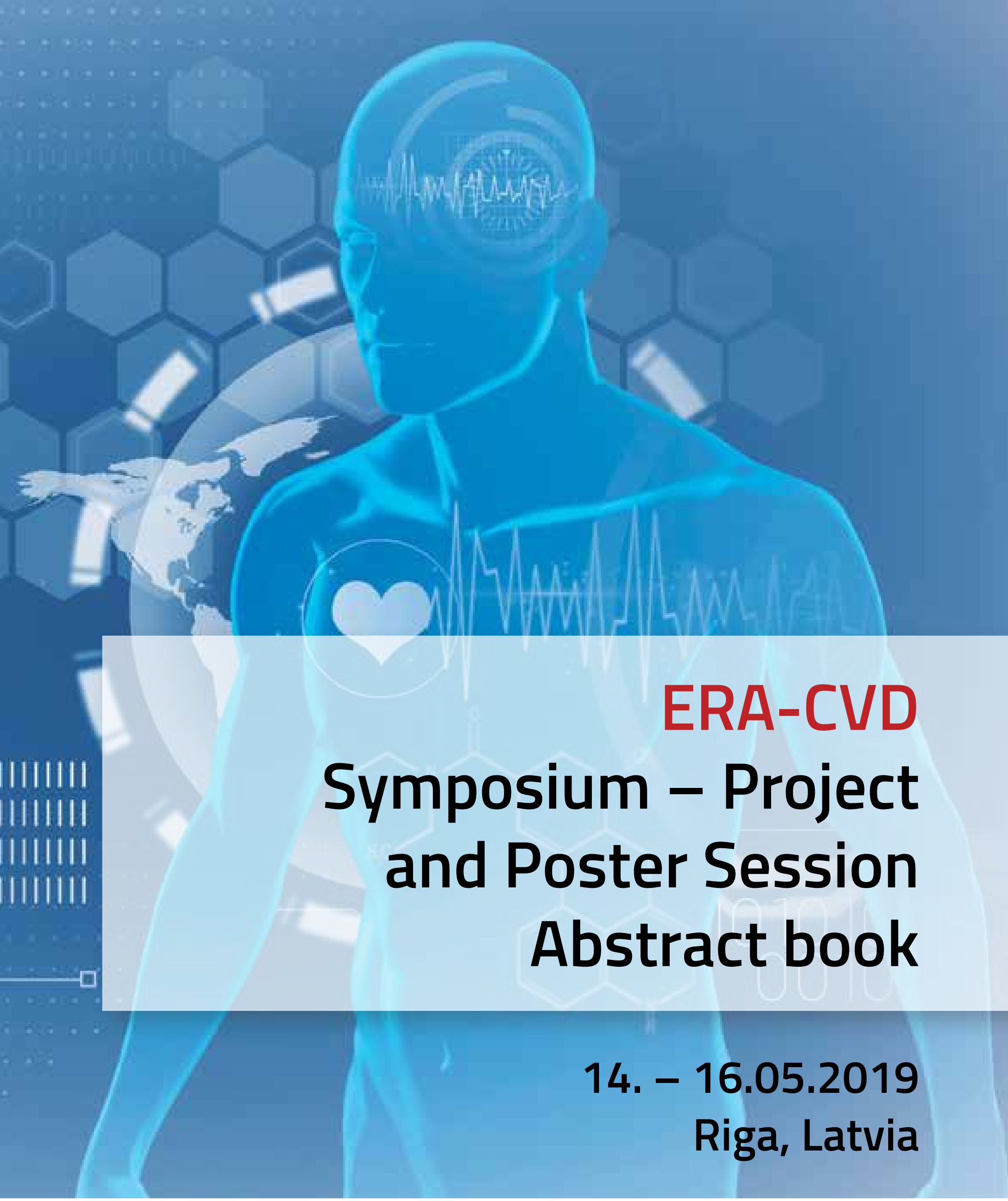


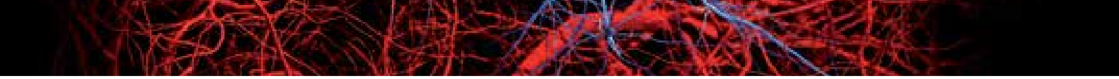
ERA-CVD

Symposium – Project and Poster Session Abstract book

14. – 16.05.2019

Riga, Latvia

ERA CVD



ERA-CVD

European Research Area Network on Cardiovascular Diseases

Symposium – Project and Poster Sessions
Abstract book

JTC 2016 Transnational Research Projects on Cardiovascular Diseases

&

JTC 2017 Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease

&

JTC 2018 Transnational Cardiovascular Research Projects driven by Early Career Scientists

ERA-Net on Cardiovascular Diseases (ERA-CVD)

Symposium

Riga, Latvia, May 14th to 16th, 2019
Agenda *(as of April 17th, 2019)*
Venue: Riga, Latvia, University of Latvia, Raina Bulv 19, Riga LV-1050

Tuesday, May 14th 2019, University of Latvia

Arrival

16:00	**Registration** (Entrance hall University) a**nd welcome coffee** · Aula minor side room **Poster mounting** in Aula minor
17:00	**Networking for Early Career Researchers (ECRs)** · Aula magna, Aula minor, Law school and Museum room
17:00 – 17:20	**Icebreaker** · Aula magna
17:20 – 17:35	**Welcome from local host VIAA** · Aula magna *Maija Bundule, State Education Development Agency (SEDA; VIAA in Latvian)* **Welcome ERA-CVD** *Wolfgang Ballensiefen (DLR-PT, Germany)*
17:35 – 18:00	**Challenges and opportunities in unravelling novel pathways in cardiovascular disease** · Aula magna *Orina Belton, University College Dublin (IE)*
18:00 – 19:30	**Open discussion in World Café format along the following topics:** *Table hosts are provided in brackets* • **Personalised Medicine Approaches for CVD Research** *Miso Sabovic (SI), Avi Israeli (IL)* • **Regenerative Strategies for CVD** *Karin Sipido (NL), Jeremy Pearson (UK)* • **Optimising Diagnosis and Prevention for CVD** *Elena Tremoli (IT), Heleen van Beusekom (NL)*

- **Development of devices and wearables**
  Pieter Doevendans (NL), Andrew Newby (UK)
- **Patient involvement**
  Daphne Bloemkolk (NL), Victoria Ridger (UK)

19:30 – 20:00	**Presentation of topics by table hosts, wrap up** *Moderation by Wiek van Gilst (NL)* · Aula magna
20:00	**Buffet-Dinner** · Aula minor and side room
21:30	**End of networking event**

Wednesday, May 15th, 2019 University of Latvia · Aula magna

08:30 – 08:45	**Welcome by *Liga Lejina*** *(State Secretary, Ministry of Education and Science, Republic of Latvia)* **Welcome by *Grzegorz Owsianik*** *(European Commission, DG Research & Innovation)* **ERA-CVD presentation and welcome** *Wolfgang Ballensiefen (DLR-PT, Germany)*
8:45 – 10:05	**Cardiomyopathy and Heart Failure (JTC 2016) – part 1** *Chairs: Gabriela Kuster-Pfister (CH), Jeremy Pearson (UK)*
08:45 – 09:05	**CLARIFY: Communication between cardiomyocytes and innate immune cells in failing hearts** *Eva van Rooij (NL), Thomas Braun (GER), Jean-Sébastien Hulot (FR)*
09:05 – 09:25	**CARDIO-PRO: Mechanisms of regenerative cardiomyocyte proliferation** *Gabriele D'Uva (IT), Gilbert Weidinger (GER)*
09:25 – 09:45	**MacroERA: Non-coding RNAs in cardiac macrophages and their role in heart failure** *Stefan Engelhardt (GER), Stephane Heymans (BL), Leon de Windt (NL)*
09:45 – 10:05	**PDE4Heart: Gene therapy with phosphodiesterases** *Rodolphe Fischmeister (FR), Viacheslav Nikolaev (GER), Finn Olav Levy (NOR)*

10:05 – 11:20 **Coffee break/Guided tour for posters with <u>even</u> numbers ·**
Aula minor, authors will be present at their posters
Guided tour by Wiek van Gilst (NL), Karin Sipido (NL)

11:20 – 12:20 **Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease (JTC 2017) – part 1**
Chairs: Andrew Newby (UK), Victoria Ridger (UK)

11:20 – 11:40 **druggable-MI-genes: Utilising myocardial infarction genes for better treatment**
<u>Heribert Schunkert</u> (GER), Andres Metspalu (EST)

11:40 – 12:00 **PROACT: Proteome analysis for the management of coronary artery disease**
<u>Jan Staessen</u> (BL), Harald Mischak (GER), Joost Schanstra (FR)

12:00 – 12:20 **PREMED-CAD: PREcision MEDicine in Coronary Artery Disease**
<u>Mahir Karakas</u> (GER), Tanja Zeller (GER), David-Alexandre Tregouet (FR), Massimiliano Caprio (It)

12:20 – 13:20 **Lunch break** · Basement cantine
Poster viewing · Aula minor, Coffee in side room

13:20 – 14:45 **'Pitch' presentation of JTC2018 projects**
Chairs: Orina Belton (IE), Andrew Newby (UK), Heleen van Beusekom (NL)

NEMO-IMMUNEagainstHF: Neural Modulation of immune system to shape cardiac remodeling to chronic arterial hypertension and counteract heart failure
<u>Sarah-Lena Puhl</u> (GER)

AIR-MI: Adaptive immune responses in myocardial infarction
<u>Gustavo Ramos</u> (GER)

MEMORY: Trained immunity in bone marrow progenitors as driver of atherosclerosis in obesity
<u>Niels Riksen</u> (NL), Andreas Schlitzer (GER), Hafid Ait-Oufella (FR)

ATHEROLNSIDE: Local immunomodulation of atherosclerosis by CD8+ T-cell based nanomedicines
Bram Slütter (NL), Remco Megens (GER), Koen Raemdonck (BL)

DENIM: Duchenne muscular dystrophy cardiomyopathy – the role of Endocannabinoids and immune regulation
Aoife Gowran (IT), Lise Lefèvre (FR)

MISsCVD: Neuroimmune guidance cues, MicroRNAs & Inflammatory responses: Sex differences in CardioVascular Diseases
Janine van Gils (NL)

DETECT ARRHYTHMIAS: Investigating determinants of atrial electrical conduction underlying arrhythmias
Sebastian Clauss (GER), Davide Rovina (IT), Fatih Kocabas (TR), Tõnu Esko (EST)

MULTIFIB: Multimodal fiber optic probe for *in vivo* highly resolved localisation of cardiac fibrosis
Iwan Wladimirowitsch Schie (GER), Richard Walton (FR), Marco Andreana (AU)

EMPATHY: Electromechanical presages of sudden cardiac death in the young: integrating imaging, modelling and genetics for patient stratification
Joost Lumens (NL), Lia Megens Crotti (IT), Christine Rootwelt-Norberg (NO)

PICASSO: Unravelling proprotein convertase subtilisin/kexin type 9 (PCSK9) mechanisms In calcific aortic valve disease: from aortic valve sclerosis to stenosis
Paolo Poggio (IT), Elvira Mass (GER), Benoit Arsenault (CA), Romain Capoulade (FR)

MEND-AGE: Dissecting mesenchymal-Endothelial cross-talk, heterogeneity and function to mend vascular ageing and atherosclerosis
Rafael Kramann (GER), Rui Benedito (ES), Agnieszka Jazwa-Kusior (PL)

HYPER DIP: Maternal hemodynamics in hypertensive disorders of pregnancy – a human and animal model under antihypertensive therapy
Julia Binder (AU), Nadine Haase (GER)

FIBER: Fibrosis treatment by enhancer targeting
Thomas Moore-Morris (FR), Paola Cattaneo (IT), Luis Luna Zurita (ES), Justus Stenzig (GER)

INNOVATION: Investigating long non-coding RNA regulated pathways driving cardiac regeneration
Christian Bär (GER), Reinier Boon (NL), Bernhard Johannes Haubner (AU), Nuno Guimarães-Camboa (IT), Bruno Bernardes de Jesus (PT)

14:45 – 16:00 **Coffee break/ Guided tour for posters with odd numbers ·** Aula minor, authors will be present at their posters
Guided tour by Wiek van Gilst (NL), Elena Tremoli (IT)

16:00 – 17:00 **Cardiomyopathy and Heart Failure (JTC 2016) – part 2**
Chairs: Anthony Gramolini (CA), Pieter Doevendans (NL)

16:00 – 16:20 **ACM-HF: Non myocyte interplay as a novel platform for mechanistic insights and therapeutic approaches in ACM heart failure**
Giulio Pompilio (IT), Angelo Parini (FR), Milena Bellin (NL), Gregor Zemljič (SL)

16:20 – 16:40 **DETECTIN-HF: Determining the role of clinical and epigenetic risk markers in dilated cardiomyopathies and heart failure**
Benjamin Meder (GER), Zofia Bilinska (PL), Andres Metspalu (EST)

