Support for Cardiovascular Cell Therapy Research at the National Heart, Lung, and Blood Institute

Denis B. Buxton, Sonia I. Skarlatos

Heart failure (HF) exerts an enormous burden both in the United States and worldwide, compounded by the lack of effective therapies for patients with end-stage HF. Current options other than medical management are limited to cardiac transplantation, severely limited by the lack of donor availability, and ventricular assist device (VAD) implantation. Although the introduction of newer VADs has reduced complications such as thrombosis and infection, mortality in patients with VADs is still significant (~25% in the first year). Since the capacity of the heart to repair itself after myocardial infarction (MI) is very limited, effective regenerative therapies for patients with large acute MI to prevent progression to HF would be highly beneficial in decreasing the morbidity and mortality associated with end-stage HF. Regenerative therapies to repair failing hearts have the potential to provide new options for patients with advanced HF who currently face low quality of life as well as poor prognosis.

National Heart, Lung, and Blood Institute Cardiovascular Cell Therapy Research Network

Clinical trials to test the ability of cell therapy to treat patients after MI were initiated a decade ago in Europe with injection of autologous skeletal myoblasts in patients undergoing coronary artery bypass surgery, followed rapidly by trials using other cell types and delivery routes. In response to the proliferation of non-US cell therapy trials, the National Heart, Lung, and Blood Institute (NHLBI) held a working group in August 2004 to assess the status of clinical studies of cell-based therapies for cardiovascular disease, to determine the gaps in knowledge and barriers that prevent the implementation of well-designed and safe clinical studies. Another charge to the working group was to identify the areas of opportunity to apply cell-based therapies for cardiovascular disease. The primary recommendation of the working group was the formation of a cardiovascular cell therapy research network, consisting of a clinical research network component (5–7 primary sites) that would have an integrated, complementary preclinical investigative component. The intent of the recommendation was to establish a stable infrastructure to conduct safe, efficient cell therapeutic clinical trials grounded in, and informed by, sound preclinical studies to improve outcomes for cardiovascular patients.

Clinical centers were chosen for their demonstrated clinical science excellence, their ability to isolate and prepare GMP grade cellular products, and their proven recruitment mechanisms appealing to patients from a variety of racial/ethnic groups. The five primary centers selected and their current principal investigators are the Cleveland Clinic (Stephen Ellis, MD), the Texas Heart Institute (James Willerson, MD), the University of Florida (Carl Pepine, MD), Minneapolis Heart Institute (Timothy Henry, MD), and Vanderbilt University (David Zhao, MD).

Three protocols were ultimately selected by the investigators, based on their potential to identify optimal cell therapy to improve ventricular function and structure.

(1) TIME (NCT00684021) is a Phase II, randomized, placebo-controlled, double-blind trial evaluating the effect of timing on the intracoronary administration of bone marrow mononuclear cells versus placebo in patients with acute MI. The trial was initiated in July 2008 and completed randomization in November 2011. Primary outcomes include global and regional left ventricular function as assessed via cardiac MRI at 6 months; results will be available in the summer of 2012.

(2) LateTIME (NCT00684060) is a Phase II, randomized, placebo-controlled, double-blind pilot trial evaluating the safety and efficacy of intracoronary administration of bone marrow mononuclear cells 2 to 3 weeks after acute MI. This pilot study is an extension of the TIME trial, with the goal of determining the best time for transplant of these cells after such an event. LateTIME was also initiated in July 2008 and completed randomization in August 2011.

(3) FOCUS (NCT00824005) is a Phase II, randomized, placebo-controlled, double-blind trial of intramyocardial injection of autologous bone marrow mononuclear cells under electromechanical guidance for patients with chronic ischemic heart disease and left ventricular dysfunction. This trial was initiated in March 2009 and completed randomization in April 2011. Primary end points include change in maximal oxygen consumption (MVO₂), change in left ventricular end systolic volume (LVESV) (via echocardiography with contrast), and reduction in perfusion defects (via SPECT).

