Proceedings: Moving Toward Cell-Based Therapies for Heart Disease

LILA R. COLLINS, CATHERINE PRIEST, INGRID CARAS, NEIL LITTMAN, LISA KADYK

California Institute for Regenerative Medicine, San Francisco, California, USA

SUMMARY

Heart disease due to myocardial infarction and the ensuing heart failure represent a major unmet medical need. Approved treatments do not prevent loss of cardiac muscle or reduce scar formation, both of which weaken heart function. Cell-based therapies currently being investigated both pre-clinically and clinically have the potential to address these underlying problems either by actually replacing lost tissue or by supplying paracrine growth factors that may have multiple beneficial effects such as reduction of inflammation, increase of blood supply, improvement in cell survival, and reduction of scar size. The best cell types, stage of disease to target, and delivery method to improve heart function are currently unclear. The California Institute for Regenerative Medicine supports multiple different cell-therapy strategies for heart disease, offering hope that improved treatments will be available for patients in the future.

SIGNIFICANCE

Heart attack and the heart failure that often follows represent an enormous financial burden and unmet medical need. Cell therapy is being actively explored to improve cardiac function for both of these forms of heart disease. The cell therapy and tissue engineering efforts supported by the California Institute for Regenerative Medicine to improve heart function after a myocardial infarction and during heart failure span a variety of novel approaches, many of which go beyond current approaches in clinical trials.

INTRODUCTION

A typical myocardial infarction (MI) results in the death of 1 billion or more cardiomyocytes. To a large extent, these cells are not regenerated but instead are replaced with a noncontractile scar, which puts additional strain on remaining cardiomyocytes and compromises heart function. Ultimately, depending upon the size of the infarct, approximately one in five MI survivors develop chronic heart failure (CHF). [1]. Drug therapies can help manage CHF but cannot repair the damage to the heart, and CHF patients face a 5-year mortality rate of 50% [2]. Although heart transplant cures the disease, supplies of donor hearts cannot meet the demand. As of this writing (May 2015), 431 heart transplants have been performed in the U.S. during 2015, whereas 4,166 people are waiting for a transplant [3]. Left ventricular assist devices, originally intended as a temporary bridge to transplant, are sometimes used as a destination therapy for patients with severe heart failure; however, the increased risk of infections or bleeding makes them unsuitable for use in all patients. Consequently, a significant unmet need remains for patients following MI that potentially could be addressed by cell therapy.

CELL THERAPY BACKGROUND

This article highlights the investments of the California Institute for Regenerative Medicine (CIRM) in cardiac regenerative medicine and the general contour of the current commercial landscape and is not intended to serve as a comprehensive review. For additional information, the reader is referred to the following recent reviews of cell therapy for MI and heart failure [4, 5].

Efforts to treat post-MI heart disease using cell therapy have been very active in the past 2 decades, with nearly 15 years of clinical trial experience. Numerous adult stem and progenitor cell types that have been tested persist only transiently after transplantation into the heart, thus any beneficial effects observed are thought to be due to paracrine mechanisms. Such cells include bone marrow-derived mononuclear cells and
mesenchymal stem cells (MSCs), adipose-derived mesenchymal stem cells, and cardiac-derived cells. Most recently, cardiac progenitors derived from pluripotent stem cells (PSCs) have entered the clinic. These trials have confirmed the safety of each of these cell types, but efficacy results have been variable from trial to trial [4, 5]. Large clinical trials designed to resolve some of the open questions about efficacy are in progress and will be discussed.

Another approach to treatment of post-MI heart failure is replacement of lost cardiomyocytes using cell types that persist in the heart. Skeletal myoblasts, for example, engraft in the heart after MI and have improved heart function preclinically, although clinical trials detected a tendency to produce arrhythmias [6]. Although not yet tested clinically, transplantation of human pluripotent stem cell-derived cardiomyocytes is a true cardiac myocyte replacement strategy. These cells have been shown to persist and have promise in preclinical models of ischemic heart disease, although close monitoring for potential arrhythmias will be required [7–9].

Although the potential for the various described cell types to regenerate the injured heart remains under debate and active investigation, the trials to date have established the clinical infrastructure for future studies and demonstrated that cell therapies can be administered safely to the heart.