16:40 – 17:00 **FAT4HEART: Heart Failure Rescue by Nutritional Approaches: relevance of mitochondrial substrate utilisation**
Borja Ibanez (ES)

17:00 – 17:15 **Coffee break – Poster viewing** · Aula minor and side room

17:15 – 17:55 **Cardiomyopathy and Heart Failure (JTC 2016) – part 3**
Chairs: Gad Keren (IL), Miso Sabovic (SI)

17:15 – 17:35 **GENPROVIC: Gene Profiling Test for Identification of Treatable Patients with Acute and Chronic Heart Failure**
Heinz-Peter Schultheiss (GER), Andrea Frustaci (IT)

17:35 – 17:55 **Cardio-Oncology: Early detection and prevention of cardiac dysfunction and heart failure induced by cancer chemotherapeutics**
Canan Nebigil (FR), Michael Wing Yan Chan (TW), Robert Passier (NL), Igor Tetko (GER), Hasan Koyuncu (TR), Laurent Désaubry (FR)

18:00 – 21:00 **Boat trip with dinner, Poster prize ceremony on board**

Thursday, May 16th,2019 University of Latvia · Aula magna

09:00 – 10:20 **Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease (JTC 2017) – part 2**
Chairs: Orina Belton (IE), Heleen van Beusekom (NL)

09:00 – 09:20 **MICROEXPLORATION: Exploring the effects of microcalcification on plaque vulnerability**
David Magne (FR), Claudia Goettsch (GER), Agnieszka Strzelecka-Kiliszek(PL)

09:20 – 09:40 **SCAN: Subclinical atherosclerosis characterisation: Nanoparticle-based molecular and cellular imaging**
Jacob Fog Bentzon (ES), Mangala Srinivas (NL), Susanne Feil (GER)

09:40 – 10:00 **OPERATION: Elucidate monocyte phenotype to predict arterial wall inflammation**
Eric Stroes (NL), Martin Satny (GER), Alberico Catapano (IT)

10:00 – 10.20 **ENDLESS: Atherosclerotic plaque erosion leading to thrombus formation. The role of extracellular vesicles in ENDotheliaL transdiffErentiation, apoptoSiS and leucocyte and platelet adhesion**
Gerard Pasterkamp (NL), Hester den Ruijter (NL)

10:20 – 10:50 **Coffee break** · Aula minor side room

10:50 – 11:35 **SRA presentation**

10:50 – 11:00 **The ERA CVD Strategic Research Agenda – the process**
Deborah Zyss, ANR (FR)

11:00 – 11:35 **The ERA CVD Strategic Research Agenda – 2019**
Wiek van Gilst (NL)

11:35 – 12:35 **Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease (JTC 2017) – part 3**
Chairs: Andrew Newby (UK), Victoria Ridger (UK)

11:35 – 11:55 **PLAQUEFIGHT: Fighting Atherosclerotic Plaques in Coronary Artery Disease Via Targeting Neuroimmune Interfaces**
Andreas Habenicht (GER)

11:55 – 12:15 **AtheroMacHete: Macrophage heterogeneity in human and murine atherosclerosis: a therapeutic opportunity?**
Erik Biessen (NL), Thomas Ulas (GER)

12:15 – 12:35 **XploreCAD: Advanced ex vivo analyses and multi-frequency ultrasound technology for improved evaluation and diagnosis of coronary plaque**
Felicia Antohe (RO)

12:35 – 13:30 **Lunch break** · Basement cantine, Coffee in Aula minor side room

13:30 – 14:30 **Interface between myocardium and non-myocyte cells in Heart Failure (JTC 2016)**
Chairs: Pieter Doevendans (NL), Gad Keren (IL)

13:30 – 13:50 **MINOTAUR: Metabolic Therapy for Managing Diastolic Heart Failure**
Simon Sedej (AU), *Wolfgang Linke (GER), Jorge Alegre-Cebollada (ES), Sylvère Durand (FR), Nádia Gonçalves (PT)*

13:50 – 14:10 **LIPCAR-HF: A prognostic marker of heart failure with impaired ejection fraction**
Florence Pinet (FR), Thomas Thum (GER)

14:10 – 14:30 **LYMIT-DIS: Targeted LYmphatic and MIcrovessel Treatments in metabolic-DISease HFpEF**
Ebba Brakenhielm (FR), Elizabeth Jones (BL), Anna Ratajska(PL), Arantxa Gonzalez Miqueo (ES)

14:30– 15:15 **Coffee break** · Aula minor side room

15:15 – 17:30 **New pathways ad therapies in Cardiomyopathy (JTC 2016) ·**
Chairs: Gabriela Kuster-Pfister (CH), Anthony Gramolini (CA)

15:15 – 16:05 **Variation: Novel RNA based therapies for treatment-resistant forms of severe cardiomyopathy caused by LMNA mutations**
Yigal Pinto (NL), Thomas Eschenhagen (GER), Lior Gepstein (IL), Gisele Bonne (FR)

16:05 – 16:25 **EXPERT: Exploring new pathways in age-related heart diseases**
Thomas Thum (GER), Leon de Windt (NL), Vicente Andrés (ES), Gianluigi Condorelli (IT), Anne-Cécile Huby (FR), Alexandrina Burlacu (RO)

16:25 – 17:15 **General discussion and wrap-up session**
16:25 Conclusions by Chairs of JTC2016
16:40 Conclusions by Chairs of JTC2017
16:55 Conclusions by Chairs of JTC2018
17:10 Conclusions & Wrap-up by EAB chair
17:25 Farewell: ERA-CVD COO
17.30 **END OF SYMPOSIUM**
18:30 Evening excursion in the Old City
19:30 Dinner

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Introduction

Cardiovascular diseases (CVD) are the largest cause of death in the European Union (EU), as they account for around 2 million deaths per year. Overall, CVD are estimated to cause the economy of the EU costs of almost 196 billion € a year. Furthermore, they are one of the leading causes of long-term sickness, chronic diseases and loss to the labor market. Therefore, CVD are a major health and socioeconomic problem in Europe.

The ERA-CVD consortium comprises members from European, associated and non-European countries. As the cardiovascular research field can only be tackled from a global perspective an effective coordination of research at a national and European level, increased cross-disciplinary interaction and research advancements are urgently needed to address the burden of cardiovascular diseases (CVDs). By gathering their forces and funding capacities, 23 partners from 18 countries hope to respond to this demand through the ERA-CVD.

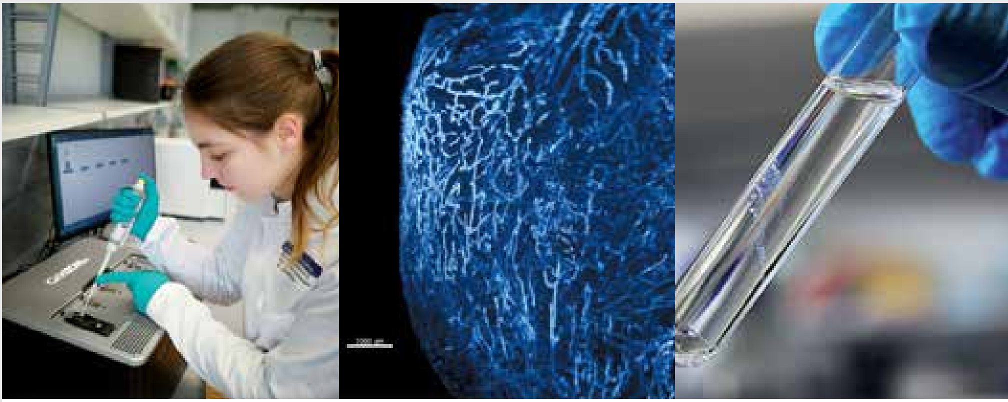
In 2016, under the umbrella of ERA-CVD, a joint transnational call (JTC-2016) was launched together with the European Commission in the field of 'Heart Failure' (HF). 22 funding organisations had agreed to fund the joint call for multinational research projects in this scientific area. The aim of the call was to facilitate multinational, collaborative research projects that address important questions relating to earlier recognition and prognosis of HF, innovative approaches to prevent and treat HF or to reverse the remodeling process, and to elucidate the role of the interface between myocardium and non-myocyte cells in pathophysiology of HF. 14 research consortia were selected for funding on the basis of ambitious scientific goals, research excellence, and clear demonstration of an added value from working together.

In 2017 ERA-CVD launched the joint transnational call (JTC-2017) on 'Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease (CAD)'. The aim of the call was to facilitate multinational, collaborative research projects that address early recognition of CAD by exploration of mechanisms leading to plaque instability, and improvement of imaging techniques as well as validation of biomarkers leading to earlier recognition of risk and/or protective factors. 10 research consortia were selected for funding on the basis of ambitious scientific goals, research excellence, and clear demonstration of an added value from working together.

In 2018 ERA-CVD launched the thematically open joint transnational call (JTC-2018) for Early Career Scientists. The aim of the call was to promote co-operation and interchange between Early Career Scientists and thus enable international collaboration and new consortia establishment in cardiovascular research. 14 research consortia were selected for funding on the basis of ambitious scientific goals, research excellence, and clear demonstration of an added value from working together.

At the ERA-CVD symposium in Riga, Latvia in May 2019 the principal investigators from the funded projects of the JTCs 2016, 2017 and 2018 discussed progresses and challenges of their research efforts. As a dedicated support measure, ERA-CVD invited (50) PhD students and postdocs to display a poster on their work within the projects. The ERA-CVD promotes young excellent scientists in early stages of their careers through a devoted set of activities like the EPCA, networking events and joint transnational calls. These activities are promoted to increase the attractiveness of cardiovascular research for early-career scientists as well as to enhance the possibilities for methodological training, networking and mobility in the context of the scientific research funded in the ERA-CVD joint transnational calls.

Besides the presentation of the strategic research agenda (SRA) for CVD the program offered the networking event with open discussions in world café format along the following topics: personalised medicine approaches for CVD research, regenerative strategies for CVD, optimising diagnosis and prevention for CVD, development of devices and wearables, and – not least – patient involvement.



Research consortia

JTC 2016 Transnational Research Projects on Cardiovascular Diseases – Heart Failure (HF)

CLARIFY:
Communication between cardiomyocytes and innate immune cells in failing hearts

Ischemic heart Failure (IHF) is a leading cause of hospitalisation and mortality in Europe. This unmet challenge asks for a better understanding of the mechanisms causing myocardial tissue damage and the development of new therapeutic approaches supporting cardiac repair. The immune system plays a decisive role for initial inflammatory responses following cardiac damage and subsequent regenerative responses. However, the interplay between immune cells and cardiomyocytes/cardiac progenitor cells within the myocardium is not well understood. We aim to explore the spatial and temporal communication between cardiac myocytes, cardiac stem cells and innate immune cells during IHF. We will use high-end transcriptional profiling involving single-cell RNA- sequencing / tomo-sequencing (genome-wide expression data with spatial resolution) combined with mouse genetics and large animal models to identify new factors involved in cellular interactions responsible for cardiac remodeling.

Furthermore, we want to understand the function of crucial mediators of cardiomyocyte-innate immune cell interactions, the Reg-family of cytokines, for post-ischemic damage control and tissue repair. Our research will supply new approaches for the manipulation of cell-cell interactions in the failing heart.

All model organisms capable of cardiac regeneration rely on immune cells to orchestrate different waves of inflammation, matrix deposition, angiogenesis and cardiomyocyte proliferation. Hence, therapeutic manipulation of local immune cell-dependent processes in the damaged heart might shift the balance between extensive scarring, minimal defect healing and regeneration.

Project Coordinator: Eva van Rooij, Hubrecht Institute, Utrecht, The Netherlands
Project Partners: Thomas Braun, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany | Jean-Sebastien Hulot, Sorbonne Universités, Paris, France
Contact: E.vanrooij@hubrecht.eu

CARDIO-PRO:
Mechanisms of regenerative cardiomyocyte proliferation

Myocardial injuries in mammals often progress to heart failure (HF) due to the loss of cardiac muscle cells (cardiomyocytes). Stimulating the very low intrinsic proliferation rate of cardiomyocytes is a promising strategy for cardiac repair in patients with heart failure. It is therefore imperative to better understand the signals that promote cardiomyocyte cell proliferation.

To identify such signals we will use comparative approaches between zebrafish, where cardiomyocyte regeneration occurs naturally, and mice, where it does not. We propose a combination of systems-, molecular- and cell-biology methodologies, both *in vitro* and *in vivo*, to explore if the difference in the regenerative capacity of lower vertebrate versus mammals relies on activation of distinct signals in response to injury, differential response of cardiomyocytes to identical signals or quantitative differences in activation of pro-regenerative signals.

