

# General Overview of the Seventh International Symposium on Stem Cell Therapy and Cardiovascular Innovations

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**Abstract** The Seventh International Symposium on Stem Cell Therapy and Cardiovascular Innovations was held in Madrid on the 6th and 7th of May 2010. Gathering for the seventh consecutive year the most relevant researchers and opinion leaders on cardiovascular cell therapy, it has become the most important worldwide event on this field. A comprehensive review of the last developments on cell therapy, surgery for heart failure and tissue engineering was made, and the results of three clinical trials were reported. The Symposium was dedicated to the memory of Professor Helmut Drexler.

**Keywords** Stem cell · Cardiovascular · Cell therapy · Tissue bioengineering · iPS · Myoblast · Cardiac repair · Myocardial infarction · Heart failure

## Introduction

Professor Helmut Drexler died of a sudden cardiac death on September 2009, having yielded a career full of scientific achievements and a huge contribution to the understanding and treatment of heart failure. Dr. Wollert and Dr. Gersh

introduced the Symposium with a recollection of Professor Drexler's scientific legacy, from molecular biology of heart failure to the BOOST trial.

## Present and Future of Embryonic-Like Cells

Embryonic stem cells show the greatest potential for organ regeneration. In animal models of infarction and cardiomyopathy, these cells have been proven to induce a significant increase of systolic function, as well as normal electrical integration. However, several issues impair their suitability for clinical application, such as their allogeneic nature and social and ethical concerns about their origin [1].

Induced pluripotent stem cells (iPS) are cells obtained from an adult human tissue, which have been modified by means of transcription factors to acquire similar characteristics to embryonic stem cells [2, 3].

These cells show identical morphology, surface markers, and capacity for proliferation and differentiation as embryonic stem cells, without the need to use human embryos to harvest them.

It has been shown that iPS cells can be made to differentiate to cells with contractile and electrophysiological properties.

These cells have been tried on preclinical models in sickle cell anemia and Parkinson's disease, and animal models of acute myocardial infarction and dilated cardiomyopathy.

In a murine model of myocardial infarction, Dr. Terzic's group, at Mayo Clinic, proved iPS cells to be useful to repair damaged myocardium, by controlled tissue integration and chimeric tissue formation. When injected into the infarcted murine myocardium, iPS cells caused an increase in ejection fraction and prevented post-infarction remodeling whereas adult fibroblasts did not [4].

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During the young investigators session, Dr. Montserrat, from Dr. Izpisua's group, commented on the process of cell reprogramming, which has been simplified since the first reports, and on the need for cell differentiation strategies and larger animal models.

Recently discovered, very small embryonic-like cells are a rare population present in adult bone marrow, which exhibit many characteristics of embryonic cells, and have been shown to suffer mobilization to peripheral blood after acute myocardial infarction in murines [5].

Dr. Tendera's group, from Katowice (Poland), studied this cell population in humans, showing these cells are present in very small proportions in peripheral blood of healthy subjects, and are rapidly mobilized during the first hours after an acute myocardial infarction [6].

During the young investigators session, Dr Wojakowski, from Dr. Tendera's group, exposed their projects in preclinical and clinical models of acute and chronic ischemia with VSELS.

## Adult Stem Cells

Although fetal stem cells hold the highest potential for organ generation, adult tissues are also a source of pluripotent cells. Four types of adult cells are the main focus of present cardiovascular research: bone marrow cells (including hematopoietic stem cells and mesenchymal stem cells), adipose-derived stem cells, skeletal myoblasts and cardiac stem cells.

**Bone Marrow Cells** The clinical applicability of bone marrow cells pluripotency has been widely proven by bone marrow transplantation for a variety of disorders over decades.

The REPAIR-AMI trial proved that intracoronary administration of bone marrow mononuclear cells after acute myocardial infarction is beneficial both in terms of ejection fraction and clinical outcomes [7].

However, their capacity to differentiate to adult cardiomyocytes is yet uncertain, although suggested by some animal experiences.

Other possible beneficial mechanisms of action include paracrine effects, vasculogenesis and vascular repair.

Dr. Simari, from Mayo Clinic, reported the existence of a vascular niche of bone marrow cells, located mainly next to the adventitia. There is experimental evidence in animals that this niche and the bone marrow population are linked, and cells from each of them can migrate and integrate into the other. Dr. Simari hypothesized this perivascular hematopoietic niche plays a role in vascular and endothelial repair, and may be the target of hematopoietic precursors delivered intravascularly for cardiac repair.