Preclinical studies have shown that the bulk of cells transplanted to the heart, regardless of cell type, fail to engraft, likely due to a combination of washout and a hostile environment, potentially limiting efficacy [10]. This limitation has spurred biomaterial and tissue-engineering efforts to enhance cell retention [11]. Taken together, these data suggest that identification of the correct cell type and method of delivery to promote retention in the heart may lead to significant benefits for patients suffering from acute MI or CHF.

**COMPETITIVE LANDSCAPE**

There is significant corporate investment in cell therapy for ischemic heart disease, and multiple companies are currently conducting clinical trials, as shown in Table 1. Notably, all corporately sponsored trials in cell therapy for heart disease are currently testing cell types hypothesized to have a paracrine mechanism of action. Although a handful of clinical trials are testing cells hypothesized to be capable of cell replacement in the heart, these efforts have not yet attracted corporate funding, likely because many questions still need to be addressed preclinically, such as, what is the correct cell type? At what stage of maturity should it be implanted? Can the cells functionally integrate without inducing arrhythmias? What methods can be used to manufacture replacement cells in sufficient numbers for large-scale clinical trials?

Although the vast majority of corporately sponsored clinical trials use bone marrow-derived cells, there are considerable differences in the cellular compositions under investigation. Therapies range from heterogeneous multicellular mixtures to selectively isolated, relatively homogeneous cell populations. Vericel (formerly Aastrom Biosciences; Cambridge, MA, http://vcel.com), for example, is conducting a phase IIb trial evaluating imxmyelocel-T, a therapy composed of bone marrow-derived MSCs, CD14-positive (CD14+) monocytes, anti-inflammatory macrophages, monocytes, granulocytes, and T and B lymphocytes. Vericel believes the cellular mixture found in imxmyelocel-T possesses the ability to promote tissue repair, immune modulation, and angiogenesis. Mesoblast (Melbourne, Victoria, Australia, http://mesoblast.com) is conducting one of the largest company-sponsored cardiovascular cell therapy trials to date, a phase III clinical trial of 1,700 patients, using a mixed population of bone marrow-derived cells, called “mesenchymal precursor cells.” Athersys (Cleveland, OH, http://www.athersys.com) is conducting a phase II trial with MultiStem, a therapy consisting of an adherent, multipotent bone marrow-derived cell population. In contrast, Caladrius Biosciences (formerly NeoStem; New York, NY, http://www.caladrius.com) has a phase II clinical trial that is evaluating a highly purified population of CD34+ cells (NBS10) obtained from bone marrow. Although each of these companies is evaluating a different population of bone marrow-derived cells, they have in common a postulated paracrine mechanism of action in which the cells, in response to local signals, release a cocktail of growth factors and other stimuli (e.g., chemokines, enzymes) that may recruit endogenous cells to the site of injury, thereby stimulating regeneration and repair of the injured tissue.

Celyad (formerly Cardio3 Biosciences; Mont Saint Guibert, Belgium, http://www.celyad.com/products/cardiology-3.htm?lng=en) is using autologous bone marrow-derived MSCs that are treated ex vivo with a proprietary cardiopoiesis process intended to reprogram the MSCs into cardiac progenitors. The company expects the cells to differentiate to heart muscle after transplantation and is currently conducting a 240-patient phase III trial.

In addition to heterogeneous versus enriched cell populations, allogeneic and autologous therapeutic strategies are being explored. From a manufacturing perspective, an allogeneic therapy is more scalable and can use the traditional pharmaceutical off-the-shelf model; however, for cells intended to persist in the heart, immune suppression may be required. Autologous cells, particularly if minimally manipulated, have the advantage of facing less restrictive regulatory requirements but tend to be less cost efficient to manufacture and distribute. Autologous therapies also largely eliminate the risk of rejection and the need for immunosuppressive drugs. Companies focused on developing an autologous therapy (e.g., Caladrius Biosciences, Cytori [San Diego, CA, http://www.cytori.com], Vericel, and Celyad) point to this as a favorable safety attribute. Not all allogeneic therapies will require immunosuppressive regimens because many cellular therapeutics do not require long-term retention of the transplanted cells to achieve their paracrine-mediated effects; however, allogeneic cell-replacement therapies that require functional engraftment for their effects may require long-term immunosuppressive drug therapy to support persistence.

At the time of publication, it is unclear which approaches may prove to have the most merit and provide the most clinical benefit to patients. Fortunately, a number of well-controlled, double-blinded mid- and late-stage clinical trials are ongoing using each of the approaches outlined above, so we may soon be one significant step closer to answering some of the questions that have challenged the field for many years.