To this end, we will identify factors that are only active in zebrafish, but not mice, by using novel unbiased approaches, in particular Tomo-Seq (genome-wide expression data with spatial resolution). We will also ask why cardiomyocytes differently respond to endogenous Bone Morphogenetic Protein (BMP) signaling, which promotes regeneration in zebrafish, but worsens outcome after cardiac injury in mice.

Finally we will explore the regulation and downstream mechanisms of Erbb2 signaling, which promotes cardiomyocyte proliferation both in zebrafish and mice, but is only weakly active in the latter. Our goal is to develop regenerative medicine strategies in the adult mammalian heart based on endogenous cardiomyocyte renewal.

Project Coordinator: Jeroen Bakkers, Hubrecht Institute, Utrecht, The Netherlands
Project Partners: Gilbert Weidinger, Ulm University, Germany | Eldad Tzahor, Weizmann Institute of Science, Rehovot, Israel | Gabriele Matteo D’Uva, IRCCS MultiMedica-MultiMedica SpA, Milan, Italy
Contact: j.bakkers@hubrecht.eu

MacroERA:
Non-coding RNAs in cardiac macrophages and their role in heart failure

Current heart failure therapy primarily addresses cardiac myocytes and vascular smooth muscle cells. However, these therapies have limited efficacy and do not effectively prevent myocardial remodeling and the functional decline observed during heart failure progression. The narrow focus on these two cell types neglects accumulating evidence that also other cardiac cell types – endothelial cells, fibroblasts and immune cells – impact on cardiac homeostasis, disease development and manifestation. Although macrophages are the largest fraction of immune cells in native mammalian myocardium, their roles in this organ are insufficiently resolved. This applies particularly to the resident type of macrophages, which is from a different (developmental) origin than monocyte-derived macrophages. The function of these resident cardiac macrophages is entirely unclear.

Non-coding (nc) RNA, such as microRNAs (miRNAs), are likely to exert critical activities in cardiac macrophages, given preliminary data from our consortium that identify miRNAs in these cells as modulators of their polarisation, but also of myocyte hypertrophy. Moreover, we find miRNAs in exosomes secreted by cardiac cells as an intercellular signal for macrophages. The laboratories involved in this joint project pursue the idea that cardiac disease progression is determined by ncRNAs that regulate macrophage function. To exploit the potential of macrophage ncRNAs for heart failure therapy, we aim to identify, characterize and manipulate macrophage miRNAs, both in myocardium and in blood, from human patients and mouse disease models.

Project Coordinator: Stefan Engelhardt, Technische Universität, Munich, Germany
Project Partners: Leon de Windt, University of Maastricht, The Netherlands | Stephane Heymans, Leuven University, Belgium
Contact: stefan.engelhardt@tum.de

PDE4HEART:
Gene therapy with phosphodiesterases to treat heart failure

Regardless of the underlying cause for heart failure (HF), patients show a neurohormonal overactivation in response to a decline in systolic function. This creates a pathophysiological "vicious circle" where chronic activation of β-adrenergic signalling plays a central role as attested by the beneficial effect of β-blockers in HF. In contrast, nitric oxide (NO) and natriuretic peptides (NPs) exert favourable effects in HF. The β-adrenergic pathway results in an increase in cAMP levels to augment cardiac function, whereas NO and NPs signal through cGMP to exert opposite effects. Cyclic nucleotide (CN) levels are tightly regulated by their hydrolysis by phosphodiesterases (PDEs). The PDE superfamily comprises several distinct isoforms that regulate cAMP and cGMP in discrete microdomains of the cardiomyocyte and ensure the specificity of CN signalling in physiological conditions. However, this organisation is lost in HF, thus causing further deterioration of the heart. While chronic PDE inhibition failed to show significant beneficial effects in HF treatment, our preliminary results indicate that it is PDE overexpression that has more promising effects. In this project we intend to further validate this hypothesis and assess whether overexpression of specific PDEs in cardiomyocytes can prevent/reverse HF. To do so, the consortium will test whether gene therapy with cardiac targeted overexpression of three different PDE isoforms can restore CN compartmentation, prevent maladaptive cardiac remodelling and constitute a new therapeutic approach for HF.

Project Coordinator: Rodolphe Fischmeister, INSERM and University Paris-Sud, Paris, France
Project Partners: Viacheslav Nikolaev, University Medical Center Hamburg-Eppendorf, Germany | Finn Olav Levy, University of Oslo, Norway | Emilio Hirsch, University of Torino, Italy
Contact: rodolphe.fischmeister@inserm.fr

ACM-HF:
Non myocyte interplay as a novel platform for mechanistic insights and therapeutic approaches in Arrhythmogenic Cardiomyopathy (ACM) heart failure

With this project we expect to elucidate mechanisms underlying the insurgence of heart failure (HF) in Arrhythmogenic Cardiomyopathy (ACM), and to identify new disease targets and potential therapeutics. ACM is a genetic disease mainly affecting young people and athletes. The disease is characterised by progressive cardiomyocyte loss (CM), fibro/adipose replacement and inflammation leading to right ventricle or biventricular heart failure and malignant arrhythmias.

We recently demonstrated a central role of cardiac mesenchymal stromal cells (C-MSCs) in the pathogenesis of ACM indicative for a potential treatment target role. Human C-MSCs undergo adipogenic differentiation, and are a source of collagen deposition. Thus, these cells represent a promising tool for *in vitro* studies. The interplay of cardiomyocytes – non-myocytes underlies ACM-HF. To mimic this axis, to investigate the cellular interplay, and to develop a platform to test potential therapeutics we will exploit an innovative patient-derived model combining CMs from induced pluripotent stem cells (hiPSC-CMs), Stromal cells and Inflammatory mediators (CSI model). We will utilize C-MSCs derived from right ventricle biopsies, hiPSC-CMs from dermal fibroblasts and peripheral nucleated blood cells from ACM patients and healthy donors (controls) to address the specific aims: 1) determine the relative contribution of each cell type to ACM pathogenesis and the underpinning mechanisms; 2) screen the ability of FDA-approved epigenetic drugs and a library of secreted factors to revert the ACM phenotype in human C-MSCs; 3) validate the capability of selected molecules to treat ACM degeneration in the CSI model. Gender and genetic background are considered in the sourcing cells. Clinical partners ensure adequate patient recruitment. Engagement activities towards patient associations and advocacy groups will provide the pertinent evidence gaps, and explain why the project questions and outcomes are relevant to patients and caregivers.

Project Coordinator: Giulio Pompilio, Centro Cardiologico Monzino-IRCCS, Milan, Italy
Project Partners: Andreas Zeiher, Goethe University, Frankfurt am Main, Germany | Angelo Parini, Institut des Maladies Métaboliques et Cardiovasculaires, INSERM, Toulouse, France | Josep Brugada Terradellas, Fundació Clínic per a la Recerca Biomèdica/ Hospital Clínic, Barcelona, Spain | Christine Mummery, Leiden University Medical Centre, The Netherlands | Bojan Vrtovec, University Medical Centre Ljubljana, Slovenia
Contact: giulio.pompilio@ccfm.it

DETECTIN-HF:
Determining the role of clinical and epigenetic risk markers in dilated cardiomyopathies and heart failure

Dilated cardiomyopathies (DCM) represent a major cause for heart failure (HF), especially in the young. Although it is estimated that 30-50% of all DCM cases are caused by a genetic predisposition, the precise mechanisms that underlie the variations in disease susceptibility and phenotype presentation including risk for HF development or sudden cardiac death (SCD) are virtually unknown.

To improve treatment and prognosis of DCM as a major cause for heart failure, there is an urgent need to improve early disease detection, to establish common standards for phenotyping and to adequately predict risk in patients and their relatives. Efforts thus far however, have concentrated mostly on smaller studies, single centers or national efforts at best.

The DETECTIN-HF consortium aims to improve this situation by applying selected clinical and molecular markers in a multi-center, multi-national cohort. To enable true translation in the field the consortium aims at three major tasks: The harmonisation of existing national registries to foster trans-national research on DCM, and second, to investigate the clinical value of selected biomarker candidates, genotypes and gender effects in this large-scale cohort, and third, to develop and validate a personalised risk model for development of HF and SCD in DCM patients. By creating a single portal and harmonisation of clinical workflows, a facility arises that is unprecedented in scale and setup and that is invaluable for research and improved clinical practice. The here established resources shall be available for other consortia within the ERA-CVD framework.

Project Coordinator: Benjamin Meder, University Hospital Heidelberg, Germany
Project Partners: Andres Metspalu, University of Tartu, Estonia | Phillippe Charron, University Hospital Ambroise Paré & Pitié-Salpêtrière, Paris, France | Zofia Bilinska, Institute of Cardiology, Warsaw, Poland | Folkert W. Asselbergs, University Medical Center Utrecht, The Netherlands
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FAT4HEART: Heart Failure Rescue by Nutritional Approaches: relevance of mitochondrial substrate utilisation

Idiopathic dilated cardiomyopathy (iDCM) is the most common cause of heart failure in the young/middle age population. A genetic basis is found in 20 – 50% of iDCM cases. Unfortunately, there is no effective therapy to reverse iDCM. The heart is a metabolically demanding organ and mitochondria are paramount to the survival and activity of cardiomyocytes. The group of investigators of this project recently demonstrated that a genetic defect leading to mitochondrial dysfunction results in altered cardiac metabolic fuel utilisation, dilated cardiomyopathy and heart failure. Interestingly, a diet with very high fat content was able to cure the disease in these mice. FAT4HEART will study the interplay between mitochondrial defects, dietary components and dilated cardiomyopathy as well as the role of specific diet fat contents as a potential therapy for dilated cardiomyopathy. The work program will uncover the nature of these cardioprotective dietary elements in small and large animal models with the ultimate aim of developing a novel therapy to treat iDCM in humans. Mice with overt iDCM will be fed diets composed of different substrates (fatty acids, carbohydrates, and proteins) and different compositions of fatty acids in order to pinpoint the dietary components responsible for cardioprotection. Porcine iDCM models will expand the translational relevance of these discoveries. Screening of iDCM patients for germline mutations affecting mitochondrial dynamics will be tested *in vitro* to evaluate the impact on mitochondrial fusion and fission. Overall, this project will provide the foundation for future clinical trial testing a simple, cost-effective therapy aimed at curbing the heart failure epidemic.

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GENPROVIC:
Gene Profiling Test for Identification of Treatable Patients with Acute and Chronic Heart Failure

Myocarditis (MC) and inflammatory cardiomyopathy (DCMi) are acute or chronic disorders of myocardium which can be caused by myocardial virus infection or immune reaction with a poor prognosis if no specific treatment is initiated immediately. In the case of MC and DCMi due to missing valuable diagnosis there are currently almost no feasible origin-oriented therapies which can recover the health of the patients. Without the exact diagnosis a specific treatment will not be possible, and patients face a very poor prognosis and a high proportion of them die within a very short period of time. The incidence of patients in Europe is 240,000–480,000 patients/ per year, which states the high unmet need.

The endomyocardial biopsy (EMB) is the gold standard for the diagnosis of MC or DCMi. The diagnostic results of the currently applied set-up of histology, immunohistochemistry and molecular biology is limited by its focal character ("sampling error"). Due to this serious diagnostic problem a high percentage of diagnoses are erroneous with the consequence of a wrong or missing therapeutic option.

In this project, the partners like to validate a gene profile (GENPROVIC) as a novel biomarker based applicable diagnostic test. This diagnostic assay will allow the identification and differentiation of patients with fulminant forms of myocarditis (sarcoidosis, autoimmunological, eosinophilic or giant cell myocarditis) or virus-induced (transcriptional active erythrovirus) heart muscle diseases in patients with or without genetic predisposition to DCM. The GENPROVIC test will combine the measurement of 30 gene transcripts extracted from EMBs. Clinical validation of GENPROVIC will be performed with 3 leading European cardiac clinics based on 300 patients. Patients (approx. 40,000 saved lives through exact diagnosis and new therapy options) and the healthcare system will benefit from the validation of GENPROVIC and its introduction into clinical routine.

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Cardio-oncology:
Early detection and prevention of cardiac dysfunction and heart failure induced by cancer chemotherapeutics

Cardiac dysfunction leading to heart failure can result from anticancer drug-induced cardiotoxicity. It is the second cause of morbidity and mortality among cancer survivors, a group that is growing due to improved cancer treatments. No reliable early markers of drug-induced cardiotoxicity have been identified, and safe, effective drugs to prevent anticancer drug-induced cardiotoxicity are not available. Taking into account the extensive economic and social costs of this condition, it is imperative to develop both early cardiotoxicity markers and efficacious drugs to protect cancer patients against cardiotoxic anticancer drugs.

The Cardio-Oncology consortium brings together cardiologists, cardiobiologists, oncologists, chemo-bioinformaticians, stem cell cardiobiologists and medicinal chemists. Together, the consortium aims to develop cardioprotective therapeutics, including *in silico, in vitro* and *in vivo* testing, and benchmarking in mouse models and human stem cells. Second, to validate and analyse predictive biomarkers of cardiotoxicity in European and Taiwanese cancer patients to detect early cardiac dysfunction, and to define the distinctive etiopathogenetic features of cardiotoxicity.