NHLBI Progenitor Cell Biology Consortium

The rapid growth of clinical trials for the treatment of acute and chronic cardiovascular diseases has not been without controversy; many investigators, both basic and clinical, have questioned the wisdom of embarking on cell therapy trials when the mechanism of action is unclear. In 2007, the NHLBI held a working group in conjunction with the NHLBI...
Cardiovascular Regenerative Medicine Symposium to seek input from leaders in the field on how the institute could provide a mechanistic underpinning for cell therapy trials and also take advantage of new developments in the field. This led to the concept of a synergistic consortium to identify and characterize progenitor cell lineages, to direct the differentiation of stem and progenitor cells to desired cell fates, and to develop new strategies to address the unique challenges presented by the transplantation of these cells. By bringing together leaders in the fields of cardiovascular, hematopoietic, and pulmonary cell biology along with stem cell biologists, the consortium would leverage expertise from different disciplines to accelerate progress in the different areas. The Progenitor Cell Biology Consortium (PCBC) was funded through an innovative 3 step process designed to foster synergy and collaboration. An initial request for applications, HL08-012 NHLBI Progenitor Cell Biology Consortium Planning Awards (R03), solicited short “white papers” proposing multidisciplinary projects at a single institution and resulted in 89 applications. Twenty-four R03 planning grants were awarded, and the successful awardees met in Bethesda to make brief presentations of their proposed work and look for complementary projects. The planning grants were then used to meet with other R03 awardees and to assemble virtual hub applications of 2 or more complementary R03s to apply for request for applications HL09-004 NHLBI Progenitor Cell Biology Consortium (U01). Seventeen U01 awards organized into 9 virtual hubs were eventually funded in Fall 2009 for 7 years. The Table lists the hubs and their areas of interest.

Another innovative feature of the PCBC is that $25 million have been set aside for competitive funding through the 7-year funding cycle of the PCBC to fund pilot studies, ancillary and collaborative studies, and cores. The funds are administered through the Administrative Coordinating Center, led by Michael Terrin, MD, at the University of Maryland, Baltimore, which publishes the Requests for Proposals through the PCBC website. Funding opportunities open to investigators not currently participating in the PCBC are announced in the National Institutes of Health (NIH) Guide and on the NHLBI public website. Proposals are reviewed by an External Advisory Committee, chaired by Dr Victor Dzau; this expedited review process provides a nimble mechanism to respond to novel findings in the rapidly moving field. The set-aside funds are also being used for skills development activities. Total funding for the PCBC over the 7 years is $170 million.

**Table. Hubs and Their Areas of Interest**

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Specialized Centers of Cell Therapy

In 2004, the NHLBI issued a request for applications to fund Specialized Centers for Cell Therapy, modeled after the NHLBI Specialized Centers of Clinically Oriented Research that the institute has used to fund research in a number of disease areas. One cardiovascular center was funded at Johns Hopkins University (JHU), with Joshua Hare as the PI, in addition to 2 centers focused on hematopoietic therapies (https://web.enmnes.com/study/scct/index.html). After relocations of investigators, Eduardo Marban is the current PI at Cedars Sinai Medical Center, with JHU and the University of Miami as additional sites. The goal of the Specialized Centers of Cell Therapy (SCCT) is to perform preclinical studies and then to develop clinical protocols and apply them to patients. The program is currently in a no-cost extension to allow completion of the ongoing trials and will not be renewed.

Preclinical studies showing safety and efficacy of autologous and allogeneic mesenchymal stem cells (MSCs) in porcine models led to the development of clinical trials at the University of Miami and JHU.

(1) PROMETHEUS (NCT00587990) is a Phase I/II, randomized, double-blinded, placebo-controlled study of the safety and efficacy of intramyocardial injection of autologous human MSCs in patients with chronic ischemic left ventricular dysfunction secondary to MI undergoing cardiac surgery for coronary artery bypass grafting. The trial was terminated early after enrollment of 11 of a planned 24 patients due to difficulties in recruiting.
(2) POSEIDON (NCT01087996) is a Phase I/II, randomized pilot study of the comparative safety and efficacy of transendocardial injection of autologous MSCs versus allogeneic MSCs in patients with chronic ischemic left ventricular dysfunction secondary to myocardial infarction. Patients receive 20 million, 100 million, or 200 million autologous or allogeneic MSCs administered transendocardially through a Biocardia helical infusion catheter. The primary outcome is incidence of treatment-emergent serious adverse events at 1 month after catheterization. Secondary outcomes include functional measurements at 6 and 13 months as well as safety at the same time points. Recruitment is complete, and initial results are expected in Fall 2012.

