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CONFERENCE REPORT

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Second International Pulmonary Hypertension/Heart Failure Symposium—Structural heart disease, right ventricular dysfunction, and stem cell therapy: The European Pediatric Pulmonary Vascular Disease Network

Philippe Chouvarine[1] | **Klea Hysko**[1] | **Stephen Y. Chan**[2] | **Paulo Oliveira**[3] | **Bradley A. Maron**[4] | **Stella Kourembanas**[5] | **Georg Hansmann**[1]

[1]Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Hannover, Germany

[2]Department of Medicine, Division of Cardiology, Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, University of Pittsburgh School of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

[3]CNC—Center for Neuroscience and Cell Biology, CIBB—Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

[4]Brigham and Women's Hospital, Harvard Medical School and the Department of Cardiology, Division of Cardiovascular Medicine, VA Boston Healthcare System, Boston, Massachusetts, USA

[5]Division of Newborn Medicine, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA

Correspondence: Georg Hansmann and Philippe Chouvarine, Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.
Email: georg.hansmann@gmail.com and chouvarine.philippe@mh-hannover.de

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INTRODUCTION

The second International Pulmonary Hypertension/Heart Failure Symposium gathered 80 basic and clinical researchers from various pulmonary arterial hypertension (PAH)-related fields of research. There were 26 speakers and 7 poster presenters. The session topics included three basic science topics and three clinical science topics. The basics science topics were: (1) angiogenesis and vascular remodeling, (2) stem cell therapy for pulmonary vascular disease and heart failure, and (3) cardiovascular metabolism and inflammation. The clinical science topics were: (1) modern imaging of pulmonary vascular disease and cardiac dysfunction, (2) diagnostics and therapeutic interventions on the right ventricular (RV)—pulmonary arterial (PA) unit and single ventricles, and (3) clinical trials and new trial designs: pulmonary hypertension, acute respiratory distress syndrome (ARDS), heart failure with combined pre- and post-capillary pulmonary hypertension (HF/CpcPH), structural heart disease. Below we provide brief background information on the three basic science subsections of the conference.

Philippe Chouvarine and Klea Hysko contributed equally to this work.

PAH is a progressive disease characterized by a plexigenic and obstructive vasculopathy involving distal pulmonary arterioles that elevates pulmonary vascular resistance leading to high pulmonary arterial pressure and subsequent right heart failure. Although vasoconstriction is a central feature in the pathophysiology of PAH in some patients, increased cellular proliferation, apoptosis resistance, and fibrosis are central endophenotypes that underpin pathogenic vascular remodeling in PAH and drive pulmonary artery pressure. Plexiform lesions, characteristic of pulmonary vascular remodeling, are high in expression of angiogenic and growth factor genes, suggesting perturbed angiogenesis.

Other causes of pulmonary hypertension that are important but distinct clinically (and pathologically) from PAH include lung or structural heart diseases that are associated with hypoxic vasoconstriction, effacement of the alveolar-capillary interface, and left atrial hypertension, respectively, as well as thromboembolic conditions that result in a unique pathophenotype defined by in situ thrombotic remodeling of medium- and small-pulmonary arterioles (e.g., chronic thromboembolic pulmonary hypertension [CTEPH]). Investigations that aim to clarify the mechanisms leading to PAH in at-risk patients are another interesting research avenue with important implications for prevention and therapy.

Research efforts on application of mesenchymal stem cells (MSCs), and their secreted byproducts for treatment of PAH have gained more attention in the recent years. At this conference, Dr. Kourembanas presented several animal model studies demonstrating beneficial effects of MSC extracellular vesicles (MEx). Dr. Hansmann presented first-in-human case of successful treatment of PAH using MSC conditioned media (MSC-CM) and MSCs in a 3-year old female with severe heritable PAH (HPAH).