The cardio-oncology project will deliver biomarker candidates and cardioprotective drug candidates that can be further developed by pharmaceutical and diagnostic companies aiming to improve cancer care.

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MINOTAUR:
Metabolic Therapy for Managing Diastolic Heart Failure

Heart failure (HF) with preserved ejection fraction (HFpEF) is the most rapidly increasing form of heart failure in the Western world. Despite high public-health importance, no treatment strategy has yet been established to reduce morbidity and mortality.

MINOTAUR is a multidisciplinary consortium of cardiologists, cardiac surgeons, systems biologists, biophysicists, biochemists and physiologists aimed at expanding our original observations that a natural caloric restriction mimetic (CRM) improves diastolic function in two different animal models with independent HFpEF risk factors, namely age and hypertension. To investigate the broader applicability of CRM treatment and its translational potential, we will extend our investigations to an animal model of HFpEF induced by metabolic syndrome, the most prevalent cause and comorbidity of HFpEF. We will concentrate our efforts on the mechanistic details by which CRMs counteract the development of HFpEF with metabolic dysfunction.

Using a wide range of state-of-the-art techniques, we will provide a comprehensive in-depth characterisation of the CRM-treated HFpEF cardiac phenotype at the molecular, cellular and whole organism level, and considering the gender aspect.

Gender-based correlation analysis of key biochemical parameters (polyamine levels and post-translational modification of titin) and diastolic dysfunction in human samples will reveal new diagnostic and prognostic tools in HFpEF. This novel information will feed future clinical trials to evaluate the potential of CRM-rich diets as an effective therapy for managing heart failure with preserved ejection fraction.

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LIPCAR-HF:
A prognostic marker of heart failure with impaired ejection fraction

The objective of this project is the development of the long noncoding (lnc) RNA LIPCAR as a new diagnostic and prognostic marker for heart failure (HF) patients. Together Partner 1 (France) and Partner 2 (Germany) have shown that the novel circulating long noncoding RNA LIPCAR is present in blood plasma of patients with cardiovascular diseases. The plasma LIPCAR levels successfully identified patients developing significant cardiac remodeling after myocardial infarction, and are associated with early cardiovascular deaths in Heart Failure (HF) patients with reduced ejection fraction (HFREF).

In this project we will measure and validate circulating levels of LIPCAR (and related lncRNAs) levels in blood samples of cohorts obtained from Parter 3 (Spain) for the LEIZARAN cohort, Partner 4 (Italy) for the GISSI-HF cohort, and Partner 5 (Poland) for acute myocardial infarction, and HF cohorts and healthy controls. Also we will evaluate LIPCAR levels for gender differences in outcome of HFREF patients, and against or combined with other biomarkers of cardiac remodeling (e.g., myocardial fibrosis) (Partner 3, Spain). We will optimize and standardize the measurement of LIPCAR for a kit development, and perform mechanistical studies to fully understand the mode of action of LIPCAR upon various stresses. Finally, we will develop strategies based on lncRNA modulation by enhancing or blocking LIPCAR on cardiomyocytes. This project will enable scientists from 5 European countries to join in an interdisciplinary team for characterizing LIPCAR as early predictor of outcome of heart failure patients with reduced ejection fraction.

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LYMIT-DIS:
Targeted LYmphatic and MIcrovessel Treatments in metabolic-DISease heart failure with preserved Ejection Fraction (HFpEF)

Heart Failure (HF) with preserved Ejection Fraction (pEF) represents a major unmet clinical need for improved diagnostics, prognostics, and development of new treatments to limit the mortality of this increasingly common disease. This type of HF with diastolic cardiac dysfunction affects more than 50% of HF patients, notably women, and is linked to metabolic syndrome (MetS). That is an increasingly common condition characterised by insulin resistance, abdominal obesity, dyslipidemia, and hypertension. The overall objective of LYMIT-DIS is to forward our understanding of the mechanisms involved in the cardiac diastolic dysfunction in HFpEF with the aim to identify and evaluate new tractable therapeutic targets.

In this integrated, multidisciplinary and translational project we will evaluate the impact of MetS-induced HFpEF in a gender-dependent manner, in both experimental models and clinical cohorts. Our focus will be to address the combined role of cardiac lymphatic and blood microvascular dysfunctions in mediating key pathological steps such as cardiac blood supply/demand imbalance, edema, and inflammation, and their effects on cardiac metabolism and fibrosis. The key aspects of our project are investigations of the impact of MetS and gender on cardiac lymphangiogenesis, angiogenesis, and microvascular glycocalyx remodeling, and evaluations of the roles of these processes in HFpEF pathogenesis. At the cellular level, we will determine how MetS alters paracrine signaling in endothelial cells, macrophages, and fibroblasts versus cardiomyocytes in search for new molecular targets against diastolic dysfunction. Further, using cutting-edge technology, we will investigate if insufficient cardiac perfusion and lymphatic transport in MetS modulate cardiac metabolism and fibrosis thereby predisposing to HFpEF.

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VARIATION:
Novel RNA based therapies for treatment-resistant forms of severe cardiomyopathy caused by lamin A/C gene (LMNA) mutations

The currently used therapies for heart failure (HF) have successfully improved symptoms and survival for most forms of HF. However, in a minority of patients these evidence-based treatments do not halt the progression of the disease which leaves these patients with therapy-resistant HF. This most often concerns young patients with heritable forms of HF, including the patients with HF caused by mutations in the LMNA gene. Patients with mutations in LMNA display a dilated cardiomyopathy with conduction disorders (DCM-CD). Mutations in LMNA are one of the major causes for inherited DCM accounting for approximately 8% of cases with inherited DCM, and even up to 33% of cases with DCM with conduction disease. Furthermore, DCM-CD caused by LMNA mutations is characterised by an aggressive clinical course compared to patients with other forms of HF.

We aim to develop a molecular therapy for these patients by using a novel approach to silence the mutated allele. The standard approach currently tested to silence a mutated allele is by short-inhibitory (si) RNAs that are directed in an allele-specific (as) manner (as-siRNAs) against the single, discrete mutation. However, since hundreds of pathogenic mutations are known in the LMNA gene, one would also need to develop hundreds as-siRNAs to treat most patients. We take a different approach as we direct our as-siRNAs against common variants in the LMNA gene. This enables us to target most of the mutated alleles after having to develop and test as few as only 4-7 different as-siRNAs.

We will assess the effect of our as-siRNAs in human induced pluripotent stem cell derived cardiomyocytes on disease specific parameters (e.g. organisation of the nuclear lamina) and on general parameters such as contractility and electrophysiology. Most promising as-siRNA strategies will be tested in a knock-in mouse model. We anticipate that this transnational approach combines the necessary expertise and cutting-edge facilities to demonstrate the Proof-of-Concept of our novel approach to further develop the strongly needed molecular therapy for carriers of mutations in LMNA that cause treatment-resistant heart failure.

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EXPERT:
Exploring new pathways in age-related heart diseases

EXPERT will identify pathways of age-related Heart Failure (HF) and will explore noncoding (nc) RNA (ncRNA)-mediated regulatory pathways of the human genome, instead of the traditional focus on protein-coding genes. EXPERT uses innovative genetic models, ncRNA gender-specific theranostic approaches, and clinical/population-based cohorts to explore age-related HF. The project will lead to novel diagnostic and therapeutic strategies for treatment of HF and other cardiovascular alterations, such as atherosclerosis and myocardial infarction.

EXPERT focuses on: 1) the growing elderly population with higher cardiovascular risks, where improvements in risk detection and preventive therapy are likely to translate into cost savings; 2) novel pathways involved in the ageing heart and related co-morbidities; 3) the development of a biomarker-based stratification of risk that translates into targeted cardiovascular therapy of the elderly, thereby reducing the overall treatment costs and avoiding side effects and associated costs; and 4) novel pathways involved in progression of subclinical atherosclerosis in asymptomatic individuals to prevent future cardiovascular ischemic events.

EXPERT will specifically address how ncRNA-mediated pathways of the healthy ageing heart are affected by genetic backgrounds, cardiovascular risk factors (e.g. diabetes) and/or co-morbidities (such as atherosclerosis, myocardial infarction, cardiac remodelling). Thus, EXPERT will lead to the validation of age-relevant novel ncRNA-related pathways, which will result in a better understanding of the complex pathophysiology of cardiac ageing and the development of new diagnostic as well as therapeutic strategies for aged individuals at risk of HF. Ultimately, EXPERT data will increase the probability of identifying new druggable, therapeutic targets and companion diagnostics with highest clinical applicability.

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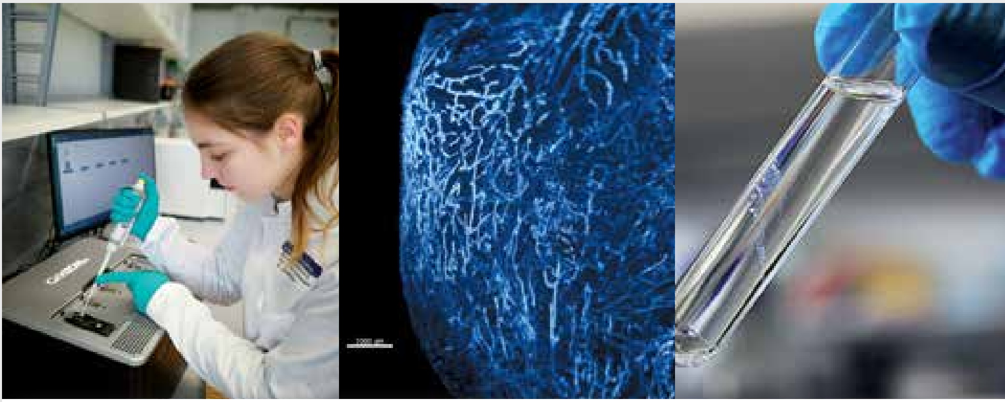

JTC 2017 Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease

Druggable-MI-genes: Utilising myocardial infarction genes for better treatment

Genome-wide association studies (GWAS) firmly associated 93 loci with coronary artery disease (CAD) risk. The mechanisms leading to a higher burden of coronary atherosclerosis are unclear at most loci.

Our consortium aims to unravel molecular alterations of genetic variants shown by GWAS to increase CAD risk, but that are currently not addressed for prevention or therapy. By exploring loci from the globally leading GWAS consortium on CAD (CARDIoGRAM) for expression signals in multi-tissue transcriptomics (RNA-seq) data from several studies (e.g., STARNET, Athero-Express™), we will first differentiate between genes/transcripts underlying genetic risk of myocardial infarction (i.e., unstable plaques) *vs.* stable CAD. Next, we will explore molecular contexts of the most promising candidates in tissue-specific co-expression networks and pathways, aiming to identify the so-called key disease drivers. In parallel, novel molecular mechanisms of plaque instability, which have been partly characterised by the applicants already, will be further scrutinised for potential therapeutic interventions. Finally, translation to human pathophysiology will be achieved by interrogating unique biobanks of human atherosclerotic plaque material, as well as population-based samples of up to 500,000 individuals with genome-wide data and in-depth phenotypic characterisation for better recognition of individuals at risk.

The overall aim of druggable-MI-genes is to lay the foundation for a more precise and genomics-based prevention of coronary atherosclerosis and plaque stability.

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PROACT:
Proteome analysis for the management of coronary artery disease

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in developed countries. Although current diagnostic and therapeutic tools have significantly improved its management, the prevalence of CAD is still on the rise due to demographic transition and increased survival rates. The socio-economic burden of CAD is thus obvious and innovative methods that will improve its management are urgently needed.

In this project, we will focus on two core aims: First, early biomarkers and mechanisms of CAD. We will validate on a large-scale previously identified urinary peptidome-based classifiers that allow early detection of CAD by using a standardised laboratory tool in two European cohorts of over 5000 patients. The classifiers combined with tissue proteomics will be used to extrapolate early CAD-mechanisms in human tissues.

The second core aim is the investigation of plaque instability mechanisms. Using a novel tandem stenosis (TS) mouse model displaying the full stages of atherosclerosis progression of human plaque pathology developed by the associate partner (BAK), plaque proteome analysis and systems biology analysis, we will determine novel therapeutic targets to stabilize the plaque and initially validate those in the TS model. To improve the translational character, these targets will be subsequently validated in human tissues. PROACT will on one hand use cutting-edge technologies to non-invasively detect the risk of developing CAD and on the other hand via an innovative systems biology approach provide potential therapeutic targets to treat plaque instability.

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PREMED-CAD:
PREcision MEDicine in Coronary Artery Disease

Cardiovascular disease (CVD) represents the most important cause of morbidity and mortality in the EU, and causes an enormous socioeconomic impact with almost 200 Billion Euros annual costs. Despite the knowledge on CVD risk factors and several biomarkers, currently existing tools fail to adequately identify subjects at short-term risk. Therefore, innovative approaches to enhance risk assessment and early identification of "at-risk subjects" are urgently needed.

