cell contact-dependent interaction with PD-L1 (expressed on the surface of hCPCs). In a subsequent study from the same lab, Boukouaci *et al.* investigated the interaction of hCPCs with NK cells [60], by coculturing hCPCs with mismatched-allogeneic NK cells in medium containing IFN-γ (in an attempt to mimic the inflammatory milieu of infarcted myocardium). The authors found that under inflammatory conditions, allogeneic hCPCs not only evade killing by NK cells, but also suppress NK-cell cytotoxicity and modulate NK-cell cytokine secretion toward an anti-inflammatory profile.

Taken together, multiple studies from independent laboratories have demonstrated that CD105/CD45$^-$/c-Kit$^-$ heart-derived cells are hypoimmunogenic (and possibly immunosuppressive) *in vitro*, and exhibit an immunologic profile that, at least theoretically, renders them attractive for allogeneic applications without requiring concomitant immunosuppression.

In vivo preclinical studies of allogeneic CDC transplantation

The favorable *in vitro* immunological profile of CDCs led to the design of animal studies exploring the potential of allogeneic CDC therapy *in vivo* (summarized in **Table 1**). Malliaras *et al.* investigated the safety and efficacy of allogeneic CDC therapy (without concomitant

immunosuppression) in infarcted rats [30]. In order to create a stringent model of allogeneic cell transplantation, inbred rats from two immunologically divergent strains characterized by complete mismatch of MHC antigens were used as cell donors and recipients. Rats underwent MI and were randomized to receive syngeneic CDCs, allogeneic CDCs, xenogeneic CDCs (human CDCs, used as a positive control for immune rejection) or vehicle in the infarct border zone. Quantification of cell engraftment revealed that syngeneic and allogeneic CDCs survived at similar levels in infarcted recipient myocardium one week after delivery, but few syngeneic and even fewer allogeneic CDCs remained at three weeks post-cell administration. Allogeneic CDCs induced a mild, local immune response that had completely subsided by six months postdelivery and was not associated with any immune-related myocardial damage. Allogeneic CDCs did not induce systemic immunogenicity, as levels of circulating proinflammatory (IFN-γ, TNF-α, IL-1b, KC/GRO) and anti-inflammatory (IL-5, IL-13, IL-4) cytokines were comparable in rats receiving syngeneic or allogeneic CDCs. With regard

Table 1. Preclinical studies of allogeneic cardiosphere-derived cells.

Study (year)	Model	Route	Cells	Immune response	Efficacy	Ref.
Malliaras *et al.* (2012)	Rat, acute MI	IM	CDCs	Mild/transient local mononuclear infiltration, no DSAs, plus cellular memory response	↓scar size, ↑function, ↑myocyte cycling, ↑angiogenesis Equal benefits of allogeneic and syngeneic CDCs	[30]
Tseliou *et al.* (2013)	Rat, acute MI	IM	CSps	No local immune response	↓scar size, ↑function, ↑angiogenesis Equal benefits of allogeneic and syngeneic CSps	[61]
Malliaras *et al.* (2013)	Pig, convalescent MI	IC	CDCs	Mild/transient local mononuclear infiltration, no DSAs	↓scar size, ↓scar mass, ↑viable mass, ↑ (global, regional) function, ↓volumes, ↑myocyte cycling, ↑progenitors, ↑angiogenesis	[40]
Yee *et al.* (2014)	Pig, chronic MI	IM	CSps	Mild/transient local mononuclear infiltration, no DSAs	↓scar size, ↑viable mass, – global function, ↓LV volumes	[62]
Gallet *et al.* (2015)	Pig, convalescent MI	IC	CSps	Mild/ transient local mononuclear infiltration, no DSAs	↓scar size, ↓scar mass, ↑viable mass, ↑ (global, regional) function, ↓volumes, ↑perfusion, ↑angiogenesis	[63]
Suzuki *et al.* (2014)	Pig, hibernating myocardium	IC	CDCs	Not assessed	↑ (global, regional) function, ↑myocyte number, ↑myocyte cycling	[41]
Weil *et al.* (2015)	Pig, hibernating myocardium	IC	CDCs vs MSCs	Not assessed	↑ (global, regional) function, ↑myocyte number, ↑angiogenesis, ↑myocyte cycling Equal benefits of CDCs and MSCs, except for ↑myocyte cycling with CDCs	[22]
Kanazawa *et al.* (2015)	Pig, acute MI	IC	CDCs	Not assessed	↓scar size, ↓'no reflow' area, – global function, – LV volumes, ↓ apoptosis	[64]
Crisostomo *et al.* (2015)	Pig, acute MI vs convalescent MI	IC	CPCs	Not assessed	– size, – on global function, ↑viability within risk area, ↓ volumes (only in convalescent MI), ↑angiogenesis (only in acute MI)	[65]