Metabolic dysregulation in PAH is another major area of research and a target of therapeutic intervention. In particular, PAH is characterized by a metabolic shift from oxidative phosphorylation to glycolysis even with adequate supply of oxygen (Warburg effect) both in the pulmonary vasculature and in the hypertrophied RV. Commonality of pathogenic processes in PAH vessels with tumorigenic processes, goes beyond the Warburg effect and includes proliferative, antiapoptotic processes in pulmonary artery smooth muscle cells (PASMCs). In the PAH RV, due to RV hypertrophy driven by pressure overload and compounded by compromised coronary perfusion, ischemia and capillary rarefication impairs myocardial oxygen supply. These changes lead to reliance on glycolysis at the expense of fatty acid oxidation, that is, "glycolytic shift." Several research projects on the metabolic abnormalities were presented at the conference.

The full conference report with summaries of all oral presentations is available as an Supporting Information. Post-hoc evaluation of the conference by the participants is provided in Table 1.

SUMMARY OF SELECTED PRESENTATION HIGHLIGHTS

The conference updated the participants on the exciting new research in cardiovascular remodeling, advances in imaging and diagnostics, promising stem cell derived therapies, and novel therapies focusing on modulation of metabolic pathways. Below we provide brief overviews of five highlight presentations.

Dr. Bradley Maron presented research showing that Neural Precursor Cell Expressed, Developmentally Downregulated 9 (NEDD9) is a potential therapeutic target for patients with CTEPH and other lung pathophenotypes characterized by hypoxia and thrombosis. Luminal pulmonary embolism or microthrombus are antecedent to CTEPH and initiate a signaling cascade that involves upregulation of hypoxia inducible factor (HIF)−1α in human pulmonary artery endothelial cells (HPAECs). In turn, HIF-1α-dependent transcriptional regulation of NEDD9 induces overexpression of a peptide within the NEDD9 substrate domain (N9p) on the HPAEC extracellular plasma membrane surface. In CTEPH, the plasma concentration of activated platelets expressing P-Selectin is increased, and P-Selectin forms a protein-protein interaction with N9p to promote platelet-pulmonary endothelial adhesion and thrombotic remodeling. The N9p peptide was sequenced by liquid chromatography-mass spectrometry, synthesized, and administered to New Zealand white rabbits. The resulting anti-N9p antibody (Ab) was isolated for further analysis (Figure 1a). Treatment with anti-N9p antibody (Ab) inhibits platelet adhesion to HPAECs in vitro and CTEPH-HPAECs ex vivo, and prevented the acute formation of platelet-PAEC aggregates as well as chronic thromboembolic remodeling of pulmonary arterioles in vivo (Figure 1b).

Dr. Stella Kourembanas presented data showing that mesenchymal stromal/stem cell extracellular vesicles (MEx) provide protection from inflammation-induced injury in multiple organs. Analysis of spatial biodistribution of MEx over the 24 h period in postnatal mice showed that MEx primarily concentrate in liver and lung. Single cell MEx-target cell interaction showed that MEx interact with nonclassical (CD45+, CD11b+ F4/80+, CD64+) myeloid cells, that is, anti-inflammatory cells