Within PREMED-CAD we will take an interdisciplinary and translational approach integrating knowledge from CAD epidemiology, imaging, bioinformatics, statistics and molecular biology. PREMED-CAD builds on existing biomarker data of distinct CAD and subclinical myocardial ischemia phenotypes, functional imaging data, epidemiological cohort data and biobanks, bioinformatical methodologies and animal models. Existing blood-based and clinical biomarkers will be integrated in a biomarker signature. This biomarker signature will be validated in atherosclerosis-prone mouse models and human cohorts. The validated signature will be translated in an ongoing existing large preventive clinical trial comprising 3,000 patients with subclinical ischemia. PREMED-CAD will enable us to pave the way for affordable tools for early recognition of individuals at immediate and short-term CAD risk, and identify the need for interventional strategies, and explore the molecular pathophysiology and signature of myocardial ischemia in a comprehensive view, and apply precision medicine to affected individuals using an already financed clinical trial.

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MICROEXPLORATION:
Exploring the effects of microcalcification on plaque vulnerability

The majority of acute coronary events are caused by atherosclerotic plaque rupture. Today, vulnerable plaques are only identified a posteriori using histopathology on the basis of a thin fibrous cap, macrophage infiltration, a large necrotic core, intraplaque hemorrhage and microcalcifications. Calcification is a well-known hallmark of atherosclerotic plaques and cardiovascular calcification has emerged as a predictor of and contributor to cardiovascular morbidity and mortality. Plaque ossification, also referred to as macrocalcification, appears to stabilize plaques.

On the other hand, histopathological evidence suggests that subcellular microcalcifications, which form earlier in the thin collagen-based fibrous cap, reduce plaque integrity and trigger rupture and subsequent acute cardiovascular events. However, the direct proof of this detrimental effect and the *in vivo* role of microcalcification on plaque vulnerability have never been addressed. We aim to determine the effects of microcalcification inhibition on plaque inflammation and remodeling, using a recently developed and characterised inhibitor of tissue-nonspecific alkaline phosphatase (TNAP). Upon prevention of microcalcification in atherosclerotic mice, we will longitudinally analyse inflammation and plaque evolution with µMRI, histology and 18F-NaF PET (positon emission tomography (PET) after 18F-NaF injection). Moreover, we aim to identify the molecular mechanism responsible for microcalcification, with particular focus on TNAP activation. Collectively, this project will provide the first data on the effects of microcalcification on plaque evolution. This research program might generate novel therapeutic and diagnostic strategies to control pathophysiological calcification response that is of high unmet clinical need.

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SCAN:
Subclinical atherosclerosis characterisation: Nanoparticle-based molecular and cellular imaging

The aim of SCAN is to expand knowledge on smooth muscle cell (SMC) plasticity in experimental and human atherosclerosis, and to develop nanoparticle-based imaging of macrophage-like SMCs as a measure of plaque progression rate and instability. Non-invasive imaging of cellular processes can become a key tool to 1) understand progression of atherosclerosis to coronary artery disease (CAD); 2) identify persons that need preventive therapy; 3) test the causal role of potential risk factors, including life style; and 4), guide development of new drugs against CAD.

Recent research has shown that the quantitative importance of the SMC lineage for the cellular content of plaques has been grossly underestimated. The partners in this consortium in particular have shown that SMCs in plaques undergo abundant conversion to a previously unrecognised, macrophage-like cell type. Given the important role of traditional fibrous cap SMCs for safeguarding against acute CAD, and the importance of inflammation in disease progression, we hypothesize that such SMC modulation has direct consequences for future risk of progression and acute CAD events.

SCAN is a collaboration between experienced experimental atherosclerosis and imaging groups in Nijmegen, Tübingen and Madrid. It brings together deep knowledge in atherosclerosis mechanisms, SMCs, and imaging modalities; genetically designed atherosclerosis models in mice and minipigs; human atherosclerotic plaque biobanks; two complementary and cutting-edge nanoparticle platforms, and state-of-the-art MRI, PET/CT, ultrasound, and optoacoustic imaging facilities.

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OPERATION:
Elucidate monocyte phenotype to predict arterial wall inflammation

Up to 70% of cardiovascular (CV) events are not prevented despite optimal lipid-lowering therapies. In search for additional strategies, immune–cell driven inflammation of the arterial wall has been recognised as a key player contributing to atherosclerotic plaque initiation and eventually destabilisation. Biomarkers capturing inflammatory activity of arterial plaques in patients have been put forward as a promising tool in CV-risk assessment. To date, traditional plasma biomarkers of inflammation have however failed to predict arterial wall inflammation. In view of the cumulative evidence supporting a role of the innate immune system in atherogenesis, there has been increasing interest in the determination of monocyte function and accompanying plasma metabolites as markers for CV-risk. Here, the phenotype of circulating monocytes is believed to serve both as an indicator of disease burden as well as prognostic marker in CV-prevention.

In this project, we propose to combine state-of-the-art genome-scale metabolic modelling on plasma monocytes with plasma proteomics to select a biomarker pattern correlating with arterial wall inflammation. Subsequently, advanced machine-learning will be applied to optimize a biomarker pattern predicting CV-risk, which will be validated in primary and secondary prevention cohorts available to this consortium. The end-result is the delivery of a clinically relevant and industrially usable integrated inflammatory plasma biomarker pattern which will enable us to accurately identify patients at residual inflammatory CV-risk.

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ENDLESS:
Atherosclerotic plaque erosion leading to thrombus formation. The role of microparticles in ENDtheliaL transdiffErentiation, apoptoSiS and senescence

The scientific community has faced an impressive shift in incidence and clinical presentation of atherosclerotic disease over the last two decades. Mortality rates due to myocardial infarctions (MI) have decreased by nearly 50% and clinical presentation deviated away from a predominance of ST-elevation myocardial infarction (MI) (STEMI) towards non ST-elevation MI (NSTEMI). In line with the shift in clinical presentation there has been a significant change in the underlying pathology of symptomatic atherosclerotic lesions that is gradually shifting from the lipid rich unstable plaque towards more stable, fibrous and less inflammatory plaques.

This paradigm shift mandates uncovering the mechanisms that accelerate a thrombotic event on top of a stable plaque. The endothelial cell plays a crucial role in leucocyte and platelet adhesion that promote luminal thrombosis, even in the absence of plaque rupture.

ENDLESS will unravel mechanisms that lead to endothelial desquamation or transdifferentiation (EndoMT) and explain the thrombotic event that is superimposed on a stable non-ruptured plaque. We will study how coronary artery disease susceptibility genes affect endothelial cell transcription and function. In addition, we will assess the role of microparticle formation in the mechanisms that disrupt the endothelial layer (erosion) and gives rise to a thrombotic event. Analyses will be executed in sex-stratified manner as thrombus on top of stable plaques has been described to be more prevalent in women. Ultimately, this may lead to new mechanisms pointing towards therapy to preserve a healthy endothelium on top on an intact stable plaque.

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PLAQUEFIGHT:
Fighting Atherosclerotic Plaques in Coronary Artery Disease via Targeting Neuroimmune Interfaces

Atherosclerosis is caused by plaques in the inner layer of arteries. Plaque instability triggers clinically significant disease including coronary artery disease (CAD). However, there is currently no causal therapeutic approach to treat CAD and the pathophysiological mechanisms of atherosclerosis largely remain to be uncovered.

We focused on possible interactions between atherosclerosis associated adventitia inflammation and the nervous system, a research area previously unexplored as atherosclerotic plaques are not innervated. Our preliminary studies showed that atherosclerosis initiates immune-neurovascular interactions with the peripheral nervous system (PNS) in Apolipoprotein E-deficient (ApoE-/-) mice via atherosclerosis-associated adventitia innervation. We found that artery tertiary lymphoid organs (ATLOs) and fat-associated lymphoid clusters (FALCs) in the adventitia of arteries interact with the PNS by stimulating axon growth and infiltrating para-aortic ganglia, dorsal root ganglia, and nerves. We also identified a putative atherosclerosis-brain-circuit (ABC) connecting the adventitia/ATLOs and distinct brain nuclei using retroviral, i.e. pseudorabies virus (PrV), tracing technologies. Thus, immune-neurovascular interactions form ABCs that sense, respond to, and may affect atherosclerosis.

To study the functional impact of ABCs, we plan to i) ablate components of the PNS; ii) study the morphopathology of ABCs; iii) determine whether PNS conductance is altered in atherosclerosis; and iv) validate our results in human cardiovascular tissues in translational approaches.

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AtheroMacHete:
Macrophage heterogeneity in human and murine atherosclerosis: a therapeutic opportunity?

Atherosclerosis-related cardiovascular diseases are the most important cause of death worldwide. As the most abundant immune cell type in the plaque, macrophages play crucial roles in the pathogenesis throughout the disease course. Due to their versatile and plastic phenotype in response to locally-produced factors, and depending on differences in their ontogeny (monocyte-derived vs. tissue-resident), macrophages are very heterogeneous and exert pro- and anti-atherogenic activity. As context and origin, and hence macrophage heterogeneity, are disease stage-dependent this complicates the design of effective macrophage-based therapies.

Here, a complimentary team of experts in plaque pathophysiology (EB), macrophage systems biology (JS), macrophage differentiation (MS) and cardiovascular medicine (PA) joins forces to tackle this challenge. We will provide a detailed transcriptional, ontogenic, contextual and functional macrophage map for several disease stages and benchmark this against known populations in other tissues. These unprecedented new insights into atherosclerotic macrophage heterogeneity in human and mouse will allow identifying gene programs and regulatory cues driving critical functions of plaque macrophages. Finally, through a network-guided systems medicine approach we will identify effective compounds based on known interactions between existing FDA approved drugs and our identified molecular networks, validate them in *in vitro* high throughput screenings, *in vivo* mouse models and provide proof-of-concept for clinical atherosclerosis.

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Xplore CAD:
Advanced ex vivo analyses and multi-frequency ultrasound technology for improved evaluation and diagnosis of coronary plaque

Atherosclerosis is considered a multifactorial disease with risk factors ranging from high-fat diet, hypertension, smoking, diabetes, to genetic susceptibility and other factors. The general consensus is that myocardial infarction, a hallmark of coronary artery disease (CAD), can be prevented if these known risk factors are taken care of in due time. Classic statin treatment has led to significant reduction of clinical events, but considerable residual risk of cardiovascular related mortality still remains.

Alarmins are host biomolecules that can initiate and perpetuate a noninfectious inflammatory response. Myocardial ischemia, the result of coronary artery occlusion, initiates systemic inflammation through the release of such alarmins.

Our hypothesis is that alarmins are critical players of CAD plaque progression, which can be directly used for targeted imaging. By analyzing blood and plaque samples from both patient and animal models, the XploreCAD project will identify potential biomarkers of coronary artery disease that will be used in combination with novel ultrasound technology for improved imaging of coronary plaque. The methodology will include high performance nano-liquid chromatography mass spectrometric analysis and immunological assays of plaques and sera isolated from atherosclerotic animals and patients. Expected results will lead to better understanding of the mechanisms related to plaque progression and plaque instability, and an improved imaging technique for plaque diagnosis.

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JTC 2018 Transnational Cardiovascular Research Projects driven by Early Career Scientist

NEMO-IMMUNEagainstHF:
NEural MOdulation of IMMUNE system to shape cardiac remodeling to chronic arterial hypertension and counteract Heart Failure

Hypertensive heart disease (HTN-HD) develops secondary to left ventricle (LV) pressure overload and neurohormonal hyperactivation induced by hypertension (HTN). Chronic HTN-HD leads to heart failure (HF) with a preserved ejection fraction (HFpEF). Despite reflecting an initial adaptive process, HFpEF progresses toward worsening of LV function, evolving toward HF with reduced EF (HFrEF).

The fate of cardiac remodeling to challenges imposed on the LV is the result of different components mutually interacting. The autonomic nervous system (ANS) is main regulator of cardiac function and ANS imbalance is a strong negative predictor of clinical outcome. We recently recognised that ANS modulates immune functions involved in cardiovascular diseases. Since immune cells and burden of inflammation in pericardial adipose tissue (PAT) control myocardium's ability to withstand HTN, we hypothesize that finely tuned neuroimmune interactions are required to shape LV adaptive responses.

Thus far, therapeutic strategies exploiting these mechanisms have been limited by the inability to distinguish between immune cells residing within or recruited to the myocardium. A major advancement has been achieved by our group by discovering that macrophages (MFs) from diverse lineages differently control adaptive vs. maladaptive responses to cardiac challenges. Our network will connect combined expertise to address how 1) ANS modulation of immune organs, 2) immune cells of different lineages, and 3) pericardial adipose tissue (PAT) modulate cardiac milieu during HTN, and influence HF development, and testing 4) how our results translate to human pathology.