↑ Increase; ↓ Decrease; – No effect; CDC: Cardiosphere-derived cell; CPC: Cardiac progenitor cell; CSp: Cardiosphere; DSA: Donor-specific antibodies; IC: Intracoronary; IM: Intramyocardial; LV: Left ventricle; MI: Myocardial infarction; MSCs: Mesenchymal stromal cells.

to development of immune memory response, no alloreactive circulating antibodies (donor-specific antibodies [DSAs]) were detected in recipients of allogeneic CDCs (suggesting no development of humoral memory response). However, mixed lymphocyte reactions demonstrated that lymphocytes isolated from recipients of allogeneic CDCs exhibited increased proliferation when cocultured with allogeneic CDCs, suggesting development of cellular memory response after allogeneic CDC therapy; whether the intensity of the memory response diminishes with time (as reported in studies of allogeneic MSCs) [20] and whether the observed memory response would result in accelerated rejection of a repeat dose of the same batch of allogeneic cells *in vivo* are issues that remain to be investigated. Importantly from a therapeutic standpoint, allogeneic and syngeneic CDCs produced indistinguishable benefits in cardiac structure (increase in viable myocardium, decrease in scar tissue) and function (improved systolic performance), which persisted for at least 6 months post-cell delivery (long after allogeneic cells had been cleared from the recipient myocardium). In addition, allogeneic CDCs stimulated endogenous reparative mechanisms (cardiomyocyte cycling, angiogenesis) and increased myocardial levels of VEGF, IGF-1 and HGF equally with syngeneic CDCs. In a subsequent study from the same lab, Tseliou *et al.* investigated the safety and efficacy of intramyocardial injection of allogeneic CSps, without concomitant immunosuppression, in immunologically mismatched infarcted rats [61]. Allogeneic CSps did not elicit a significant local immune response *in vivo* and exhibited similar levels of cell engraftment as syngeneic CSps at 1 and 3 weeks postdelivery. Importantly, allogeneic and syngeneic CSps produced similar durable functional and structural benefits post-MI, and promoted comparable upregulation of beneficial paracrine factors (VEGF, IGF-1 and HGF) in the peri-infarct area.

The encouraging results obtained from the aforementioned small animal studies prompted the investigation of allogeneic CDC therapy in clinically relevant large animal models of MI and chronic ischemic cardiomyopathy. Malliaras *et al.* investigated the safety and efficacy of intracoronary infusion of allogeneic CDCs (without concomitant immunosuppression) in immunologically mismatched pigs with convalescent MI [40]. Yucatan minipigs underwent induction of MI and 2–3 weeks later were randomized to receive intracoronary infusion of mismatched allogeneic CDCs or vehicle in the infarct-related artery. Allogeneic CDCs induced a mild local lymphoplasmacytic infiltration in interstitial and perivascular spaces of the peri-infarct area, but did not result in any rejection-related myocardial damage. With regard to development of immune memory response, no circulating alloreactive antibodies (DSAs) were detected in recipients of allogeneic CDCs. In terms of efficacy, allogeneic CDCs produced significant structural benefits (increase in viable myocardium and decrease in scar mass), improved global and regional cardiac function and stimulated endogenous regenerative mechanisms (cardiomyocyte cycling, recruitment of endogenous cardiac progenitors, angiogenesis). Importantly, histological analysis of explanted hearts ruled out myocyte hypertrophy as a contributor to the increase in viable myocardium observed after allogeneic CDC therapy; myocyte size was actually smaller in the risk region of CDC-treated animals compared with controls, a finding consistent with cardiomyocyte hyperplasia and attenuation of remodeling-associated cardiomyocytes after cell therapy.