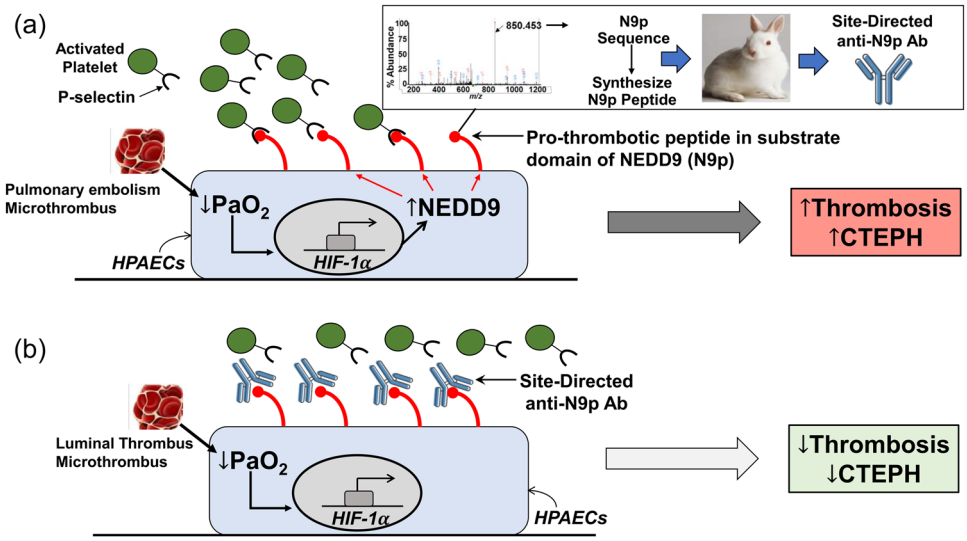
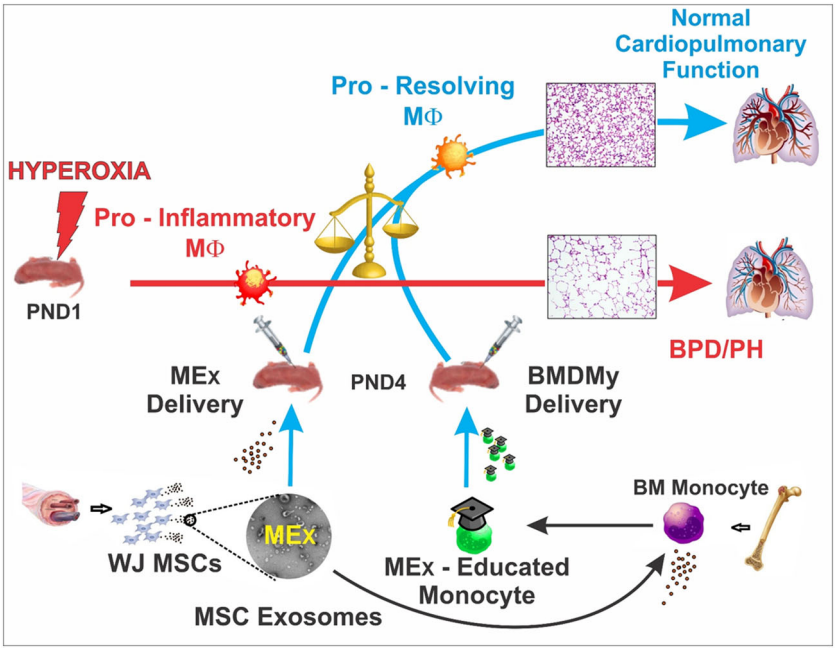


FIGURE 1 Targeting NEDD9 to inhibit chronic thromboembolic pulmonary hypertension (CTEPH). (a) In CTEPH, the plasma concentration of activated platelets expressing P-Selectin is increased, and P-Selectin forms a protein-protein interaction with N9p to promote platelet-pulmonary endothelial adhesion and thrombotic remodeling. (b) Treatment with anti-N9p antibody (Ab) inhibits platelet adhesion to HPAECs in vitro and CTEPH-HPAECs ex vivo, and prevented the acute formation of platelet-PAEC aggregates as well as chronic thromboembolic remodeling of pulmonary arterioles in vivo.

