A REPORT FROM THE FIRST REMEDIC WORKSHOP ON 'HEART REGENERATION' AUGUST 15-17 2008

SUMMARY

This workshop on heart regeneration was planned during the kick-off meeting of the Research Networking Programme (RNP) on Regenerative Medicine (REMEDIC) in Strasbourg 13 May 2008. Attended by 22 participants, including several PhD students, with high-level invited speakers including Professor Anthony D. Ho, University of Heidelberg in Germany, this was a productive and stimulating first REMEDIC workshop, addressing several critical issues in heart regeneration.

Accumulating clinical and experimental evidence indicates that mesenchymal stem cells (MSCs) are promising cell types in the treatment of cardiac dysfunction. They may trigger production of reparative growth factors, replace damaged cells or create an environment favor endogenous cardiac repair. However, identifying mechanisms which regulate the role of MSCs in cardiac repair is still unclear. To achieve the maximal clinical benefits, *ex vivo* manipulation could further enhance MSCs therapeutic potential. Three companies in the EU area made a contribution to the meeting, which also served as a platform to discuss substance to a proposal to the Regenerative Medicine Call of the 7th Framework Programme of the European Union. The next workshop was also discussed, and a proposal for a joint meeting on bone regeneration with the European Orthopaedic Research Society at the Rizzoli Institute in Bologna, was welcomed. A formal application will be submitted to the Steering Committee in October 2008. A summary report of the 'Heart Regeneration' workshop described here will appear in the Journal of Cellular and Molecular Medicine (in press).

SCIENTIFIC CONTENT AND PROGRAMME

The programme of the meeting is shown below and there was active participation by most participants with lively discussions and questions.

Friday 15 August 2008	
12.00 - 12.15	Welcome and Opening by the REMEDIC Chair, **Professor Yrjö T. Konttinen**.
12.15 - 13.00	Presentation of the Monzino Cardiology Centre, and 'Endothelial progenitor cells: the long way to clinical translation', **Professor Maurizio Pesce**, Milan, IT.
13.00 - 14.00	Lunch
	Session Chair, **Professor Dominque Pioletti**.
14.00 - 14.45	Presentation of the Bristol Heart Institute, University of Bristol, UK (Professor Paolo Madeddu); presentation of the Istituto Dermopatico dell'Immacolata, Rome, IT (Professor Mauricia Capogrossi), **Professor Maurizio Pesce**, Milan, IT.
14.45 - 15.30	Presentation of the Department of Medicine, University of Heidelberg, DE; and 'Characterisation and standardisation of MSCs preparations', **Professor Anthony D. Ho**, Heidelberg, DE.

15.30 - 16.15	'Role of intravenously administered bone marrow cells in left ventricular remodeling after myocardial infarction', PhD Student **Kathrin Odoerfer**, Vienna, AU. 'Immunomodulatory effect of intravenously administered bone marrow cells', DMV Thesis Student **Verena Spielberger**, Vienna, AU.
16.15 - 16.30	Coffee
	Session Chair, **Professor Andrey Zaritskey.**
16.30 - 17.15	Presentation of the companies by company representatives: *Miltenyi Biotec*, **Dr. Sabrina Hörper** *Cardio 3*, **Dr. Jozef Bartunek** *Cellartis*, **Dr. Nan Ma** (on behalf of Dr. Peter Sartiby)
17.15 – 17.30	Roundtable Discussion – 'Regenerative Medicine from the Company Perspective' Miltenyi Biotec/**Dr. Dirk Balshuesemann**, Cardio3/**Dr. Jozef Bartunek**
17.30-17.45	Summation of the day, **Professor Yrjö T. Konttinen.**
18.15 -	Dinner
Sat 16 August	
	Session Chair, **Dr. Peter Donndorf.**
9.00 - 9.45	Presentation of the Department of Cardiac Surgery and Foundation of Regenerative Medicine; and 'Intramyocardial stem cell therapy for myocardial ischemia - clinical introduction', **Professor Gustav Steinhoff**, Rostock, DE
9.45 - 10.30	'MSC markers and their analysis using qRT-PCR', **Dr. Mari Ainola**, Helsinki, FI.
10.30 - 11.15	'Engineering stem cell homing and targeting', **Dr. Nan Ma**, Rostock, DE.
11.15 - 12.00	PhD student **Catalin-Gabriel Manole**, Bucharest, RO, and PhD student **Staffan Dånmark**, Bergen, NO, present themselves and their research. 'Analysing peripheral endothelial homing of circulating bone marrow stem cells regulated by eNOS and SDF-1 using intravital microscopical methods', PhD student **Peter Donndorf**, Rostock, DE.
12.00 - 13.00	Lunch
	Session Chair, **Professor Reinhold G. Erben.**