**CIRM CARDIOVASCULAR DISEASE PORTFOLIO**

As shown in Table 2, CIRM supports a variety of cell and gene therapy-based projects aimed at achieving cardiac repair and regeneration. CIRM’s portfolio in ischemic heart disease is diverse and consists of projects in various stages of development, including early preclinical proof of principle for novel technologies and phase II clinical trials. Approaches include paracrine regeneration, direct cardiomyocyte replacement, cardiovascular progenitor (CVP) transplantation, and tissue-engineering efforts to enhance cell retention and transdifferentiation efforts. Some of
the advantages and challenges of these approaches are discussed in this section.

The most advanced cardiovascular disease program being funded by CIRM is a phase II clinical trial (ALLSTAR), sponsored by Capricor Therapeutics, Inc. (Beverly Hills, CA, http://capricor.com) to develop an allogeneic cardiac-derived stem cell product, cardiosphere-derived cells (CDCs), for use in patients with residual left ventricular dysfunction following MI. This therapeutic is thought to act through a paracrine mechanism, activating endogenous reparative pathways, and is hypothesized to have greater cardiac reparative potential than non-cardiac-derived cellular products. A head-to-head comparison of four different cell populations including CDCs, derived from different sources and tested in the same animal model, provides support for this notion [12]. The ALLSTAR trial that is under way is designed to test the safety and efficacy of intracoronary infusion of allogeneic cardiosphere-derived cells (CAP-1002) in patients that had an MI within the prior 12 months and that have a large infarct (scar) size (>15%), as measured by magnetic resonance imaging (MRI). The overall primary efficacy endpoint will be scar size reduction at one year, assessed by MRI.

A CIRM-funded program led by Dr. Joseph Wu (Stanford University, Stanford, CA) is working to develop a cellular therapy to repair the heart muscle of people with end-stage heart failure. The group is using human embryonic stem cells (hESCs) as source material to derive functional heart muscle cells (cardiomyocytes). These cells could both replace damaged tissue and secrete factors to stimulate healing of the heart muscle wall and slow further decline of heart function. The cardiomyocytes are derived from a highly expandable, allogeneic cell population and are intended to be an off-the-shelf therapeutic that could be administered into the ventricular heart muscle of a patient after MI. Although the allogeneic therapy would likely require use of immunosuppression, it would offer a promising strategy for improving outcomes in many more patients than could be matched for heart transplant. Preliminary studies have shown the feasibility of this approach with similar cells in rodent models of acute or chronic heart failure. The team is currently focused on performing the activities necessary to request approval from the U.S. Food and Drug Administration (FDA) to initiate clinical testing of these hESC-derived cardiomyocytes (hESC-CMs).

The goal of another CIRM-funded program, directed by Dr. Reza Ardehali (University of California Los Angeles [UCLA], Los Angeles, CA), is to identify PSC-derived CVP cells that can differentiate into mature cardiomyocytes and functionally integrate following transplantation into the heart. PSC-derived CVPs isolated at sequential stages of differentiation will be tested in a preclinical animal model of MI for the ability to integrate and improve heart function so as to identify the cell type that is most effective for repair. This strategic contrast two approaches in which differentiated cardiomyocytes are transplanted, in that a CVP is expected to give rise to multiple lineages of heart cells that are lost during a myocardial infarction (i.e., cardiomyocytes,
The J. David Gladstone Institutes (San Francisco, CA, http://gladstoneinstitutes.org) is intended to be a scalable, off-the-shelf therapeutic. The team under their CIRM award. Similar to the hESC-CM strategy, this function is being evaluated in relevant preclinical models by the future, provide a source of off-the-shelf allogeneic iPSC-CMs for multiple patients and different time windows following an MI.

As mentioned, previous clinical trials testing a variety of bone marrow-derived cells for treatment of post-MI heart damage have not demonstrated definitive improvements in heart function, potentially owing to poor donor cell retention and survival after intramyocardial or intracoronary injection. On the basis of this hypothesis, a CIRM-funded team led by Dr. Walter Boyd (University of California Davis, Davis, CA) aims to improve on these methods by seeding allogeneic bone marrow-derived MSCs onto a porcine small intestinal submucosa extracellular matrix (ECM) bioscaffold that is already FDA approved and used clinically for heart repair. This combination therapy is hypothesized to be superior to injection of cells in suspension because the matrix will provide structural integrity and help retain the cells at the site of transplant, allowing more sustained localized delivery of beneficial paracrine factors secreted by the MSCs. Furthermore, the matrix itself may retain ECM signaling functions that result in improved neovascularization of the region where it is transplanted [14]. This program is focused on demonstrating the potential efficacy of this combination product in preclinical models of ischemic heart disease after MI.