FIGURE 2 MSC EVs (MEx) promote cardiopulmonary health and protect from injury through reprogramming of myeloid cells. Exposure of neonatal mice to hyperoxia results in lung inflammation, alveolar simplification, and associated pulmonary hypertension (PH), key findings of bronchopulmonary dysplasia. Treatment with either MEx or with bone marrow derived myeloid cells (BMDMy) pretreated with MEx (MEx-educated monocytes) prevents and reverses hyperoxia-induced cardiopulmonary injury by promoting an anti-inflammatory, pro-resolving macrophage phenotype.



contributing to vascular homeostasis. Proteomic analysis of bone marrow (BM)-derived monocytes showed that the MEx treatment reprograms monocytes to non-classical (Ly6Cneg) phenotype. RNA-seq and ATAC-seq analyses showed transcriptomic reprogramming (modulation of the Ccl2-Ccr2 axis) by MEx, which promotes the immunosuppressive monocytic phenotype. The in vivo administration of MEx-educated BM-derived monocytes to hyperoxia exposed newborn mice exerted protective effects on cardiopulmonary system and improved functional exercise capacity (which was not true for the non-MEx-treated monocytes) (Figure 2).

Dr. Georg Hansmann presented research from his group on the first-in-human umbilical cord mesenchymal stem cell (HUCMSC)-derived treatment of severe PAH (compassionate use therapy). The case was a 3-year