Yee *et al.* investigated the safety and efficacy of allogeneic CSps delivered by percutaneous NOGA-guided injections without concomitant immunosuppression, in immunologically mismatched pigs with chronic ischemic cardiomyopathy [62]. First, the investigators performed a dose-ranging study in infarcted minipigs, in order to optimize CSp dosage. The superior CSp dose (150 million CSp-forming cells, based on the results of the dose-ranging study) was then tested in a subsequent pivotal study; Yucatan minipigs underwent MI creation and 8 weeks later were randomized to receive transendocardial dose-optimized injection of allogeneic CSps or vehicle in the peri-infarct area. Allogeneic CSps exhibited a favorable safety profile *in vivo*; no excess myocardial inflammation and no development of alloreactive antibodies (DSAs) were observed in treated animals. In terms of efficacy, cardiac MRI revealed that transendocardial injection of allogeneic CSps decreased scar size, increased viable mass and attenuated left ventricular dilatation (but had no effect on global systolic function).

In a recent study, Gallet *et al.* investigated the safety and efficacy of intracoronary infusion of allogeneic CSps in pigs with convalescent MI [63]. Yucatan minipigs underwent induction

of MI and 1 month later were randomized to receive dose-optimized intracoronary infusion of allogeneic CSps (without concomitant immunosuppression) or vehicle in the infarct-related artery. Allogeneic CSps induced a mild local mononuclear infiltration in the peri-infarct area, but did not result in any rejection-related myocardial damage or in robust DSAs. Cardiac MRI revealed that intracoronary infusion of allogeneic CSps decreased scar size, increased viable mass, attenuated remodeling, improved global and regional myocardial function, and increased myocardial perfusion (measured both by MRI and by coronary flow reserve) compared to the control group.

In a study from the Canty lab, Suzuki *et al.* investigated the efficacy of global intracoronary infusion of allogeneic CDCs, with concomitant cyclosporine immunosuppression, in a porcine model of hibernating myocardium (from chronic occlusion of the left anterior descending artery) [41]. Allogeneic CDCs were infused in all three major coronary arteries, as the researchers aimed to target both the hibernating and the remote remodeled myocardium. Global intracoronary infusion of allogeneic CDCs did not raise safety concerns and improved global and regional cardiac function over a 4-week follow-up period. In agreement with previous studies [30,40,43], CDC administration stimulated resident myocyte cycling, increased myocyte nuclear density and reduced myocyte size; these findings are consistent with CDC-induced upregulation of resident myocyte proliferation resulting in generation of new smaller myocytes. Recently, the same group performed a head-to-head comparison of global intracoronary infusion of allogeneic bone marrow-derived MSCs versus allogeneic CDCs (with concomitant cyclosporine immunosuppression) in the aforementioned porcine model of hibernating myocardium. Both allogeneic MSCs and CDCs improved regional cardiac function, decreased myocyte size and increased myocyte density to the same extent compared with control animals over 4 weeks of follow-up. Allogeneic CDCs were superior to allogeneic MSCs in promoting cycling of resident cardiomyocytes, as manifested by a twofold increase in the number of $Ki67^+$ and $H3P^+$ myocytes in CDC-treated hearts compared to MSC-treated hearts [22].