13.00 -13.45	Presentation of the "Victor Babeş" National Institute of Pathology group; and, 'Interstitial Cajal-like cells (ICLC) or Popescu's cells', **Professor Laurentiu Popescu**, Bucharest, RO.
13.45 - 14.30	Presentation of the Berlin-Brandenburg Center for Regenerative Therapies group; Pharmicell Europe; and 'Immune interactions of stem cells (MSC)', **Dr. Andreas Kurtz**, Berlin, DE.
14.30 - 15.15	'Replicative senescence of mesenchymal stem cells: a continuous and organised process', **Dr. Wolfgang Wagner**, Heidelberg, DE.
15.15 - 16.00	Discussion / Coffee
16.00 -	Dinner
Sun 17 August	
	Session Chairs, PhD Students **Staffan Dånmark** and **Catalin-Gabriel Manole**.
9.00 - 9.45	Presentation of the Institute of Bioengineeing group; and 'Experience on the development of GLP/GMP platform to use foetal and autologous cells for clinical applications', **Professor Dominiqe Pioletti**, Lausanne, CH.
9.45 - 10.30	Presentation of the Federal Centre for Heart, Blood and Endocrinology group; 'Preclinical data on our project on acute myocardial infarction', **Professor Andrey Zaritskey**, Saint Petersburg, RU.
10.30 - 11.15	General Discussion on Heart Regeneration, **Professor Gustav Steinhoff**, Rostock, DE.
11.15 - 12.15	Lunch
12.15 - 13.00	Presentation of the Institute of Pathophysiology group; and 'Marker tolerant animals as a new tool in regenerative medicine, with special emphasis on heart', **Professor Reinhold G. Erben**, Vienna, AU.
13.00	Workshop Close

The main content of the presentations and discussions is summarised below using the text written based on the joint expertise of the ESF RNP REMEDIC and some outside experts. This text was created before the meeting and after the meeting revised according to the comments of peer reviewers and is now in press in Journal of Cellular and Molecular Medicine. It will be soon freely and fully available on the internet. The core content of the meeting discussions are described below.

DISCUSSIONS ON THE SCIENCE AND CLINICAL ASPECTS OF HEART REGENERATION

Accumulating clinical and experimental evidence indicates that mesenchymal stem cells (MSCs) are promising cell types in the treatment of cardiac dysfunction. They may trigger production of reparative growth factors, replace damaged cells or create an environment favor endogenous cardiac repair. However, identifying mechanisms which regulate the role of MSCs in cardiac repair is still unclear. To achieve the maximal clinical benefits, ex vivo manipulation could further enhance MSCs therapeutic potential. Heart disease remains the No. 1 killer in developed countries and often leads to damage or loss of functional heart tissue. Cell therapy may provide an effective intervention for repair of cardiac injury due to the self-renewing and multipotent nature of specific cells. Mesenchymal stem cells (MSCs) contain a population of cells that are self-renewing, and are capable of differentiating into multiple mesodermal tissues, including bone, cartilage, fat and muscle, including heart muscle.