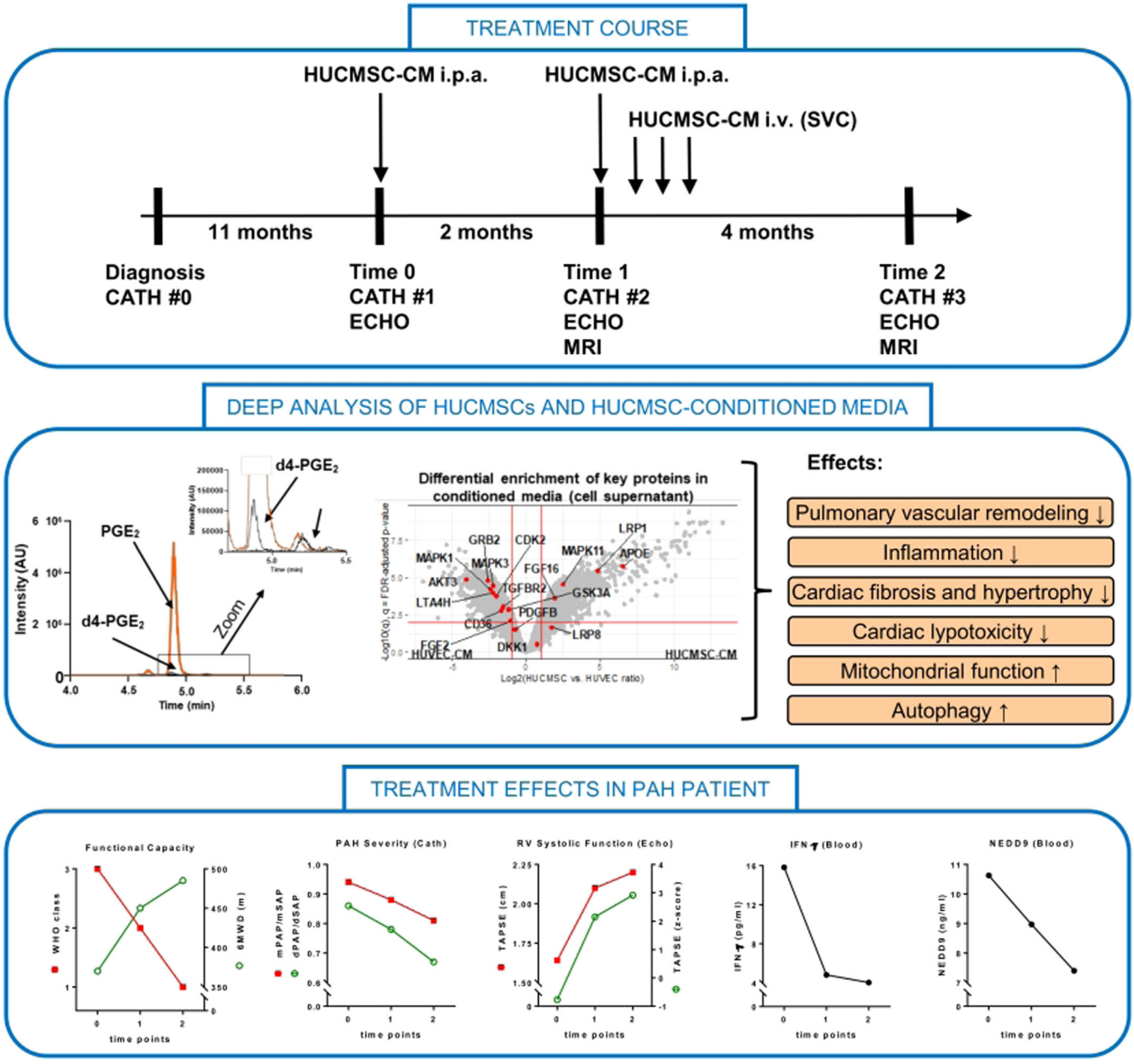


FIGURE 3 First-in-human umbilical cord mesenchymal stem cell-derived treatment of severe pulmonary arterial hypertension (PAH) has lasting beneficial effects. A 3-year old female with severe HPAH, HHT (Osler's D.) and ACVRL1 gene mutation was given 100 ml of HUVEC-CM in the right PA and 100 ml in the left PA at Time 0 (11 months after the diagnosis), the same treatment was repeated 2 month later (Time 1), followed by daily SVC infusions (200 ml of HUCMSC-CM over 60 min on three following days). Cath and Echo measurement were taken at Time 0, Time 1, and 4 months later (Time 2). Prostaglandin analysis of the HUCMSC-CM used for treatment showed high concentration of PGE2 and negligibly small expression of other PGs. The following multi-cord scRNA-seq analysis (3 HUCMSC vs. 2 HUVEC) and label-free quantitative discovery proteomic analysis (5 HUCMSC vs. 4 HUVEC) identified key differentially expressed molecules involved in the pathways that are likely conferring their beneficial effects for the observed clinical cardiovascular improvements. The treatment induced body growth and improved functional capacity (FC, 6MWD), PH risk scores, pulmonary hemodynamics, and RV systolic function. Blood plasma concentrations of NEDD9 (PAH severity marker), ICAM-1 (vascular injury marker), SAA and interferon (IFN)-γ (inflammation marker) were reduced after the treatment. SAA, serum amyloid A.

old female with severe heritable PAH, hereditary hemorrhagic telangiectasia (HHT) (Osler's D.) and ACVRL1 mutation. HUCMSCs (Wharton's Jelly) were isolated at the time the patient's younger sister was delivered by Cesarean section and sub-cultured. Subsequently the conditioned medium secreted by these HUCMSCs (HUCMSC-CM) was harvested. Using cardiac catheterization, the patient was given 100 ml of

TABLE 1 Post-hoc evaluation of the second International Pulmonary Hypertension/Heart Failure Symposium by the participants

Scientific content	Ranking	%
Overall rating for scientific content	Excellent	89.5
	Very good	10.5
	Good	
	Fair	
	Poor	
	No response	
Overall quality of plenary speakers' presentation content	Excellent	73.5
	Very good	26.5
	Good	
	Fair	
	Poor	
	No response	
Overall workshop quality and content	Excellent	100
	Very good	
	Good	
	Fair	
	Poor	
Overall panel quality and content	Excellent	79
	Very good	16
	Good	5
	Fair	
	Poor	
	No response	
Overall scientific quality of posters/abstracts	Excellent	68.5
	Very good	16
	Good	5
	Fair	
	Poor	
	No response	10.5

Note: Affiliations of participants were: Academia/research institute (73.5%), industry (10.5%),and unknown (16%). Primary job positions were: Student (16%), post-doc (10.5%), early-stage investigator (26.5%), established or senior investigator (21%), and unknown (26%).