As discussed earlier in this review, a major advantage of allogeneic cell therapy is that it is not bound by the timing constraints of autologous cell therapy (as the former does not necessitate patient-specific tissue harvesting and cell processing). Therefore, the use of allogeneic 'off-the-shelf' cellular products enables timely application of cell therapy in the acute MI setting. In patients with acute MI, intracoronary administration of allogeneic cells immediately after coronary reperfusion appears particularly attractive both from a practical standpoint (it obviates the need for a second catheterization procedure for cell infusion) as well as from a cardioprotective standpoint (since most cardiomyocytes die during the first 24 h post-MI [66], interventions beyond this timeframe have minimal potential for myocardial salvage). A caveat of this therapeutic approach is aggravation of microvascular obstruction (which occurs naturally after reperfusion in acute MI) via coronary microembolization of administered cells [67]. Kanazawa *et al.* investigated the safety and efficacy of intracoronary infusion of allogeneic CDCs in pigs with acute MI [64]. Yucatan minipigs underwent MI and were randomized to receive intracoronary infusion of allogeneic CDCs or vehicle solution 30 min postreperfusion. Dose-optimized infusion of allogeneic CDCs was well-tolerated in the setting of acute MI (no significant changes in TIMI flow post-cell infusion were observed). Histology of infarcted hearts (explanted 48 h post-MI) demonstrated that infusion of allogeneic CDCs decreased infarct size, microvascular obstruction (i.e., 'no reflow' area, measured after thioflavin dye administration) and cardiomyocyte apoptosis in the risk region compared with control animals. The fact that structural benefits were detected this early (48 h post-cell delivery) suggests that the decreased infarct size observed in treated hearts should be attributed to an acute cardioprotective effect conferred by allogeneic CDCs rather than to CDC-induced myocardial regeneration. Whether this acute cardioprotective effect translates into longer-term benefits remains to be investigated.

Crisostomo *et al.* investigated the optimal timing of allogeneic heart-derived cell therapy early post-MI, by comparing delivery of allogeneic cells (without concomitant immunosuppression) in the acute MI setting versus 1 week post-MI [65]. Intracoronary infusion of allogeneic CPCs (either postreperfusion or 1 week post-MI) did not raise significant safety concerns. In terms of efficacy, both cell-treated groups exhibited attenuation of remodeling (as assessed by cardiac MRI), increased amount of

viable myocardium within the risk region and more mature vessels in the infarct border zone compared with control animal. Timing of cell delivery did not seem to significantly affect the therapeutic efficacy of the administered allogeneic CPCs, except for a trend toward greater attenuation of left ventricle dilatation observed in animals treated at 1 week post-MI compared with those treated immediately post-MI.

Taken together, preclinical studies have demonstrated that allogeneic CDC transplantation without immunosuppression is safe and produces durable structural and functional benefits, indistinguishable from syngeneic transplantation, in animal models of ischemic cardiomyopathy. The persistence of benefit, despite ephemeral cell survival, is rationalized by the indirect mechanism of action of transplanted cells [30,31]. It appears that allogeneic CDCs, possibly due to their hypoimmunogenic profile, can survive in recipient myocardium long enough to stimulate endogenous regenerative and reparative pathways, resulting in sustained benefit without the requirement for stable engraftment of transplanted cells.

Clinical studies of allogeneic cardiosphere-derived cells transplantation

The preclinical work described above motivated the testing of allogeneic human CDCs as potential 'off-the-shelf' therapeutic agents for heart disease. Two studies of allogeneic CDC therapy are currently underway, the ALLogeneic heart STem cells to Achieve myocardial Regeneration (ALLSTAR) [68] trial and the Dilated cardiomYopathy iNtervention with Allogeneic MyocardIally-regenerative Cells (DYNAMIC) trial [69].

ALLSTAR is a Phase I/II trial designed to investigate the feasibility, safety and efficacy of intracoronary infusion of allogeneic CDCs in patients with anterior MI (within the prior 1 year) and substantial scar burden (scar size: >15% of the left ventricle). In the recently completed open-label Phase I portion of the study (aiming at assessing feasibility and preliminary safety), 14 patients (left ventricular ejection fraction [LVEF]: ~42%, infarct size: ~25%) underwent intracoronary infusion of 12.5 million or 25 million allogeneic CDCs and were followed over a 1-year period [70]. Intracoronary infusion of allogeneic CDCs did not raise safety concerns; no prespecified primary safety endpoint (acute myocarditis, major adverse cardiac event, death due to arrhythmias or sudden unexpected death) occurred. With regard to development of immune memory response against the transplanted cells, four patients were found to have pre-existing DSAs against donor CDCs prior to infusion (one resolved and three persisted during follow-up). *De novo* DSAs developed in four subjects (three resolved and one persisted during follow-up), while no *de novo* cellular immune memory responses were detected. In terms of efficacy, cardiac MRI revealed that patients who received 25 million cells to which they had no pre-existing DSAs (n = 8) exhibited a significant decrease in infarct size (15% relative reduction) and a trend toward improvement in ejection LVEF (by ~4%) over the 1-year follow-up period. On the basis of the encouraging Phase I findings, ALLSTAR has proceeded to the randomized, double-blind Phase II portion of the trial, aiming at assessing safety and efficacy. In this portion of the study (which is currently recruiting), 260 patients without pre-existing DSAs against donor CDCs will be randomized (in a 2:1 fashion) to undergo intracoronary infusion of 25 million allogeneic CDCs or placebo solution and will be followed over a 1 year period. Two strata will be enrolled: recent MI, defined as index MI 28–90 days prior to treatment (n = 130), and chronic MI, defined as index MI 90–365 days prior to treatment (n = 130). Comparisons between the two strata may provide insight into the optimal timing of cell administration post-MI. The primary efficacy endpoint of the Phase II portion of ALLSTAR is infarct size reduction, as measured by MRI.