MSCs can be easily isolated by plastic adherence and rapidly expanded ex vivo. MSCs may repair injured myocardium by activating multiple mechanisms. After transplantation, they may trigger production of reparative growth factors as produce many growth factors, so that growth factors and cytokines are locally produced. They can suppress local inflammation but they can also replace damaged cells. Furthermore, they can contribute to creation of an environment, which favors endogenous cardiac repair. Thus, they have been identified as promising cells in the treatment of many cardiovascular diseases with heart tissue damage as the common denominator. Discussion focused mainly on MSC transplantation for cardiac repair. MSCs isolation, characterisation and standardization from different sources such as bone marrow, adipose tissue, umbilical cord blood and mobilised peripheral blood were discussed. Various mechanisms of cardiac repair to which MSCs contribute, with emphasis on ex vivo manipulation of the MSCs prior to transplantation, were presented. Finally, the clinical aspects of MSCs to cardiac repair were discussed.

Detailed information on the following topics will soon be available in the website of the Journal of Cellular and Molecular Medicine.

- MSC isolation, characterisation and standardisation
    - Isolation from different sources
    - Isolation under different culture conditions
    - Characterisation of MSC
    - Standardisation of MSC
- Mechanisms of cardiac repair
    - Differentiation of MSCs towards cardiomyocytes
    - Paracrine effect of MSCs
    - MSCs integration into the injured myocardium
- Ex vivo Manipulation of MSCs
    - Pre-Treatment of MSCs with growth factors
    - Genetic engineering
    - Hypoxia Preconditioning
    - Pharmacological interventions
    - Delivery approach and dosage
    - Preclinical application on cardiovascular disease

ASSESSMENT OF THE RESULTS AND IMPACT

Since 2002, numerous clinical trials have been performed to test the safety and efficacy of bone marrow-derived mononuclear cells (MNC) transplantation for the treatment of myocardium infarction. Although most of these studies were not double-blinded or randomly designed, the initial results confirmed the safety of such a cell therapy and thereby aroused the enthusiasms to attempt mesenchymal stem cells (MSC)

transplantation. However, compared with MNC and other bone marrow-derived progenitor cells, which were isolated or sorted directly from fresh whole bone marrow, MSC usually need some time in a cell culture to allow their cell purification and expansion so that adequate numbers can be achieved prior to transplantation. The microbial contamination during cell culture could cause a failure of the cell preparation and the xenogeneic serum additions in the culture media could cause unexpected alteration of human MSC and subsequent host immune rejection. Therefore, there is a need for an animal serum-free replacement in the clinical scale propagation of human MSC. Secondly, the mean diameter of cultured MSC in the injection suspension was 20μm, which was much larger than MNC (mean diameter 10–12μm), and hence a direct intracoranary infusion of MSC might result in iatrogenic coronary embolisms and microinfarctions. Considering the above facts, unlike its MNC counterparts, the clinical application of MSC for cardiac repair is still in its initial stages and only a few small phase I-II clinical studies have been reported.

In 2004, a cardiologists group from China firstly infused autologous MSCs intracoronarily for patients with acute myocardial infarction. In Chen's study, MSCs were cultured for 10 days and eventually 5-6 x 10^9 MSCs were obtained and infused through the targeted coronary artery after a successful percutaneous coronary intervention. Serial cardiac echocardiographic and positron emission tomograpic monitoring demonstrated improvement of cardiac function 3 months after cell transplantation. However, no further information was presented to clarify the culture process and eventual myocardial injuries after intracoronary cell infusion.

In another study from Greece, MSCs were in vitro cultured for 7 days with 10% fetal calf serum under Good Laboratory Practice conditions, and then 1-2 x 10^6 cells were infused through a stented coronary artery. Before cell transplantation, the cell samples were analyzed and 66±19% of them cell in the culture were positive for the biomarkers of MSC. Furthermore, the authors selected into their series 5 patients who had implantable cardioverter defibrillator (ICD) to investigate the possible proarrhythmic potential of MSC treatment. At 16–36 months follow-up, interrogation of the ICD failed to detect sustained or non-sustained ventricular arrhythmia in any of the MSC treated patient. Therefore, the authors concluded that MSC transplantation did not appear to have any arrythmogenic potential in this small series.