A collaborative effort led by Dr. Wu (Stanford) and Dr. Wolfram Zimmermann (University of Göttingen, Göttingen, Germany) is developing an allogeneic, cardiomyocyte-based patch approach to support failing hearts. The team is incorporating hESC-CMs into contractile patches of engineered heart tissue, and the patches are then applied to the surface of the heart following an MI. The patch is intended to provide contractile and mechanical support for a weakened ventricular wall. The technology can retain delivered hESC-CMs at the heart, where they have the opportunity to engraft with the host cardiomyocytes. A first generation of this approach using rat cardiomyocytes slowed progression of heart failure and displayed graft-host coupling in vivo [15]. Studies to date with the human version have demonstrated prolonged survival of hESC-CMs in vivo and enhanced cell retention over cells delivered as a suspension. The impact of these patches on left ventricular function is being evaluated in relevant preclinical models by the team under their CIRM award. Similar to the hESC-CM strategy, this patch is intended to be a scalable, off-the-shelf therapeutic.

Finally, CIRM is funding a project led by Dr. Deepak Srivastava at the J. David Gladstone Institutes (San Francisco, CA, http://gladstoneinstitutes.org) that uses a gene therapy-mediated cell-fate switch to achieve cardiac regeneration. By transducing cells with specific cardiogenic (transcription) factors, fibroblasts can be induced to acquire the features of cardiomyocytes. Induced cardiomyocytes have gene expression signatures similar to native cells and can display cardiomyocyte-like calcium signaling, morphology, and beating [16]. Dr. Srivastava’s team has demonstrated that this reprogramming process is more efficient in vivo than in vitro and that the cells can mature and improve cardiac function in mice following an MI [17, 18]. Under this CIRM award, the group has recently identified factors capable of reprogramming human fibroblasts toward the cardiac lineage in vitro [19] and has achieved reprogramming of large animal fibroblasts in vivo, which would enable large animal safety and efficacy studies. They are also optimizing gene-delivery methods to achieve in vivo reprogramming and cardiac functional recovery in small and large animal models. This approach, if successful, is potentially transformative. It provides a potential source of new cardiomyocytes and could obviate a roadblock to cardiomyocyte cell-replacement therapy and the need to optimize cell delivery and achieve long-term cellular integration. The approach also has the advantage of off-the-shelf potential without the immune suppression that will likely be required for most allogeneic approaches.

**Conclusion**

The unmet medical need faced by heart failure patients is clear. A therapy that could either prevent progression to heart failure after MI or improve the function of failing hearts could have a major impact on the standard of care. Such a therapy could also have an economic impact because cardiovascular disease represents a multibillion-dollar annual economic burden in the U.S. alone [20, 21]. Given the urgency and magnitude of the need, concurrent pursuit of alternative strategies is a rational approach.

Ongoing phase III clinical trials should provide a more definitive answer as to the efficacy of bone marrow-derived cell therapies, whereas other approaches, such as cardiac-derived progenitors and hESC-derived cardiac progenitors and hESC-CMs, will soon enter or have begun phase I and II clinical trials. One of the challenges facing the field is the size and duration of clinical studies required to definitively assess efficacy of cardiac therapies. Endpoints typically include long-term metrics such as survival, so approval of these therapeutics can require registration trials of thousands of patients followed over multiple years.

CIRM is investing in a wide variety of new cell therapy approaches to treatment of post-MI cardiovascular disease, including both paracrine and cell replacement mechanisms of action, and taking advantage of novel tissue engineering and cell reprogramming strategies. Complementing CIRM’s therapeutic portfolio for cardiovascular disease, CIRM is also supporting relevant research in the areas of tissue engineering and animal modeling. Taken together, this CIRM-funded cardiovascular portfolio has the potential to enhance existing approaches and produce a lasting and positive effect for patients facing ischemic heart disease.

**Author Contributions**

L.C. and L.K.: conception and design, manuscript writing; C.P., I.C., and N.L.: manuscript writing.

**Disclosure of Potential Conflicts of Interest**

The authors indicated no potential conflicts of interest.
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