DYNAMIC is a Phase II trial designed to investigate the feasibility and safety of intracoronary infusion of allogeneic CDCs in patients with ischemic or non-ischemic dilated cardiomyopathy, systolic dysfunction (LVEF: <35%) and advanced heart failure (NYHA III/IVa). Patients with advanced heart failure may represent a more fertile target for cell therapy trials [71] since: they have significantly greater room for improvement compared with patients with MI; and results from several clinical trials to-date indicate that sicker patients are the ones who benefit the most from cell therapy [4,72–74]. In the open-label Phase IIa portion of DYNAMIC (which is currently in follow-up), 14 heart failure patients underwent intracoronary infusion (in all three major coronary arteries) of allogeneic CDCs; there have been no safety concerns to-date, and the patients will be followed

over a 1-year period. A placebo-controlled Phase IIb portion of DYNAMIC is anticipated. Prespecified primary safety endpoints of DYNAMIC include decreased TIMI flow post-cell infusion, acute myocarditis, major adverse cardiac events, death due to arrhythmias or sudden unexpected death. Patients are monitored for development of immune memory response against the transplanted allogeneic cells; this is of particular interest, as development of robust and persistent immune memory responses in patients with advanced heart failure could potentially complicate future heart transplantation. Functional testing (to gauge efficacy) includes cardiac imaging by computed tomography.

Potency assays

A critical step in the clinical translation of cell therapy, as mandated by regulatory authorities, [75] is development of relevant potency assays (i.e., tests to measure potency of manufactured cellular products). This is particularly important for allogeneic cell therapy, as development of appropriate potency assays would enable prospective identification of donor cells with greater potential for clinical efficacy. However, the fact that cell therapy products appear to have complex, multifactorial and not fully characterized mechanisms of action presents difficulties in establishing which cellular attributes are most relevant to measuring potency. Given that potency assays should ideally represent the cellular product's mechanism of action; [75] and multiple lines of evidence suggest that the mechanism of action of adult cells is indirect (i.e., is mediated by paracrine mechanisms) [30,31], it appears logical that *in vitro* biological assays measuring paracrine factor secretion may serve as appropriate potency assays for cellular products. CDCs, in particular, are potent secretors of several reparative cytokines [76] and miRNAs, [46,47] all of which (alone or in any combination) could potentially underlie the salutary effects of CDC therapy. Out of all identified paracrine factors, *in vitro* secreted SDF-1 by CDCs appears to be associated with *in vivo* potency, since: a) retrospective analysis of the properties of human CDCs administered in CADUCEUS revealed a positive correlation between *in vitro* SDF-1 secretion by CDCs and *in vivo* therapeutic outcome; [53] b) human CDCs that secrete higher levels of SDF-1 *in vitro* exhibit superior functional potency when injected in infarcted immunocompromised mice; [53] and c) mechanistic studies in fate-mapped mice demonstrate that CDCs amplify innate regeneration by endogenous progenitors, in part through the secretion of SDF-1 by transplanted cells [44]. Based on the above, *in vitro* SDF-1 secretion by CDCs merits prospective testing as a predictor of *in vivo* therapeutic efficacy. However, it must be noted that it is highly unlikely that the totality of CDC-induced benefits is mediated through SDF-1 secretion alone. Other potentially relevant potency assays of allogeneic CDCs include: a) surface expression of CD90 (as increased CD90 expression has been associated with decreased potency both in small animal studies and in CADUCEUS [27]); b) exosome secretion capacity (as exosomes appear to be critical agents of CDC-induced cardioprotection, regeneration and angiogenesis) [45–47]; c) miRNA secretion capacity (with miR-146a [46,47], miR-210 [46] and miR-132 [46] emerging as potentially attractive candidates); d) surface expression of PD-L1 (as PD-L1 appears to play a critical role in the immunosuppressive/immunomodulatory effects of CDCs [56]; therefore, allogeneic CDCs with increased expression of PD-L1 could potentially evade immune rejection more efficiently); and e) therapeutic efficacy in small animal models (i.e., administering human CDCs derived from different donors in immunodeficient animal models and selecting the ones that produce the greatest structural/functional benefits in animals for human applications). In any case, the clinical data collected from ongoing trials of allogeneic CDCs in conjunction with extensive *in vitro* testing of the administered allogeneic cell products will likely help better define which cellular attributes are predictors of greater clinical efficacy (and should therefore be measured as indicators of cell potency).