(3) The Cedars Sinai group is focused on cardiac stem cells (CSCs), and initial work funded by the SCCT supported development of the cardiosphere method to culture cardiac progenitor cells from heart biopsies. Preclinical studies using intracoronary infusion of cardiosphere-derived stem cells (CSCs) in a porcine model of ischemic cardiomyopathy demonstrated safety and efficacy of the method, leading to the initiation of a clinical trial at Cedars Sinai and JHU: CADUCEUS (NCT00893360) is a Phase I, randomized, dose escalation study of safety and efficacy of intracoronary delivery of CSCs in patients with ischemic left ventricular dysfunction and a recent myocardial infarction. The primary end point is safety at 6 months, defined by cardiac-related death, cardiac tumor formation, or composite major adverse cardiac events. Secondary end points include regional and global function and additional safety measures.

Other Cell Therapy Research Support at NHLBI

The NHLBI supports a very robust portfolio of basic, preclinical, and clinical research in cardiovascular cell therapy through a variety of mechanisms, including small business mechanisms, investigator-initiated applications, and applications submitted in response to Funding Opportunity Announcements. A search via the National Institutes of Health RePORTER system (http://report.nih.gov/) for grants on cardiac and cardiovascular cell therapy yielded approximately 100 funded grants for 2010 in addition to the programs discussed above, constituting an additional investment for the fiscal year of approximately $50 million.

Research Supported by NHLBI

Progenitor Cell Biology

Fundamental research to understand progenitor cell biology and harness it toward cell therapy represents a major focus of NHLBI support, both within and outside the PCBC. The generation of hiPSCs by expression of transcription factors remains slow and inefficient, but PCBC-supported researchers at the University of Pennsylvania have developed a novel method to improve speed and efficiency of reprogramming. Transduction of mouse or human fibroblasts with lentivirus expressing the miR302/367 cluster of microRNAs resulted in a 2–order of magnitude increase in reprogramming efficiency compared with transcription factor–based reprogramming.

The generation of patient-specific cardiomyocytes for regenerative therapies after MI is a very active area. In work supported by the PCBC, Ieda et al showed that fibroblasts can be directly differentiated into cardiomyocytes by a combination of 3 transcription factors, Gata4, Mef2c, and Tbx5. More recently, work from the same laboratory has extended these findings into mice in vivo, using local delivery of the 3 transcription factors by retroviral-mediated gene transfer to the infarct border zone in a mouse MI model. Delivery of the transcription factors resulted in reprogramming of cardiac fibroblasts to cardiomyocytes and resulted in decreased infarct size and improved cardiac function. Co-delivery of the proangiogenic peptide thymosin β4 resulted in further improvements in scar area and cardiac function. Complementary results were reported in investigator-initiated research, using a combination of miRNAs 1, 133, 208, and 499 to reprogram cardiac fibroblasts to cardiomyocytes. Transient transfection of synthetic miRNAs into mouse cardiac fibroblast in vitro resulted in upregulation of mature cardiac markers, sarcomeric organization, and spontaneous calcium flux; the miRNA-mediated reprogramming was enhanced 10-fold by cotreatment with the small-molecule JAK inhibitor. Injection of lentivirus encoding the miRNAs into ischemic myocardium after left anterior descending coronary artery ligation resulted in in vivo reprogramming of cardiac fibroblasts to cardiomyocytes.

Transplantation of allogeneic ESC and iPES faces the barrier of rejection by the immune system. To overcome this hurdle, Pearl et al blocked leukocyte immunostimulatory molecules and used bioluminescence imaging to track the longitudinal survival of implanted cells transduced with a double-fusion reporter gene construct carrying firefly luciferase (Fluc) and enhanced green fluorescent protein (eGFP). They found that induction of immune tolerance with 3 costimulatory receptor-blocking antibodies (CTLA4-Ig, anti-LFA-1, and anti-CD40L) prevented rejection of allogeneic mESCs. Co-stimulatory blockade also prevented rejection of xenogeneic hESCs, and, in both cases, brief treatment (>6 days) provided long-term protection. Since differentiation of stem cells leads to upregulation of major histocompatibility complex expression, they also tested survival of in vivo spontaneously differentiated cells isolated from an explanted hESC-derived teratoma and in vitro differentiated hESC-derived endothelial cells. Costimulatory blockade provided extended survival of in vivo–differentiated cells and maintained survival of in vitro–differentiated hESC-ECs to the same extent as implantation in NOD/SCID mice (Figure 1).

