overexpressing cardiomyocytes. The Myc levels than induce cardiomyocyte competition are within the homeostatic range that supports normal heart anatomy and physiology, which provides a window for intervention in cell replacement strategies.

Gene therapy for inherited pediatric cardiomyopathy

Lucie Carrier, *Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf and DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck*

The annual incidence of newly diagnosed cardiomyopathies determined over a 4-year period in New England and Central Southwest is 1.13 per 100,000 children younger than 18 years and 8.34 per 100,000 infants younger than 1 year. These cases were mainly dilated (DCM) and hypertrophic cardiomyopathy (HCM; *Lipshultz et al. NEJM 2003*). **Pediatric cardiomyopathy** is genetically determined and two-thirds of cases are caused by sarcomere gene mutations, predominantly in *MYH7* and *MYBPC3*, encoding b-myosin-heavy chain (b-MHC) and cardiac myosin-binding protein C (cMyBP-C), respectively (*Morita et al., NEJM 2008*). In addition, patients with more than 1 mutation develop a more severe phenotype. Specifically, biallelic truncating *MYBPC3* mutations (26 cases reported so far) cause neonatal cardiomyopathy, heart failure and sudden death within the first year of life (reviewed in *Tardiff et al., Cardiovasc Res 2015*). Over the last years, our group evaluated several **gene therapy** approaches to target the cause of the disease *in vivo*, in cardiomyocytes and in engineered heart tissue derived from homozygous *Mybpc3*-targeted knock-in (KI) mice that carry the c.772G>A transition, resulting in 3 different aberrant mRNAs and proteins. These homozygous KI mice genetically mimic the situation of neonatal forms of cardiomyopathy in humans. **Spliceosome-mediated 5'-*trans*-splicing** was induced between the endogenous mutant *Mybpc3* pre-mRNA and an engineered pre-*trans*-splicing molecule (PTM) carrying a WT-*Mybpc3* cDNA sequence. PTMs were packaged in adeno-associated virus (AAV) for specific transduction of cultured cardiomyocytes and the heart *in vivo*. Full-length repaired *Mybpc3* mRNA represented 33% and 0.15% of total *Mybpc3* transcripts in cardiac myocytes and in the heart, respectively. Repaired cMyBP-C protein was detected by immunoprecipitation in cells and *in vivo* (*Mearini et al., Mol Ther – Nucl Acids 2013*). **Exon skipping** enhancing expression of alternative spliced mRNA was induced by AONs that mask exonic splicing enhancer motifs in exons 5 and 6. AONs were inserted into modified U7snRNA and packaged in AAV. Transduction of cardiac myocytes or systemic administration in newborn KI mice markedly increased Var-4 mRNA and protein, reduced aberrant mRNAs, and rescued the cardiac phenotype in mice (*Gedicke-Hornung et al., EMBO Mol Med 2013*). More recently, we showed successful long-term ***Mybpc3* gene replacement** with correction of both haploinsufficiency and production of poison peptides in engineered heart tissue and in the heart of homozygous KI mice. We provide evidence that gene therapy prevents cardiac hypertrophy and dysfunction over a 34-week period (*Mearini et al., Nat Commun 2014*). In the absence of alternative treatment options, except heart transplantation, *MYBPC3* gene therapy is a realistic treatment option for this subset of infants with severe pediatric cardiomyopathy. Next steps towards patients will be presented, including gene corrections with *trans*-splicing or CRISPR/Cas9 in cardiomyocytes derived from iPSC from patients with cardiomyopathies.

Transplantation of Stem Cells in the Myocardium

Philippe Menasché, Département de Chirurgie cardio-vasculaire, Université Paris Descartes & INSERM U-970, Hôpital Européen Georges Pompidou 20, rue Leblanc 75015 Paris, France

Stem cell-based therapy is currently tested in several trials of chronic heart failure. So far, however, the clinical outcomes have not matched the high expectations raised by the experimental studies, and the main question, now, is to understand the reasons of these suboptimal results and to determine whether and how this therapy could be used more broadly. For addressing this question, three main conditions need to be met. The first is to better decipher the mechanism of action of the grafted cells: structural integration within the recipient myocardium to generate a new tissue or, more likely, release of biomolecules that foster endogenous repair processes (stimulation of angiogenesis, reduction of fibrosis, recruitment of endogenous stem cells). Of note, regardless of the mechanism, there is increasing evidence that the best outcomes seem to be achieved by cells phenotypically close to the target tissue. This rationalizes the use of cardiac-committed cells among which the pluripotent embryonic stem cells are particularly attractive since their initial undifferentiated state can be leveraged to derive a cardiac progeny, which has led us to test them in the ongoing ESCORT trial. The second condition is to optimize the retention of cells so that they have time enough to release the factors underlying their protective effects. This can be achieved by dedicated delivery catheters and/or combination of cells with polymers, yielding injectable mixtures which gel *in situ* or epicardially-delivered patches, thereby highlighting the importance of tissue engineering to complement the action of cells; focus on early retention, as opposed to sustained survival, should also allow for a more liberal use of allogeneic cells which feature multiple advantages over patient-specific products while their expected rejection may no longer be a limiting issue since this rejection only requires to be delayed, not fully avoided, thereby allowing a shortened, and consequently better tolerated, immunosuppression regimen (1 month in our clinical protocol). The third condition is to develop fully automated industrial-scale cost-effective manufacturing technologies and quality control processes, hence the interest of cells derived from extensively qualified allogeneic cell banks. One step further, the long term objective of cell therapy could be to use the cells exclusively for producing the factors accounting for their cardioprotective effects and then to only administer them to the patient. The production process would then be closer to that of a biological pharmaceutic, thereby facilitating an expended clinical use, a consideration which has to be taken into account in the changing landscape of heart failure where new drugs and devices may take an increasing place.