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AIR-MI:
Adaptive Immune Responses in the wounded heart: novel diagnostic and therapeutic opportunities

Ischemic heart disease causes tremendous morbidity, mortality, and economic burden. Inflammatory processes determine the remodeling response of the infarcted heart and thus the healing outcome; however, they still remain poorly understood.

We previously demonstrated that myocardial infarction (MI) and ageing trigger distinct immune responses and revealed a crucial and unprecedented role for adaptive immunity in the myocardial injury response. However, to date there are no established means to qualitatively assess ongoing myocardial repair processes and predict their outcome. We cannot timely identify and treat patients with inadequate healing that deteriorates myocardial function until they reach a critical stage of heart failure (HF). Herein, we put forward that exploiting adaptive immune phenomena offers exciting opportunities to address these unmet needs.

In our joint initiative we will apply cutting edge techniques to assess the T-cell repertoires in a well-defined (available) cohort of patients exhibiting comparable initial MIs, but contrasting cardiac healing outcomes at 1 year. By integrating clinical and immunological data we expect to identify T-cell signatures bearing prognostic value to discriminate "good" *vs* "poor" healers. Furthermore, we plan to devise T-cell-based therapeutic interventions to foster cardiac repair in experimental models of MI. These experiments are a requisite to develop novel diagnostic and therapeutic approaches and ultimately translate them to clinical applicability.

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MEMORY:
Trained iMmunity in bonE MarrOw progenitors as dRiver of atherosclerosis in obesitY

Due to the worldwide adoption of Western lifestyle, obesity is no longer an exception. Obesity strongly predisposes to atherosclerotic cardiovascular disease, even after correction for metabolic complications such as insulin resistance or dyslipidemia. Once obesity has developed, losing weight is difficult and mostly transient. Innate immune cells are key drivers of atherosclerosis, and we recently reported that these cells can build a long-lasting memory ("trained immunity") which contributes to atherogenesis. In mice, a Western type diet leads to atherogenic reprogramming of bone marrow progenitors of these cells that persists long after switching to a normal diet.

The aim of this project is to understand how myeloid cell progenitors are reprogrammed to an enhanced atherogenic state in obesity-induced atherosclerosis. This provides tools to identify obese patients with imminent cardiovascular risk, and allows the development of drugs to halt atherosclerosis.

We will extensively interrogate myeloid cells and bone marrow progenitors for a trained immune phenotype in patients with obesity induced atherosclerosis. This will uncover pathways that we subsequently block in *in vitro* models of trained immunity. Effective compounds will be tested in animal models of Western type diet-induced atherosclerosis to prevent or reverse trained immunity and atherosclerosis. Finally, we will take the most effective drugs that are suitable for human use, back to the patients with obesity induced atherosclerosis to explore whether trained immunity can be reversed.

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AtheroInside:
Local immunomodulation of atherosclerosis by CD8+ t-cell based nanomedicines

Atherosclerosis is the most prominent underlying pathology of cardiovascular disease and an important cause of major adverse cardiovascular events (MACE). Chronic inflammation drives growth and destabilisation of atherosclerotic plaques. Recent clinical trials assessing the effect of systemic administration of anti-inflammatory drugs show a promising decrease in MACE, but the trade-off is systemic immune suppression. A better understanding of the local inflammatory processes in the lesion and specific targeting of anti-inflammatory drugs to the lesion will allow for local intervention, without systemic immune suppressive effects.

Pilot data show CD8+ T-cells inside the atherosclerotic lesion are markedly different from their counterparts in the circulation and have a local anti-inflammatory effect. We will apply state-of-the-art techniques such as mass cytometry, optoacoustic-, superresolution-, and multi photon microscopy to human plaques to study CD8+ T-cells and their interactions with foam cells to identify their protective pathways which may allow for therapeutic intervention. Importantly, as our data indicate that CD8+ T-cells readily accumulate in the lesion, we propose to also use these cells as delivery vehicles for local drug delivery to the plaque by loading them *ex vivo* with liposomes encapsulating the anti-inflammatory agent rapamycin. We anticipate that this project will provide novel mechanistic insights in the local inflammatory pathways and novel targeted strategies to image and stabilize atherosclerotic lesions via pharmacological intervention.

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DENIM:
Duchenne muscular dystrophy cardiomyopathy; the role of ENdocannabinoids and IMmune regulation

Muscular dystrophy (MD) is an X-linked neuromuscular disease manifesting as muscle atrophy and cardiomyopathy in young boys. Carrier females (CF) are also often diagnosed with advanced stage cardiomyopathy. The existing therapy has limited effect and many patients die of heart failure. Therefore discovering new therapies for MD cardiomyopathy (MDC) is a clinical imperative.

Altering the endocannabinoid system (ECS) dramatically impacts cardiomyopathy, however its role in MDC remains unexplored. The Italian Unit uncovered the involvement of the ECS in MDC. Cardiomyocytes derived from MD patients' induced pluripotent stem cells (iPS-CM) have an increased ECS tone, inflammation and membrane-damage which were ameliorated by ECS modulators. Since evidence suggests that immune-regulators contribute to MD, we will incorporate immune-regulatory cells (cardiac stromal cells and macrophages) to our model forming a tri-cell (3C) MDC model. The French and Italian Units will use the 3C-MDC model to evaluate the immune-modulatory potential of ECS targeting. The Italian Unit will accrue clinical data from MD patients and CF, and correlate them with cellular phenotypes. To gain insight on the impact of ECS modulators *in vivo,* the German Unit will assess the effects of molecules targeting specific ECS components in a mouse model of MDC. The Italian Unit will recruit CF and use engineered isogenic iPS-CM to address gender and genetic differences. We expect to provide proof-of-concept data supporting ECS modulation to improve MDC bolstering the pursuit of clinical trials.

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MISs-CVD:
Neuroimmune guidance cues, MicroRNAs & Inflammatory responses: Sex differences in CardioVascular Diseases

Cardio-vascular disease continues to be a leader of morbidity and mortality. Despite advances in the diagnosis, prevention and treatment of cardiovascular disease, the risk of coronary artery disease in women, increases dramatically after menopause. Although sex hormones are known to play a role in this lipid- and inflammatory-driven disease, the underlying mechanisms are largely unclear. Neuroimmune guidance cues (NGCs) are key regulators of cell movement and positioning, and have emerged as significant players in inflammation.

We have previously reported a role for Netrin-1 on macrophage emigration from plaques as well as key regulation of lipid metabolism by microRNA (miRNA)-33. Recently, we have un-covered sex-biased miRNAs targeting NGCs and hypothesise that these miRNAs provide missing mechanistic links in the endothelial-monocyte interactions and macrophage retention in the vascular wall during atherosclerosis in women vs men.

In this MISs-CVD project we aim to better understand sex-specific biology in the vessel wall. We study specifically sex-biased non-coding microRNAs that can regulate proteins that 'guide' white blood cells playing a role in the development of arteriosclerosis. We seek to fully characterise the clinically relevant sex-biased miRNAs and NGCs in monocytes and aorta specimens from healthy subject and patients with atherosclerosis. We will also use primary endothelial cells and assess direct interactions between the sex-biased miRNAs and NGCs and unravel the functional impact of promising miRNAs on endothelial permeability, monocyte and macrophage trafficking and atherosclerosis using an innovative 3D vessel-on-a-chip platform and *in vivo* mouse models. This will provide crucial understanding of the gender differences in atherosclerosis and may deliver gender specific therapeutic avenues to combat atherogenesis in both men and women.

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DETECT ARRHYTHMIAS: Investigating DETerminants of atrial Electrical ConducTion underlying ARRHYTHMIAS

Arrhythmias are common, affecting millions of Europeans and are a major healthcare challenge as they are associated with substantial morbidity and mortality. Underlying mechanisms are complex and still not fully understood resulting in insufficient therapeutic strategies. A growing body of evidence suggests that electrophysiologic properties are not exclusively determined by cardiomyocytes but also by mechanisms involving the complex cardiac environment including genetic factors, other cell types such as stem cells or immune cells, or the surrounding epicardium.

Therefore, we hypothesise that factors beyond the cardiomyocyte determine electrical conduction properties, and thus play a key role both in physiologic generation and propagation of electrical impulses through the heart and in pathophysiologic mechanisms leading to arrhythmias. To validate this hypothesis DETECT ARRHYTHMIAS will integrate *in vitro* and *in vivo* experiments in a multidisciplinary translational approach including electrophysiology, basic science, stem cell biology, genetics, and immunology.

DETECT ARRHYTHMIAS will undertake a comprehensive evaluation of genetic mechanisms, will investigate the role of the epicardium, and will study inflammatory mechanisms underlying atrial conduction in patients, mouse models, and patient-derived iPS cells. DETECT ARRHYTHMIAS will have ground-breaking impact on our understanding of mechanisms underlying both physiology and arrhythmia as well as on the translation from bench to bedside by catalysing the development of innovative and pioneering therapies.

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MultiFib:
Multimodal Fibre optic probe for highly resolved *in vivo* localisation of cardiac Fibre

Sudden cardiac death (SCD) accounts for 350,000 deaths each year in Europe alone. A major cause of SCD is a lethal cardiac rhythm disturbance, ventricular fibrillation (VF). Symptomatic patients can be treated with implantable defibrillators that deliver painful shocks to arrest arrhythmias. Yet, only a minority of patients are eligible to targeted curative treatments, such as ablation therapy. This is due to an unmet capability to identify and localise the combined electrical, structural, or biochemical substrates that predispose to VF.

The project MultiFib aims at the development of a multimodal imaging fibre probe for a combined morphological and biochemical characterisation of arrhythmogenic substrates at optical resolutions as a guidance tool for cardiac ablation. MultiFib will for the first time integrate optical coherence tomography, second harmonic generation, and Raman spectroscopy in to a single optical fibre probe, enabling structural and biochemical mapping in conjunction with conventional electrical measures for an unprecedented assessment of myocardial properties and identification of pathological substrates. It should also provide a means to assess in real-time ablation efficacy. As a result, the availability of such a probe will substantially increase the eligibility for patients requiring treatment for cardiac electrical diseases to curative ablation therapy.

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EMPATHY:
Electromechanical presages of sudden cardiac death in the young: integrating imaging, modelling and genetics for patient stratification

In the young (<40 years), sudden cardiac death (SCD) is often the first manifestation of a genetic cardiac disease causing lethal arrhythmias. Both, male gender and physical exercise are known risk factors for SCD in this vulnerable population. Current mechanistic understanding of how gender-related and environmental factors such as exercise interact with a pro-arrhythmic substrate and trigger life-threatening arrhythmias is limited. This lack of knowledge makes the identification of those at risk for SCD extremely difficult.

The EMPATHY project aims to unravel the complex pro-arrhythmic electro-mechanical interactions in the apparently healthy yet vulnerable hosts of genetic cardiac diseases by combining three different but highly complementary scientific fields, being clinical cardiac imaging (OSLO), genetics and cellular electrophysiology (MILAN), and multi-scale computational modelling (MAASTRICHT). We expect that the integrative EMPATHY approach will reveal novel genetic and electro-mechanical signatures of arrhythmogenic diseases, and thus enabling earlier disease recognition, personalised therapeutic intervention, and effective prevention of SCD in the young.

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PICASSO:
Unravelling proprotein convertase subtilisin/kexin type 9 (PCSK9) mechanisms in calcific aortic valve disease: from aortic valve sclerosis to stenosis

Calcific aortic valve stenosis (CAVS) is a fatal pathology that affects 2–3% of the population. Yet, drug therapies are ineffective, leaving the urgency to fully characterise the etiology of aortic valve calcification (AVC) to develop innovative therapies.

The proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to and targets the low-density lipoprotein receptor (LDLR) for lysosomal degradation. It was recently shown that carriers of the PCSK9 R46L loss-of-function genetic variant have low levels of LDL and lipoprotein (a) and reduced risk of coronary heart disease and CAVS. However, the mechanisms through which altered PCSK9 activity could protect or cause CAVS are lacking.

Our preliminary data suggest that PCSK9 plays an active role in CAVS. Therefore, we will determine whether PCSK9 is a causal risk factor for CAVS using genetic epidemiology. Further, we will shed light on the molecular underpinnings through which PCSK9 causes CAVS in humans using state-of-the-art *in vivo* as well as *in vitro* models in combination with next-generation sequencing. As a complementary and potential preclinical model, we will determine the role of PCSK9 in the development of AVC in mice.

The overarching aim of this research program is to provide the required biological evidence using a highly translational approach to support PCSK9 as a therapeutic target for the prevention and treatment of patients with CAVS.