During the young researchers' session, Dr. van Ramshorst from Leiden presented the results of their clinical trial involving patients with "no-option" coronary artery disease. Direct intramyocardial injection of bone marrow mononuclear cells guided by the NOGA system improved myocardial perfusion, left ventricular function, and anginal symptoms compared with placebo injection [8].

**Adipose-Derived Stem Cells** These cells, located in the peripheral fat tissue, present advantages such as being easily and safely harvested by means of a liposuction. There is experimental evidence that these cells can trans-differentiate to cardiomyocytes and form vascular structures [9, 10].

In animal models of infarction these cells have been proven capable of improving ventricular function [11, 12].

According to Dr. Prosper, from Pamplona (Spain), mesenchymal stem cells have a beneficial effect on infarcted myocardium by means of different paths, such as a trophic effect on vascularization, remodeling modulation, and in vivo contribution to vascular cells and myofibroblasts.

The results of the PRECISE trial, involving treatment with adipose mesenchymal cells for no-option coronary patients, were presented during the meeting, and are discussed later on this paper.

**Resident Cardiac Stem Cells** The human adult heart lodges a small population of cells with progenitor capacity. These cells have shown ability for in vitro expansion [13–15], and in animal models of infarction they have shown improvements of ventricular function. However, in the clinical setting these cells appear to have a limited natural potential for cardiac repair, and have the inconvenience of being scarce and difficult to collect. Dr. Nadal-Ginard advocated the use of these cells on an allogenic, "off-the-shelf" basis, as a paracrine tool to stimulate cardiac repair by local resident cells.

## Remaining Questions and Challenges on Stem Cell Therapy

**Mechanisms of Action. Are Cells Really Necessary?** Although several types of cells have shown capacity to improve cardiac function in animal models of myocardial infarction and in human trials, it is far from clear that this benefit is caused by cardiomyocyte regeneration. As pointed out by Dr. Dimmeler, from Frankfurt, potential mechanisms of action of progenitor cells include myocyte differentiation, cell fusion, differentiation to vascular structures, and paracrine effects on vascular homeostasis, apoptosis, inflammation, scar remodeling and resident cell activation.

It is an acknowledged fact that homing and survival of different progenitor cells administered to the myocardium is scarce. Yet, Dr. Dimmeler presented experimental evidence that such homing is important, and clinical benefit depends largely on the persistence of those cells.

By means of an animal model of myocardial infarction in which animals were treated with progenitor cells carrying a suicide gene, it was possible to prove that suppression of engrafted cells also suppresses the benefits in left ventricular ejection fraction (LVEF) and vascularization these cells provide.

*Delivery, Homing, and Survival* Delivering cells into the myocardium in an efficient and safe way which is appropriate for each clinical setting is still a major practical issue. Cells have been delivered by five different paths: intravenous, intracoronary, via coronary sinus, via direct epicardial injection and by transendocardial percutaneous injection. Intravenous and intracoronary paths are preferred in acute myocardial infarction whereas myocardial injection (epicardial at the time of surgery or transendocardial by a percutaneous procedure) is usually the choice for chronic ischemia.

Despite the growing number of specific delivery devices available, homing and survival of most cell types in the myocardium is very low. Dr. Dib, from San Diego, commented on these issues and presented different innovative approaches to the problem of homing and survival, like shock wave and 3D myocardial matrix transplantation.

#### The Future of Clinical Trials

Dr. Nadal-Ginard believes resident cardiac stem cells have the best characteristics for myocardial repair research. These cells are expandable, and can differentiate to cells from three embryonic layers in vitro. For a cell type to be of practical use in clinical research and practice, it should be readily available, easy to apply, safe and affordable. That is why Dr. Nadal-Ginard defends the use of allogeneic cardiac stem cells, which according to his experience with pigs have a very high homing rate and are well tolerated.

From a clinical perspective, Dr. Zeiher analyzed the next challenges in cardiac cell therapy human trials. Concerning acute myocardial infarction trials, Dr. Zeiher stressed the importance of selecting patients at high risk, who were the only to have any benefits in the REPAIR-AMI, REGENT and FINNCELL trials [7, 16, 17].

He also advocated for a large scale trial with clinical endpoints in myocardial infarction.