HUCMSC-CM in the right pulmonary artery (PA) and 100 ml in the left PA at Time 0 (11 months after the diagnosis), and 2 months later (Time 1), followed by daily SVC infusions (200 ml of HUCMSC-CM over 60 min on 3 subsequent days). Cath and Echo measurements were taken at Time 0, Time 1, and 4 months later (Time 2). MRI was performed at Time 1 and 2 (Figure 3). The treatment induced body growth and improved functional capacity (FC, 6-min walk distance [6MWD]), PH risk scores, pulmonary hemodynamics, and RV systolic function. Single cell RNA sequencing (scRNA-seq) of the HUCMSCs showed four cell clusters one of which had heightened expression of regeneration and anti-inflammation markers. Prostaglandin analysis of the HUCMSC-CM used for treatment showed high concentration of PGE_2 and negligible expression of other prostaglandins. Blood plasma concentrations of NEDD9 (PAH severity marker), ICAM-1 (vascular injury marker), serum amyloid A and IFN-γ (inflammation marker) were reduced after the treatment. Subsequent multi-cord scRNA-seq analysis (3 HUCMSC samples vs. 2 human umbilical vein endothelial cell (HUVEC) samples) and label-free quantitative discovery proteomic analysis (5 HUCMSC vs. 4 HUVEC) identified key differentially expressed molecules involved in the pathways that are likely conferring their beneficial effects for the observed clinical cardiovascular improvements (Figure 3). The caregivers (parents) of the treated patient gave written informed consent for compassionate use of therapy, bioanalysis and publication of the data.

Dr. Chan presented research from his group on a long noncoding RNA (lncRNA) axis that regulates endothelial reprogramming in pulmonary hypertension. At a fundamental level, cellular adaptation in hypoxia in the pulmonary vasculature relies upon genomic, epigenetic, and metabolic programs, but the overarching molecular regulators are incompletely defined. Dr. Chan's group identified a number of lncRNAs that are dysregulated in the lungs of mice with PAH. One, in particular, was dysregulated in endothelium and mapped partially to a human homolog that neighbors a protein-coding gene controlling histone methylation, a crucial epigenetic mark for determination of chromatin structure and transcriptional activation. Through a combination of gain- and loss-of-function assays in cultured pulmonary arterial endothelial cells, a master transcription regulator of hypoxia, HIF-2α, was found to drive the expression of this lncRNA-protein coding gene pair, together promoting histone methylation and alterations of endothelial phenotypes consistent with PAH. Furthermore, a noncoding single nucleotide variant (SNV) embedded at this genomic locus was found to control HIF-2α binding and its activity. Importantly, through multi-center discovery and validation cohorts of PAH versus control patients, a novel and significant association was found between this SNV and the risk of developing PAH. Finally, via CRISPR/Cas9 genome editing technology, mice deficient in this lncRNA were protected from multiple PH subtypes, a result

phenocopied by the improvement of PAH in rats treated with an inhibitor of histone methylation. The implications of these findings were discussed, relevant to our fundamental understanding of hypoxic signaling in endothelial pathobiology and to therapeutic applications of these genetic and epigenetic insights.

Dr. Paulo Oliveira presented research from his group regarding the role of mitochondria in cardiovascular disease. He provided background on mitochondrial dynamics and function and the progression toward mitochondrial dysfunction and heart failure. In the context of cardio-oncology, he then talked about Doxorubicin-induced acute and cumulative cardiac toxicity leading to mitochondrial dysfunction and heart failure. Dr. Oliveira introduced two distinct rat model of Doxorubicin-induced sub-chronic or acute mitochondrionopathy, which are used by his group. Interestingly, mitochondrial alterations, that is, inhibition of ADP-stimulated respiration and loss of calcium loading capacity were observed in both models even in the absence of echocardiographic, histopathological or ultra-structural changes in one of the models. He then presented several studies from his group regarding mechanisms of delayed anthracycline cardiac toxicity caused by Doxorubicin such as increased p53/Bax expression and translocation to mitochondria or apoptosis-inducing factor release and consequent nuclear translocation in cardiomyoblasts. Dr. Oliveira showed results of his group regarding altered regulation of nuclear epigenetics in response to Doxorubicin treatment in the heart, which included changes in mitochondria-relevant transcripts, decreased mtDNA copy number, increased relative histone deacetylases activity, and decreased percentage of methylated DNA. Finally, he presented some of his more recently published data demonstrating how Doxorubicin treatment alters the expression of the circadian molecular clock, and regulation of metabolic and mitochondrial-related genes in the heart of a juvenile anthracycline toxicity mouse model.