Basic Research in Cell Therapy

Regeneration of myocardium after MI, either by supplying exogenous cells or by strategies designed to enhance endogenous repair, is another area of very active support. Modification of progenitor cells to improve survival when transplanted in the hostile post-MI environment has been an area of active interest. Modification of cells with prosurvival kinases such as Akt and Pim enhances the regenerative potential of progenitor cells for cardiac repair, and so recent work from the Sussman laboratory has examined whether overexpression of nuclear Akt would enhance the efficacy of cardiac progenitor cells (CPCs). Nuclear Akt overexpression promoted rapid proliferation and secretion of paracrine effectors, and injection of Akt overexpressing CPCs into mice
with acute MI resulted in greater recruitment of endogenous c-kit+ cells as well as survival of exogenous c-kit+ CPCs. However, the cells displayed impaired commitment to the cardiovascular lineage and were unable to improve cardiac function. Temporal control of Akt expression through an inducible expression system to facilitate proliferation followed by lineage commitment could be helpful in this context.

The majority of cell transplantation studies have focused on treating acute MI, but helping the large population of patients with existing HF, where infarcted tissue has been replaced by scar, is a major need. In a study supported by several NHLBI grants, Tang et al administered GFP-labeled CSCs via intracoronary infusion in rats 1 month after coronary occlusion and reperfusion. They demonstrated beneficial structural and functional effects of the CSCs compared with vehicle infusion. Although they found some GFP-labeled cells expressing cardiomyocyte, endothelial, and smooth muscle markers, the number of these cells was modest, and paracrine effects causing proliferation of endogenous CSCs were suggested to be the main mechanism involved in the beneficial effects.

**Preclinical Research**

Although rodent models have been invaluable for basic research in cardiovascular cell therapy, translating the findings obtained in these models toward clinical application requires the use of larger animal models such as pig and dog that more closely approximate human physiology and pathophysiology. The CADUCEUS trial uses CDCs rather than cardiospheres because the larger size of cardiospheres (50–200 μmol/L) would be anticipated to cause embolization during intracoronary injection. However, direct injection of cardiospheres into peri-infarct myocardium provided more functional improvement than injection of CDCs in mice with acute MI. To compare the efficacy in a more clinically relevant model, cardiospheres and CDCs were injected into the peri-infarct zone in pigs with heart failure 4 weeks after MI. Whereas both cell preparations provided similar benefits on ejection fraction compares with placebo, echocardiographic and hemodynamic indexes of efficacy improved more with cardiospheres, and adverse remodeling was more attenuated with cardiospheres than with CDCs (Figure 2).

Short-term survival from cardiac transplantation has been improved by advances in immunosuppressive therapy, but accelerated cardiac grant vasculopathy still results in an annual mortality rate of 5%. D’Alessandro et al recently demonstrated in a dog model that progenitor cells isolated from the explanted heart and expanded can be infused back into the coronary arteries of the transplant recipient, resulting in the generation of functional immune-compatible chimeric myocardium and blood vessels within the transplanted heart. This NHLBI-supported study raises the intriguing possibility that over time, newly formed myocardium from the original heart could replace donor tissue, reducing the need for immunosuppression.

**Clinical Studies**

As discussed above, several clinical trials supported by the Cardiovascular Cell Therapy Research Network (CCTRN) and SCCT programs have now finished recruitment, and publication of the results is ongoing. For LateTIME, 6-month cardiac MRI efficacy data were presented at the 2011 Scientific Sessions of the American Heart Association in Orlando in November 2011. The results were simultaneously published in the Journal of the American Medical Associa-
tion. Intracoronary infusion of autologous bone marrow mononuclear cells, 2 weeks to 3 weeks after PCI, did not improve global or regional function at 6 months in patients with MI and left ventricular dysfunction.24 A sister trial (TIME) will further examine the timing of intracoronary infusion of these cells (3–7 days after MI) to help provide a more complete picture of changes in circulating progenitor cells derived from bone marrow in the postinfarction milieu. Results of the TIME trial are anticipated in the summer of 2012.

The safety and efficacy of intramyocardial injections using bone marrow cells in an HF population are explored in the FOCUS trial; 6-month efficacy data were presented at the American College of Cardiology Annual Meeting in Chicago in March 2012, and the results were simultaneously published in the Journal of the American Medical Association.25 Transendocardial injection of autologous bone marrow cells compared with placebo did not improve LVESV, MVO2, or defect reversibility on SPECT. Further exploratory analysis showed a significant improvement in left ventricular ejection fraction associated with treatment.25 These findings provide evidence for further studies to determine the relationship between the composition and function of bone marrow product and clinical end points.