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MEND-AGE:
Dissecting Mesenchymal-ENDothelial cross-talk, heterogeneity and function to mend vascular AGEing and atherosclerosis

Ageing increases cardiovascular events through cell senescence with accelerated atherosclerosis, fibrosis, endothelial dysfunction, oxidative stress (ROS), and inflammation. Resident mesenchymal cells (MCs), i.e. fibroblasts and pericytes, may not only drive fibrosis, but their cross-talk with smooth muscle, immune and endothelial cells (ECs) also affects angiogenesis, vascular tone, calcification and inflammation.

Indeed, our consortium identified migrated MC driving atherosclerotic plaque calcification. Yet underlying drivers and the possibility to rejuvenate function of MCs and ECs in the ageing vasculature are not fully explored. Compelling data of this consortium shows that 1), MC are recruited from adventitia and drive fibrous cap development and calcification, 2), MCs are highly heterogenous, 3), EC are prone to senescence, and 4), EC cross-talk activates MCs.

Our joint expertise in ageing, fibrosis, angiogenesis, ROS and atherosclerosis and access to single-cell-sequencing, unique multicolor reporter mice and human samples will allow us to investigate how ageing affects EC / MC heterogeneity and cross-talk driving atherosclerosis. We will analyse adventitial cell heterogeneity, fate and cross talk in arterial ageing and atherosclerosis, identify ageing-induced drivers of vascular disease, and target ageing-induced drivers in animal models to ameliorate ageing-associated vascular pathology. This passion that drives this project is driven by a simple emerging hypothesis: It is possible to treat atherosclerosis and plaque rupture by synergising endothelial-mesenchymal cross-talk.

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HyperDiP:
Maternal hemodynamics in hypertensive disorders of pregnancy – a human and animal model under antihypertensive therapy

Hypertensive disorders of pregnancy (HDP) have been the leading cause of maternal and neonatal morbidity and mortality worldwide in the last decades. Recently, the common belief of preeclampsia (PE), defined as hypertension and proteinuria after 20 weeks of gestation, and being a placental disorder was challenged. A number of studies reported short and long term cardiovascular changes in pregnancies affected by HDP suspecting a cardiovascular origin of the disease. Impaired cardiac output (CO), increased systemic vascular resistance and diastolic ventricular dysfunction were detected by echocardiography. Even though there are new insights into the pathophysiology of the disease, the concepts of therapy have not changed. Evidence for an improvement of antihypertensive therapy in women with HDP reflected by a decrease in adverse maternal and neonatal outcome is lacking.

Therefore we aim to investigate maternal hemodynamics and cardiovascular parameters in women with HDP compared to a healthy pregnant control group matched for gestational age and in a preeclamptic rat model, with the special focus on changes in maternal hemodynamics due to antihypertensive treatment that has not been accessed in this specific setting before. In this prospective observational case control study over a three year period we challenge the idea of blood pressure being the suitable target for antihypertensive therapy in women with HDP and aim to get new insights into the pathophysiology of the disease by using invasive procedures and assessing the structural morphology of the heart in our rat model.

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FIBER:
Fibrosis Treatment by Enhancer Targeting

Fibrosis results from the activation, accumulation and persistence of resident cardiac fibroblasts (CFs). The currently largely uncharacterised changes to the cardiac fibroblast epigenome driving this pathologic process represent potential therapeutic targets.

FIBER is a transnational multi-disciplinary 3-year program involving young researchers and a physician from 4 European countries aiming to identify novel therapeutic targets against cardiac fibrosis, a major pathologic component of most cardiac diseases. FIBER will use state-of-the art genetic tools, innovative *in vivo* and *in vitro* approaches, as well as gender specific patient tissue analysis in order to characterise epigenetic features of cardiac fibroblasts responsible for cardiac fibrosis. FIBER will assist the development of epigenetic therapeutic approaches targeting fibrosis in heart disease patients. Specifically, FIBER aims to 1) generate transcriptome, chromatin accessibility, histone mark, DNA methylation and transcription factor (TF) binding profiles for activated versus quiescent CFs. 2), identify enhancer elements and TFs involved in regulating the fibrotic response, and 3), disrupt candidate enhancer activity using gene editing approaches in human cell lines and in mouse. 4), analyse candidate enhancer activity in human patient samples and engineered heart tissue (EHT). In order to achieve these objectives, we will exploit genetic tools that have been well established by FIBER partners, as well as develop novel tools including a state-of-the-art *in vitro* human cardiac fibrosis EHT-based system.

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INNOVATION:
Investigating long non-coding RNA regulated pathways driving cardiac regeneration

INNOVATION is an interdisciplinary 3-years program that brings together 5 European nations aiming to exploit RNA biology for the stimulation of cardiac regeneration and thus, ultimately combating heart failure. The adult mammalian heart is void of significant regenerative capacities, while neonatal hearts fully regenerate without scarring after myocardial infarction (MI).

INNOVATION will identify new pathways that distinguish regenerative neonatal mouse hearts from non-regenerative adult hearts. In contrast to the traditional focus (which lies on protein-coding genes), INNOVATION will explore long non-coding RNA (lncRNA)-mediated regulatory pathways. To do so, this project will use state-of-the-art molecular tools paired with innovative *in vitro* and *in vivo* models which will lead to the discovery of novel regeneration-associated lncRNAs. Human conserved lncRNAs will subsequently be exploited to devise therapeutic RNA strategies for the stimulation of cardiac regeneration in adult mice and human cell models.

INNOVATIONs specific objectives are: 1) generation of a gender and cell-type specific atlas of conserved lncRNAs to identify regeneration-specific expression patterns in the heart; 2) validate regeneration-related cardiovascular pathways by testing lncRNA interventions in a neonatal mouse model of MI; 3) define the pro-regenerative role of lncRNA in human cardiac cells and engineered heart tissue; 4) develop therapeutic lncRNA-based strategies to boost cardiac regeneration after MI in adult mice, aimed at a potential translation into patients.

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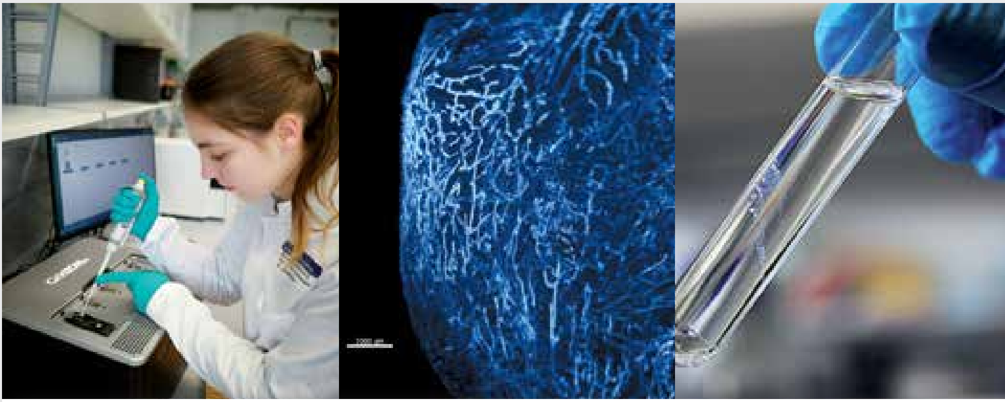

Poster Abstracts

No.	Call	Consortium	Name ECR	Abstract Title
1	JTC 2016	ACM-HF	Viviana Meraviglia	Generation of iPSC from patients with Arrhythmogenic Cardio-myopathy and their differentiation into cardiomyocytes in the context of Consortium ACM-HF
2	JTC 2016	ACM-HF	Elena Sommariva	Characterisation of Cardiac Mesenchymal Stromal Cells form Arrhythmogenic Cardiomyopathy patients, in the context of Consortium ACM-HF
3	JTC 2016	ACM-HF	V. Douin-Echinard	Characterisation of immune derived mediators from PBMC of Arrhythmogenic Cardiomyopathy patients, in the context of Consortium ACM-HF
4	JTC 2016	ACM-HF	Andraž Cerar	Impairment of myocardial perfusion correlates with heart failure progression in patients with noncompaction cardiomyopathy
5	JTC 2016	CARDIO-PRO	Hessel Honknoop	Single-cell analysis uncovers that metabolic reprogramming is essential for cardiomyocyte proliferation in the regenerating heart
6	JTC 2016	CARDIO-PRO	Simone Redaelli	Dissecting the molecular mechanisms of BMP signaling function in heart regeneration

No.	Call	Consortium	Name ECR	Abstract Title
7	JTC 2016	Cardio-oncology	Pavel Karpov	Design of PKR1 agonists – new agents for treating cardiotoxicity
8	JTC 2016	CLARIFY	Bas Molenaar	Characterisation of intercellular communication in ischemic heart disease by single-cell RNA sequencing
9	JTC 2016	MINOTAUR	Mahmoud Abdellatif	Spermidine improves diastolic heart failure in rats with cardio-metabolic syndrome
10	JTC 2016	MINOTAUR	Maria Rosaria Pricolo	Therapeutic effect of the caloric restriction mimetic spermidine on diastolic heart failure: the contribution of titin oxidative posttranslational modifications
11	JTC 2016	MINOTAUR	F. Koser	Unbiased screen of the cardiac proteome and effect of metabolic therapy on titin properties in a rat model of heart failure with preserved ejection fraction (HFpEF)
12	JTC 2016	DETECTIN-HF	Maris Alver	Identifying dilated cardiomyopathy-affected individuals in a population-based biobank based on high-coverage sequencing data and electronic health records
13	JTC 2016	DETECTIN-HF	G.T. Trusz-kowska	Genetic testing in Polish dilated cardiomyopathy patients as a part of ERA-CVD DETECTIN-HF

No.	Call	Consortium	Name ECR	Abstract Title
14	JTC 2016	EXPERT	Federica De Majo	Dichotomy between the trans-criptomic landscape of naturally versus accelerated aged murine hearts
15	JTC 2016	EXPERT	Rosa M. Nevado	Role of microRNAs in cardiac alterations in Hutchinson-Gilford progeria syndrome
16	JTC 2016	EXPERT	Christina Pagiatakis	The novel long non-coding RNA Chheaf-1 regulates early-phase cardiac hypertrophy
17	JTC 2016	EXPERT	Evelyn Gabriela Rusu	Influence of ageing on the quality and dynamics of mouse cardiac fibroblasts in culture
18	JTC 2016	EXPERT	Livier De La Rosa Vargas	Metabolic regulation of heart failure caused by imbalanced mitochondrial dynamics
19	JTC 2016	FAT4HEART	Eduardo Oliver	Metabolic flexibility of the failing myocardium through high fat die
20	JTC 2016	FAT4HEART	Rocio Villena-Gutiérrez	Dietary restriction prevents dilated cardiomyopathy progression by enhancing mitophagy
21	JTC 2016	LIPCAR-HF	Nicola Pianca	The impact of glucocorticoids on Neuregulin-1/ERBB2 signalling in cardiomyocyte proliferation and heart regeneration

No.	Call	Consortium	Name ECR	Abstract Title
22	JTC 2016	LIPCAR-HF	Francesca Fumagalli	Post-resuscitation treatment with inhaled argon improves outcome after a prolonged untreated cardiac arrest in a porcine model
23	JTC 2016	MacroERA	Deepak Ramanujam	MicroRNA-21 in cardiac resident macrophages regulates pressure overload-induced cardiac remodeling
24	JTC 2016	MacroERA	Marida Sansonetti	Cardiomyocyte-derived exosomal miR-200c favors a shift towards pro-inflammatory M1 cardiac macrophages and accelerates heart failure
25	JTC 2016	MacroERA	P. Carai	MiR-223-3p inhibition modulates cardiac inflammation in Viral Myocarditis
26	JTC 2016	PDE4HEART	Aurélia Bourcier	Cardiac gene therapy of heart failure with phosphodiesterase PDE4B in mice
27	JTC 2016	PDE4HEART	Nikoleta Pavlaki	Gene therapy with phospho-diesterase 4B in a murine model of pressure overload-induced heart failure
28	JTC 2016	PDE4HEART	Jean Piero Margaria	Gene therapy with PDE2A and PDE4B in a mouse model of heart failure

No.	Call	Consortium	Name ECR	Abstract Title
29	JTC 2016	PDE4HEART	Bernadin Ndongson-Dongmo	Development of a rat model of heart failure with preserved ejection fraction (HFpEF) for evaluation of phosphodiesterase gene therapy
30	JTC 2016	VARIATION	Charlotta S. Behrens	Modelling of LMNA-cardiomy-opathy in human iPSC-derived engineered heart tissue for testing novel therapies
31	JTC 2016	VARIATION	Anke J. Tijsen	Allele-specific shRNAs directed against a common variant to treat cardiomyopathy due to LMNA mutations
32	JTC 2016	VARIATION	Assad Shiti	Optical mapping of human induced pluripotent stem cell derived cardiac cell sheets
33	JTC 2017	Athero-MacHete	Pieter Goossens	Spatial mapping of macrophage heterogeneity in murine atherosclerotic plaques
34	JTC 2017	Athero-MacHete	Nico Reusch	Deciphering cell-to-cell communication in perturbed whole blood using single-cell RNA sequencing
35	JTC 2017	MICROEX-PLORATION	Hoda Alibiglou	The role of tissue-nonspecific alkaline phosphatase in microcalcification formation