In chronic angina, existing small trials have set the ground for a phase III clinical trial with NOGA (BDS, Cordis Corporation, Johnson and Johnson) guided transendocardial injection of bone marrow cells or CD34+,

aimed at achieving approval of such therapy in routine clinical management of stable refractory angina.

In chronic postinfarction heart failure, cell therapy has so far achieved little improvement of ventricular function, and no solid data are available concerning mortality. Yet, comparison of data available with model-predicted mortality suggests a beneficial effect, which is yet to be experimentally confirmed.

In his adjourn, Dr. Fernández-Avilés reminded that although iPS cells and cardiac stem cells are very promising, nowadays bone marrow cells and adipose-derived cells are realistic and effective tools.

#### Innovations for Advanced Heart Failure

Surgery for heart failure (Dr. Yacoub, London): from coronary revascularization to left ventricular assistance and heart transplantation, cardiac surgery offers a wide range of possible interventions on the natural history of heart failure. Special emphasis must be put on combination therapies and a multidisciplinary approach. Dr. Yacoub presented his findings on the negative effect of long term ventricular unloading on T tubule structure and function, especially on calcium-induced calcium release, which affects electromechanical coupling [18].

Heart transplantation (Dr. Mallidi, Stanford): since the first successful procedure four decades ago, over 75,000 patients worldwide have received a heart transplant. Mortality and morbidity have improved much since the first years, as better strategies have been established to fight rejection and infection, but still heart transplant must face new challenges. Aging population, more complex and morbid patients, increasing sensitization are some of the issues that need new solutions. Age, diabetes, renal failure and pulmonary hypertension are no longer necessarily excluding conditions. From a technical point of view, shortening ischemia, preserving normal anatomy and improving organ protection must be goals for the future. In postoperative care, special consideration must be given to immunosuppressive strategies, infection prophylaxis and rejection detection and control. Gene expression analysis is an interesting alternative to biopsy in low risk patients [19].

Ventricular assistance (Dr. Krabatsch, Berlin): from 1987 to 2010, the German Heart Institute has treated over 1,500 patients with some ventricular assistance device (VAD), and currently has 120 patients on this therapy. Over the last 15 years, the proportion of elderly patients on VAD has been increasing to almost 30%. Current indications for VAD include postoperative complications, acute myocarditis, and end-stage heart failure. Previous to the procedure, a strategy must be established as to the patient's long term fate (transplant, destination VAD, weaning), which in addition

to the patient's characteristics (age, size, comorbidity...) will determine the type of device and procedure. Thromboembolic events and infections are still a major problem.

### The Challenge of Tissue and Organ Engineering

Selecting the ideal type of cell for cardiac repair or regeneration, and delivering it in a safe and efficient way to maximize the rate of retention and survival are not easy tasks, as discussed above.

Yet, probably the greatest challenge of all in cell therapy is achieving integration and interaction of the cells delivered with the complex and damaged environment of the myocardium.

It has been often argued that while the heart hosts a population of resident cardiac stem cells, these seem rather inefficient at repairing large amounts of necrosis, such as happens in a myocardial infarction. One reason for this could be the difficulty for these cells to migrate and integrate in the hostile environment of necrosis and inflammation which follows a myocardial infarction.

In order to improve cell integration and contractile and electrophysiological function, different strategies have been tried to create bioartificial myocardial tissues, which can be developed in vitro and then patched to the heart in vivo.

The bioengineering approach consists of utilizing biological scaffolds made of collagen, silk, polylactic acid, polyglycolic acid or other large molecules, to which cardiomyocytes are attached to form a 3D muscular structure.

The biological assembly approach consists of culturing myocytes in a high-density suspension of cells and extracellular matrix materials, which supports aggregation of myocytes to form a micro-tissue.

The cell sheet approach consists of superposing several monolayers of cultured myocytes, which then generate compact muscular tissues [20].

All these approaches, however, still have problems to be solved, such as vascularisation, achieving a capacity to generate cells in large numbers for clinical use, immunological response, etc. Dr. Zimmerman, from Goettingen, commented on these approaches.

*The decellularization–recellularization strategy* Dr. Taylor, from Minnesota, believes it is virtually impossible to generate an artificial 3D structure which resembles all the functional and morphological complexity of the human heart. That is why years ago she set herself to the task of decellularizing the heart, eliminating all of its immunoreactive components, and then using the remaining structure as a natural scaffold to rebuild a functioning heart to be used

on an allogeneic basis for therapy. So far, Dr Taylor's group has worked with animal organs, achieving successful decellularization of any tissue or organ. They have also proved that the decellularized heart can be perfused and recellularized, and that it is capable of beating.