Conclusion & future perspective

During the next few years, significant insight is expected to be gained regarding the safety and efficacy of allogeneic CDCs both from preclinical studies (currently underway in multiple laboratories worldwide) and, particularly, from ongoing clinical investigations of allogeneic CDC therapy. Results from ongoing trials should be available within 1–2 years and will help answer pivotal questions regarding allogeneic CDC therapy: is intracoronary infusion of allogeneic CDCs safe and effective in patients with MI (ALLSTAR) or advanced heart failure (DYNAMIC)? Do allogeneic CDCs induce

humoral or cellular immune memory responses in human recipients? If so, are said immune responses transient or persistent? This is particularly relevant to patients with advanced heart failure (DYNAMIC) as it could potentially complicate future heart transplantation. What is the optimal time of cell administration post-MI? Is application of allogeneic cells earlier in the remodeling process associated with increased therapeutic benefit (comparison of recent MI stratum versus chronic MI stratum in the ALLSTAR trial)? Does targeting a sicker patient population (as the one enrolled in DYNAMIC) maximize the potential of allogeneic therapy to produce clinically meaningful benefits, like reductions in mortality or rehospitalizations? Will the collected clinical data combined with the results obtained from *in vitro* testing of the administered allogeneic cell products help better define which cellular attributes are predictors of greater clinical efficacy? In addition, new basic studies are needed to better elucidate the mechanism of action of CDCs and to investigate whether large-scale *ex vivo* expansion negatively impacts upon their regenerative potential. The latter is of particular importance, as it has been suggested that serial passaging of MSCs alters their immunophenotype (increases expression of MHC II molecules) [77,78], attenuates their immunomodulatory/immunosuppressive properties, [79] decreases their stemness/multipotentiality [80,81] and attenuates secretion of paracrine factors [80].

In any case, potential establishment of clinical safety and efficacy of allogeneic CDCs in conjunction with generation of highly standardized, 'off-the-shelf' allogeneic cellular products would certainly catalyze broad clinical adoption of cell therapy.

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EXECUTIVE SUMMARY

- Autologous cardiosphere-derived cells (CDCs) for heart repair have yielded positive results in early-phase clinical testing (CADUCEUS and TICAP clinical trials).
- Autologous therapy is associated with significant technical, timing, economic and logistic constraints, which could be overcome by the use of allogeneic cells.
- CDCs exhibit a favorable immunologic antigenic profile and are hypoimmunogenic (and possibly immunosuppressive) *in vitro*.
- Allogeneic CDC transplantation without immunosuppression in immunologically mismatched recipient animals is safe and produces sustained functional and structural benefits through stimulation of endogenous regenerative pathways.
- Allogeneic human CDCs are currently being tested clinically in the ALLSTAR and DYNAMIC clinical trials.
- Potential establishment of clinical safety and efficacy of allogeneic CDCs combined with generation of highly standardized, 'off-the-shelf' allogeneic cellular products would facilitate broad clinical adoption of cell therapy.

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