OTHER PRESENTED RESEARCH TOPICS AND FUTURE DIRECTIONS

Other presentations were based on cutting-edge research dealing with the following open questions:

1. What are the effects of mechano-biology on chromatin folding and gene regulation in vascular cells?
2. Do vascular cell types undergo expansion/reduction during the remodeling process?
3. Can biomarkers in circulating endothelial cells be used for diagnostics and identification of therapeutic targets?
4. Can organotypic vasculature from induced pluripotent stem cells (iPSCs) be applied for studies of cardiopulmonary diseases?
5. Can single nucleus RNA-seq be successfully applied for studying significantly heterogeneous (in cell size) cardiac cell type subpopulations in cardiac pathologies?
6. Can altered regulation of nuclear epigenetics in response to chemotherapy (e.g., Doxorubicin treatment) in the heart be prevented?
7. Can novel lipid-, nutritional-based therapies be applied to reverse myocardial lipid accumulation and reduced fatty acid oxidation in cardiac diseases?
8. Can ERBB2-YAP and AGRIN regenerative signals be utilized to induce dedifferentiation-redifferentiation cycle for cardiomyocyte regeneration and protection against ischemic injury?
9. How does myocardial contractility affect organelle morphology and function in active cardiomyocytes?
10. How does the combination of advanced imaging modalities (cine arterial spin labeling-cardiac magnetic resonance imaging; synchrotron-based phase contrast microcomputed tomography) with already established methods (e.g., immunohistochemistry, laser microdissection) improve the understanding of PAH and cardiomyopathies?
11. When should we opt for transcatheter procedures (atrial septostomy/atrial flow regulators; reverse Potts shunt) as a mean to offload the right ventricle in severe PAH?
12. Does the RV of pediatric or adult patients with severe PAH recover after interventional treatment or lung transplantation?
13. How can we ameliorate detection and management of RV dysfunction?
14. Can the European Pediatric Pulmonary Vascular Disease Network pediatric PH risk score help in PH patient care and PH clinical trials?
15. How can we improve evaluation of safety and efficacy of pharmacotherapy in PAH clinical trials (e.g., Imatinib)?

COMPOSITION OF PARTICIPANTS

Affiliation of the participants was: Academia/research (73.5%), industry (10.5%), unknown (16%). Job titles were as follows: Student (16%), post-doc (10.5%), MD (16%), MD/PhD (10.5%), established or senior investigator (21%), and unknown (26%).

AUTHOR CONTRIBUTIONS

Philippe Chouvarine and Klea Hysko wrote the manuscript and supplement. Stephen Y. Chan, Paulo Oliveira, Bradley A. Maron, Stella Kourembanas, and Georg Hansmann provided materials for the selected presentation highlights section. Georg Hansmann organized the conference. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

S. Y. C. is a director, officer, and shareholder of Synhale Therapeutics. The remaining authors declare no conflict of interest.

ETHICS STATEMENT

Not applicable.

ORCID

Bradley A. Maron https://orcid.org/0000-0002-6784-764X
Klea Hysko https://orcid.org/0000-0003-4897-7905
Georg Hansmann https://orcid.org/0000-0003-0709-3935

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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