During the Symposium, the first complete decellularization of a human heart was achieved by the cooperative work of Dr. Taylor's and Dr. Fernández-Avilés's teams, at the Hospital Gregorio Marañón, in Madrid. This work is the product of the SABIO project, a cooperative endeavor of the University of Minnesota and the Hospital Gregorio Marañón. The next years of this exciting project will be devoted to continuing this line of work, with the aim of obtaining a recellularized functional human heart.

### Late Breaking Clinical Trials in Stem Cell Therapy

In this seventh edition of the Symposium, a new section included the presentation of the preliminary results of the most relevant stem cell randomized controlled trials which have finished enrolment and are waiting for publication. These included the first-in-man experiences with adipose-derived stem cells (ADSC) after acute myocardial infarction and in chronic ischemic myocardium, and the first-in-man trial with mesenchymal cells in chronic heart failure patients.

Dr. Dib presented the results at 3-month follow-up of the first US study to assess the feasibility and the safety of transendocardial delivery of allogeneic mesenchymal cells in subjects with heart failure. Twenty patients with left ventricular dysfunction (LVEF  $\leq 40\%$ ) and symptoms of heart failure (NYHA class  $\geq II$ ), received 25 million MSC ( $n=15$ ) or placebo ( $n=5$ ). The procedure was safe and feasible with no adverse events. Both groups showed a trend towards an improvement in quality of life parameters (6-min walk test and NYHA class). Finally, a reduction in overall arrhythmias and an improvement in end-systolic left ventricular volume/LVEF were observed in treated patients. Interestingly, histology revealed no evidence of immune rejection in a case of a patient who died in a vehicle accident 7 months after cell injection.

From Texas Heart Institute, Dr. Perin commented the results of the PRECISE trial. The PRECISE trial is a prospective, double blind, placebo-controlled trial that randomised 27 patients with end-stage coronary artery disease not amenable for revascularization and with moderate-severe left ventricular dysfunction to receive freshly isolated ADSC ( $n=21$ ) or placebo ( $n=6$ ) with the NOGA XP™ delivery system (BDS, Cordis Corporation, Johnson and Johnson). ADSC harvesting and injection were safe, with no peri-procedural complications and no

evidence of cell therapy related adverse events. An improvement in functional capacity as demonstrated by METS and  $\text{VO}_2$  Max and a decrease of infarct size as measured by MRI in ADSC-treated patients suggest potential efficacy which should be explored in a larger trial.

Finally, Dr. Duckers presented the results of the APOLLO trial, aimed to investigate the same source of freshly isolated ADSC, but in this case in patients with AMI and LV ejection fraction impairment. With a prospective, double blind, placebo-controlled design, 14 patients were randomised to ADSC ( $n=10$ ) or placebo ( $n=4$ ), which were delivered through intracoronary infusion after appropriate infarct related artery repair with stent implantation. The trial concluded that the liposuction procedure was safe in patients with normal aPTT and that intracoronary infusion of 20 million ADSC did not affect coronary flow. No ventricular arrhythmias were observed. Also, a trend towards better myocardial perfusion, LVEF and a reduction in the infarct size were noted.

### Posters Presentation

For the second year, the Meeting hosted a poster forum in which young researchers for all over the world had the opportunity of exposing their work. Three awards were given to the best presentations:

Dr. Martinez-Fernández, from Dr. Terzic's group, showed how different reprogramming strategies for iPS genesis render cells with different differentiation ability, which opens the door for individualized or at least organ specific iPS protocols.

Dr. Pelacho, from Dr. Prosper's group, presented their work with adipose-derived stroma vascular cells in a murine model of myocardial infarction, showing a long-term functional benefit, and cell contribution to vascular structures and myofibroblasts.

Dr. Windmolders, from Hasselt University (Belgium), presented her experience with isolation of cardiac stem cells from human atrial appendage. She also showed how these cells can be expanded and differentiate to cardiomyocytes.

### Conclusions

This year's meeting was dedicated to the memory of the late Professor Helmut Drexler. Bone marrow progenitors and adipose-derived mesenchymal cells are the most solid types in current cardiovascular research. Yet, an eye must be kept on promising options like iPS and resident cardiac stem cells. Tissue bioengineering is a major task, but one that will be accomplished.

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