The preliminary results of first clinical trail of MSC transplantation for cardiac repair in United States were reported recently at the American College of Cardiology's Innovation in Intervention: i2 Summit [Osiris therapeutics announces positive results in groundbreaking stem cell trial to treat heart disease. Available at http://investor.osiris.com/events.cfm. Accessed at June 17, 2008]. In the 53- patient, double-blind, placebo-controlled study, the allogeneic MSCs were taken from young healthy volunteers and intravenously injected like i.v. drugs. During the 6 months follow-up, the heart and lung function improved significantly and arrhythmic events were four times less frequent than in the placebo group. The investigators reported that such allogeneic cell products did not need time-consuming cell manipulations just prior to the treatment, but they are readily available to satisfy the needs of clinical community.

In the above mentioned American study, MSCs were administrated through a standard intravenous route. Although this route is much more convenient for the patients than the intra-arterial coronary route, pilot animal studies have demonstrated that intravenous route is much less site-specific and lead to much more extensive systemic engraftment than other more targeted delivery approaches [63]. It is generally accepted that even after intracoronary infusion only 1–3% of delivered cells actually engraft within the infarcted zone. Since the end of 2007, National Heart, Lung and Blood Institute (NHLBI) initiated another multi-center study to inject high or low dosage MSC preparations directly into the damaged myocardium that did not receive a bypass graft during open-chest coronary bypass procedure [Safety and effectiveness of human mesenchymal stem cell injections for repairing heart damage in people who have had a heart attack and are undergoing surgery for coronary artery bypass grafting (PROMETHEUS) Available at http://clinicaltrials.gov/ct2/show/NCT00587990 Access at June 17, 2008]. Although the cardiac surgeons can identify the myocardial scar and thus choose appropriate injection sites, the locally-injected cells still tend to

migrate to extracardiac organs, especially to the spleen. In the future clinical studies, the comparison of various delivery routes and methods to improve the local cardiac retention of the cells should be addressed.

Recently, a series of randomly designed clinical studies confirmed the safety and feasibility of MNC transplantation; however, they also showed that such un-cultured or non- sorted cell preparations did not have any effects on or only slightly improved heart function. MSCs currently emerge as a promising cell resource in clinical application to repair the damaged heart. According to the registered data from www.clinicaltrials.gov, a web-based service of National Institute of Health of United States, there are three ongoing phase I-II trials using MSC for cardiac applications and totally around 140 patients with myocardial infarction have been recruited across Europe and America to these studies. Compared with freshly isolated MNCs, MSCs are easy to label for the subsequent in vivo tracking using magnetic resonance imaging and other imaging techniques. Furthermore, the time period used for cell culture offers a platform for MSC-targeted gene therapy. MSCs can be engineered with various target genes to augment angiogenesis, inhibit apoptosis and reverse myocardial remodeling.

COMPANY CONTRIBUTION

There was input from three different EU companies to the workshop, namely Cardio3, Cellartis, and Miltenyi Biotec. They had sent representatives to the meeting to present the company, participate in a roundtable discussion about the companies' point of view on regenerative medicine and to participate in the general discussions. A short presentation of the participating companies is given below:

Cardio3: The company focuses on the treatment of human myocardial diseases based on bone marrow derived stem cells, their growth and differentiation to cardiomyocytes. Cardio3's first product is a therapy for congestive heart failure (CHF) which involves the isolation of cells from a patient, subsequent in vitro modification, proliferation and reinjection of stem cells into the patient's heart muscle.

Further development projects are concerned with the application of their products, e.g. the identification of optimal injection sites and aspects of their product in general, e.g. dose ranging studies. More information is available at www.cardio3.net.

Cellartis: Cellartis AB is a Swedish/British biotechnology company focused on human embryonic stem (hES) cells for drug discovery, toxicity testing and regenerative medicine with the main objective to develop hepatocytes and cardiomyocytes from these cells. More information at www.cellartis.com.

Miltenyi Biotec: This company was founded in 1989, has grown to over 1100 employees in 18 countries. The company develops, manufactures, and sells more than 1000 products and services; particularly in the fields of cell biology, immunology, regenerative medicine, and molecular biology. Miltenyi Biotec is committed to the advancement of scientific understanding and medicine by providing products and services for biomedical research and cellular therapy. Miltenyi Biotec developed MACS® Technology, the gold standard method in magnetic cell separation. More information at www.miltenyibiotec.com.

For further information on REMEDIC go to www.esf.org/remedic.