Results from the CADUCEUS trial were published recently, demonstrating intracoronary treatment with CDCs to be safe in patients 1.5 to 3 months after MI.26 Regional (but not global) function was improved in CDC-treated subjects, including a 63% increase in systolic thickening. These functional changes were accompanied by significant decreases in scar size and increases in viable cardiac mass measured by late gadolinium enhancement on MRI. A significant correlation was found between scar shrinkage and increased viable mass, consistent with replacement of scar to viable muscle mass.

Interim results from another Phase I randomized trial using cardiac-derived stem cells, Cardiac Stem Cell Infusion in Patients With Ischemic Cardiomyopathy (SCIPIO), were also published recently.27 The trial, supported in part by NHLBI, assessed the efficacy of intracoronary administration of 10^6 purified, c-kit–positive, autologous CSCs in patients with chronic postinfarction LV dysfunction. The stem cell therapy was safe, with no adverse events attributed to the treatment. In patients receiving cells, ejection fraction was increased 8% at 4 months and 12% at 12 months after administration, whereas control patients had no functional improvement (Figure 3). Infarct size was significantly reduced in treated patients, as assessed by late gadolinium enhancement on MRI. CSC-treated patients also showed significant improvements in New York Heart Association functional class and quality of life.27

Future Directions

In light of the growing numbers of patients with HF, the application of cell therapy to cardiovascular disease can be expected to remain a very active research area in coming years. CSC biology and therapy, differentiation of hESC and hiPSC to cardiac phenotypes, and direct differentiation of fibroblasts and other somatic cells to cardiomyocytes are among the areas in which particular growth can be anticipated. Strategies to improve survival of transplanted cells, for example, through genetic modification of the stem cells or provision of support matrices to provide a prosurvival environment, are also likely to find their way into preclinical models for eventual translation.

In terms of NHLBI programs, the two major basic and translational programs, the CCTRN and PCBC, are ongoing. The CCTRN was initiated in January 2007 to provide the organizational, technical, and clinical infrastructure necessary
to conduct early, proof-of-concept studies designed to add new knowledge in the field of cell-based therapies for the treatment of cardiovascular diseases. Because of the CCTRN’s accomplishments in the last 5 years and its collaborative partnerships with academic leaders, the NHLBI decided to renew the CCTRN (CCTRN 2) in a competitive manner for another 7 years. In addition to supporting cell-based therapies for acute MI and HF, CCTRN 2 will also support studies on angina and peripheral arterial disease. As of March 2012, the NHLBI funded 7 Regional Clinical Centers, 12 satellites, and 1 data coordinating center (University of Texas School of Public Health, Lemuel Moye, MD, PhD). The Regional Clinical Centers are Indiana Regional Cell Therapy Center (Michael Murphy, MD), Minneapolis Heart Institute (Timothy Henry, MD), Texas Heart Institute, Stem Cell Center (James Willerson, MD), the University of Florida (Carl Pepine, MD), the University of Louisville (Roberto Bolli, MD), the University of Miami School of Medicine (Joshua Hare, MD), and Stanford University School of Medicine (John Cooke, MD, PhD). There are also 4 Research Skills Development programs; 2 for clinical research investigators and 2 for clinical research coordinators. The PCBC is currently in its 4th year of funding, with an additional 3 years of funding planned. The use of ancillary studies will permit addition of new investigators to the PCBC to meet the changing needs of this rapidly developing field.

Summary

Cell therapy approaches have great potential for preventing the development of HF after MI and for treating patients with existing HF. The NHLBI PCBC will continue to fund research on progenitor cell biology and the development of new therapies. The renewal of the CCTRN will provide a vehicle for the clinical testing and translation of new therapies. In addition, the NHLBI expects to continue to fund a robust portfolio of investigator-initiated grants covering the spectrum of cardiovascular cell therapy research, from basic progenitor cell biology through the development of therapies in small animal and preclinical models, to clinical trials to test the safety and efficacy of cell therapy approaches.

Disclosures

None.

References


Figure 3. Echocardiographic analysis of CSC-treated patients and controls. A. Left ventricular ejection fraction (measured by use of 2-dimensional echocardiography) at 4 months after baseline in control and CSC-treated patients. B. Ejection fraction at 4 months and 12 months after baseline in the CSC-treated patients who had 1 year of follow-up. C. Change in ejection fraction from baseline at 4 months and 12 months in CSC-treated patients. From Bolli et al,27 with permission from Elsevier.


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