Helene Puccio
Abstract missing

Sudden death prevention in patients with muscle diseases: a great challenge
Karim Wahbi

Cardiac and respiratory involvements are associated with a great morbidity and mortality in patients with neuromuscular diseases. Sudden death represents one of the most frequent causes of death in this population, particularly in diseases associated with primary involvement of the cardiac electrical system leading to conduction defects or ventricular tachyarrhythmias, such as myotonic dystrophy type 1, laminopathies or mitochondrial diseases. Preventive strategies for sudden death, mainly based on prophylactic implantations of permanent pacemakers or cardiac defibrillators, should be applied in these patients to improve their long-term prognosis. Since the natural history of cardiac involvement and mechanisms underlying sudden death differ from a disease to another, these strategies have to be specific for each muscle disease. Regarding the low prevalence of muscle diseases, the identification of risk factors for sudden death is complex and, moreover, the benefit associated with preventive measures is particularly difficult to demonstrate and can hardly be based on randomized trials. We will discuss several disease-specific approaches for the prevention of sudden death.

Cardiac involvement in dystrophinopathies: best practice and current dilemmas in care.
John p. Bourke, MD- Consultant Cardiologist, Freeman Hospital, Newcastle upon Tyne, UK

Life expectancy has improved progressively for patients with Duchenne muscular dystrophy- mainly due to better integration of multi-disciplinary care teams. However, poor care during the early years after diagnosis cannot be compensated for by improved care later. This presentation will review why the *International Standards of DMD Care* require a cardiologist be fully integrated into every neuromuscular genetics team.
Progressive left ventricular dysfunction from about age 13 years is almost universal in patients with DMD. This cardiomyopathy- culminating ultimately in sudden arrhythmic death or heart failure, has become the latest barrier to prolonging high quality survival in DMD-adults. Timely information about cardiac involvement allows parents to decide whether to have their child start cardio-active medicines ahead of or with the onset of demonstrable left ventricular dysfunction. Drugs of established benefit include ACE-inhibitors (eg: perindopril, ramipril), angiotensin-receptor blockers (eg: losartan, irbesartan), beta-adrenergic blockers (ie: bisoprolol, carvedilol) and aldosterone antagonists (ie: eplerenone). Ivabridine can be used for sinus tachycardia when beta-blockers are poorly tolerated. The evidence that these readily available, well tolerated, inexpensive drugs slow the course of cardiomyopathy will be summarised.
The risk of arrhythmias increases as left ventricular function declines and fibrosis increases. In other forms of cardiomyopathy, implantable cardioverter defibrillators [ICD] are deployed routinely to prevent sudden death. To date these have rarely been offered to adults with advanced DMD because the effect on prognosis and quality of life is unknown. This presentation will make the case for ICD-therapy in DMD.
Because of its multi-system implications, DMD-patients are rarely considered for cardiac transplantation. However, a range of intra- or extra-vascular left ventricular assist devices (eg: *Heart Mate II* or similar or *Sunshine Heart*) are now used selectively in other contexts. These may also be acceptable to some DMD-patients with heart failure symptoms despite optimal therapies and prolong survival. Precise genetic characterisation of DMD-patients may allow 'disease modification' through exon-skipping, gene manipulation or 'read-through' therapies. Research to date is focused on the effects of these therapies on skeletal muscle. However, because of differences in tissue uptake, evaluation of whether they improve or aggravated cardiac involvement will be needed. The cardiologists role in the muscle team is to provide cardiac surveillance and deploy cardio-active therapies in a timely way to minimise the symptomatic and prognostic impact of DMD-cardiomyopathy. Increasingly in selected cases, this will involve adding ICD and left ventricular assist device therapies.

Symposium- Parallel Symposium SMA/ALS/STEM CELLS

• *Georg Haase (FRANCE)* • *Alexandre Henriques (FRANCE)* • *Cecile Martinat (FRANCE)*

ALS and SMA Disease Modeling and Therapy Development using pure FACS-isolated Motor Neurons.
Georg Haase
Cf Abstract P02-23 page 35

Glucosylceramide and glycosphingolipids are part of the response to motor unit stress in ALS.
1. *Schmitt F, Hussain G, Dupuis L, Loeffler JP, Henriques A.*
A plural role for lipids in motor neuron diseases: energy, signaling and structure. *Front. Cell. Neurosc* 2014 Feb 20;8:25.
2. Henriques A, Croixmarie V, Priestman DA, Rosenbohm A, Dirrig-Grosch S, D'Ambra E, Huebecker M, Hussain G, Boursier-Neyret C, Echaniz-Laguna A, Ludolph AC, Platt FM, Walther B, Spedding M, Loeffler JP, Gonzalez De Aguilar JL. Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase. *Hum. Mol. Genet.* (2015) doi: 10.1093/hmg/ddv439

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset disease characterized by upper and lower motor neuron degeneration, muscle wasting and paralysis. Growing evidence suggests a link between changes in lipid metabolism and ALS [1, 2]. We have recently studied the lipidome in SOD1 mice, an animal model of ALS [3]. We found that sphingolipid species, and particularly glucosylceramide, were drastically rearranged in SOD1 mice, before overt neuropathology. GM3,