No.	Call	Consortium	Name ECR	Abstract Title
36	JTC 2017	MICROEX-PLORATION	Lilianna Grochocka	Annexin A6 participates in the tissue nonspecific alkaline phos-phatase-induced calcification of human coronary artery smooth muscle cells
37	JTC 2017	PLAQUE-FIGHT	Sarajo Mohanta	Tripartite cardiovascular neuroimmune circuits regulate atherosclerosis
38	JTC 2017	PREMED-CAD	Apurva Shrivastava	Long non-coding RNA RP1-79C4.4 as novel candidate gene for Atrial Fibrillation/Coronary Artery Disease?
39	JTC 2017	PREMED-CAD	Henri Weidmann	Molecular assessment of iron deficiency in cardio-vascular disease
40	JTC 2017	PROACT	Zhen-Yu Zang	A Novel Urinary Biomarker Predicts 1-Year Mortality after Discharge from Intensive Care
41	JTC 2017	SCAN	Kim Cortenbach	An in vitro "circulatory system" to study the mechanical stability of therapeutic nanoparticles
42	JTC 2017	SCAN	Maria Muñoz-Hernando	Enzyme-driven multimodal detection of atherosclerosis with iron oxide nano-particles

No.	Call	Consortium	Name ECR	Abstract Title
43	JTC 2017	SCAN	Maria Teresa Kristina Zaldivia	Identification of markers associated with transdifferentiated vascular smooth muscle cells to evaluate plaque stability
44	JTC 2018	DENIM	Elisa Castiglioni	Duchenne muscular dystrophy cardiomyopathy – the role of ENdocannabinoids and IMmune regulation
45	JTC 2018	DETECT AR-RHYTHMIAS	Erik Abner	Analysis of the MEIS1 genomic locus in correlation to heart arrhythmias in the Estonian population
46	JTC 2018	EMPATHY	Aurore Lyon	Differentiating the effects of exercise-induced beta-adrenergic stimulation and stretch on calcium and force dynamics using a novel electromechanical cardiomyocyte model
47	JTC 2018	HyperDiP	Kristin Kräcker	Alterations in cardiac structure and function caused by preeclampsia
48	JTC 2018	HyperDiP	Pilar Palmrich	Maternal hemodynamics in hypertensive disorders of pregnancy – a human and animal model under anti-hypertensive therapy

No.	Call	Consortium	Name ECR	Abstract Title
49	JTC 2018	INNOVATION	Anne Bührke	Investigating long non-coding RNA-regulated pathways driving cardiac regeneration
50	JTC 2018	INNOVATION	Francisco Santos	Exploiting the role of long noncoding RNAs in the direct conversion of fibroblasts into functional cardiomyocytes
51	JTC 2018	MEND-AGE	Anne Babler	Identifying cellular and molecular mechanisms of vascular sclerosis development
52	JTC 2018	PICASSO	Ilaria Massaiu	Involvement of proprotein convertase subtilisin/kexin type 9 (PCSK9) in aortic valvular calcification

ACM-HF: Generation of iPSC from patients with Arrhythmogenic Cardiomyopathy and their differentiation into cardiomyocytes

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Arrhythmogenic cardiomyopathy (ACM) is a genetic disease characterised by fibro-fatty replacement of the myocardium, arrhythmias and sudden cardiac death. ACM inheritance is mainly autosomal dominant and often caused by mutations in genes encoding proteins of the desmosomes, with PKP2 being the most common causal gene.

Specific aim of our work was the generation of induced pluripotent stem cells (hiPSCs) from ACM patients and healthy donors and their differentiation into cardiomyocytes (hiPSC-CMs). hiPSCs were generated from one male and one female ACM patient carrying distinct PKP2 mutations by reprogramming of dermal fibroblasts using non-integrating methods (episomal plasmids and Sendai virus). hiPSCs from 2 healthy donors were also taken along, one control hiPSC line was already available in our laboratory and a new one was generated by reprogramming of cardiac stromal cells (C-MSCs) using the Sendai virus. All hiPSC lines differentiated efficiently into cardiomyocytes which expressed sarcomeric, ion channel, calcium handling and desmosomal genes at similar levels. PKP2 expression was instead reduced in ACM hiPSC-CMs compared to control.

Ongoing work includes correction of PKP2 mutations in the ACM hiPSC lines to generate isogenic pairs using CRISPR/Cas9 technology to distinguish disease-relevant phenotypes from background-related variations. Mutated and corrected hiPSC-CMs will be further characterised for ACM-phenotype based on apoptosis, necrosis, fat accumulation and BNP secretion. These hiPSC-CMs will be used for building a novel integrated human cellular model including primary C-MSCs and inflammatory cytokines (within the ACM-HF Consortium) to evaluate the interplay and the relative contribution of CMs and non-myocytes in ACM cardiac remodelling.

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ACM-HF: Characterisation of Cardiac Mesenchymal Stromal Cells form Arrhythmogenic Cardiomyopathy patients

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Arrhythmogenic Cardiomyopathy (ACM) is a genetic disease, characterised by progressive cardiomyocyte (CM) loss, fibro-adipose replacement and inflammation leading to heart failure and arrhythmias. It has been recognised that CM-non myocytes interplay underlies ACM-HF.

Within the study of this interplay, we aim to characterise Cardiac Mesenchymal Stromal Cells (C-MSCs), crucial contributors to ACM pathogenesis and thus potential treatment targets. 37 ACM patients have been recruited. Cardiac biopsies, skin biopsies and blood samples were collected. Clinical and genetic characteristics were stored in a dedicated database shared within the consortium. C-MSCs were obtained from right ventricle biopsies of enrolled patients and matched healthy controls (HC) and characterised for ACM phenotypes.

Firstly, we confirmed exaggerated lipogenesis and increased expression of adipogenic genes in ACM cells compared to HC, as previously described[1]. We detected higher levels of TGFβ in ACM patients' plasmas. Accordingly, C-MSCs were found more responsive to TGFβ treatment in the pro-fibrotic process. ACM C-MSCs revealed more prone both to apoptosis and necrosis. Accordingly they secreted more BNP than HC cells. Transcriptome analysis revealed enrichment in cell adhesion, cytokine and protein secretion and calcium-related factors. Moreover, we found 5 microRNAs regulated in ACM, for which validation is started. Methylome analysis is ongoing to understand if epigenetics plays a role in determining the transcriptional layout.

This characterisation will be instrumental for the experiments of co-culture with other cell types (cardiomyocytes and inflammatory cells). Moreover, the set-up has been completed for drug screening of a library of epigenetic modulators and a library of secreted factors.

This work was supported by a grant ERA-CVD Joint Transnational Call 2016 to ACM-HF consortium.

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ACM-HF: Characterisation of immune derived mediators from PBMC of Arrhythmogenic Cardiomyopathy patients

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Arrhythmogenic Cardiomyopathy (ACM) is an inherited disorder of the heart that causes sudden cardiac death and progressive heart failure (HF) in the young. Several evidences concerning the role of inflammation in the pathogenesis of ACM have been reported. Besides fibro-fatty replacement and myocyte degenerative changes, inflammatory patchy infiltrates could be found in myocardial histological analysis of ACM patients. Therefore, cells other than cardiomyocytes and stromal cell progenitors could participate to ACM pathogenesis and clinical onset, making a contribution to the alteration of cardiac microenvironment homeostasis. Notably, the potential contribution of inflammatory cells on cardiomyocyte (CM) death, development of fibro-fatty replacement and heart failure in ACM patients has not been studied in detail to date.

For this purpose, one arm of the "ACM-HF" European project is focused on inflammatory process in ACM, in order to characterise the inflammatory mediators involved in the cardiomyocyte – non myocyte interplay in ACM pathogenesis. To this end, peripheral blood mononuclear cells (PBMC) from ACM patients were phenotyped by flow cytometry and re-stimulated *in vitro* to assess leukocyte activation and secreted cytokines with specific focus on the activation state of monocyte and memory CD4 T cell subsets. Preliminary results show that ACM patients have higher effector memory CCR4+ CD4 T cells and higher percentages of memory ICOS+ CD25+ FOXP3+ regulatory CD4 T cells. Concerning monocytes, we observed a significant induction of CCR2 expression by non-classical monocytes in ACM patients whereas frequency of the different subsets was not modified.

Identification of the secreted factors enriched in memory CD4 T cells and monocytes from ACM patients is an essential step to develop an innovative patient-derived model combining **C**Ms from induced pluripotent stem cells, **S**tromal cells and **I**nflammation (**CSI** model) to study the cellular interplay in ACM pathogenesis and screen potential therapeutics.

ACM-HF: Impairment of myocardial perfusion correlates with heart failure progression in patients with noncompaction cardiomyopathy

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Introduction: Non-compaction cardiomyopathy (NCC) is a congenital heart disease characterised by an arrest of the myocardial compaction process. Although NCC patients have impaired formation of microvasculature, the functional impact of these changes remains undefined.

Methods: We sought to analyse a potential correlation between myocardial ischemia and heart failure progression in NCC patients. We enrolled 41 (28 male, 13 female) patients. Echocardiography determined left ventricular end diastolic volume (EDV), ejection fraction (LVEF), with global longitudinal strain (GLS) post-processing. Serum levels of NT-proBNP have been measured. Myocardial SPECT at rest and on stress defined significant myocardial ischemia as summed difference score (SDS)≥2.

Results: Myocardial ischemia has been shown in 11 patients (27%, Group A), 30 patients (73%) showed no significant ischemic changes (Group B). Groups did not differ in sex, age, serum creatinine or bilirubine levels. When compared to Group B, Group A had significantly lower LVEF (35±15% vs. 53±11% in Group B, P<0.001), higher EDV (188±52mL vs. 136±52mL, P=0.007), lower GLS (-9.9±5.2% vs. -14.5±4.1%, P=0.001) and lower NT-proBNP levels (1691±1883pg/mL vs. 422±877pg/mL in Group B, P=0.006). Overall, higher SDS was associated with lower LVEF (r=-0.48, P=0.001), higher EDV (r=0.39, P<0.012) and higher levels of NT-proBNP (r=0.66, P<0.001).

Conclusions: In patients with NCC, the presence of myocardial ischemia is associated with worse left ventricular function, dilation of left ventricle and more pronounced neurohumoral activation. Further studies are needed to investigate whether treatment approaches targeting myocardial ischemia, such as CD34+ cell therapy, may halt the progression of disease in this patient cohort.

CARDIO-PRO: Single-cell analysis uncovers that metabolic reprogramming is essential for cardiomyocyte proliferation in the regenerating heart

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Opposite to mammals, zebrafish have a remarkable capacity to regenerate the heart. They do so by cell cycle re-entry of cardiomyocytes located next to the injury area, also called the borderzone. These cardiomyocytes then migrate into the injury and restore cardiac function. Mechanisms responsible for cardiomyocyte proliferation in zebrafish hold great potential to boost cardiac regeneration in the mammalian situation.

To identify some of these mechanisms, we made use of a transgenic nppa:mCitrine line which marks cardiomyocytes in the borderzone. We FACS sorted nppa:mCitrine high and low cells and performed single cell RNA-sequencing on zebrafish cardiomyocytes 7 days post injury. This allowed us to identify two main clusters of cardiomyocytes: a first cluster of differentiated, adult cardiomyocytes; and a second cluster of dedifferentiated, immature borderzone cardiomyocytes. Pseudotime analysis on these clusters indicated that cardiomyocytes shut down mitochondrial gene expression and subsequently upregulate glycolytic genes during dedifferentiation. Further studies confirmed a glycolytic switch in borderzone cardiomyocytes. More importantly, we found that blocking this glycolytic switch by 2DG impaired proliferation of cardiomyocytes in the borderzone. Mechanistically we find that Nrg1/ErbB2 signals upstream of this glycolytic switch in zebrafish and mouse cardiomyocytes. Also, pharmacologic inhibition of glycolysis impairs Nrg1/ErbB2 induced proliferation in cultured neonatal mouse cardiomyocytes.

Altogether our data point to a general mechanism in which cardiomyocytes make a glycolytic switch during proliferation. This switch is essential for both Nrg1/Erbb2 induced proliferation and physiological heart regeneration in zebrafish. Further studies into this metabolic reprogramming hold great potential to help boost mammalian heart regeneration.

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CARDIO-PRO: Dissecting the molecular mechanisms of BMP signalling function in heart regeneration

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In contrast to adult mammals, zebrafish display a remarkable capability to regenerate the heart in response to various insults, namely cryoinjury, ventricular resection and genetic ablation of cardiomyocytes. In zebrafish, cardiac muscle regeneration occurs by proliferation of pre-existing cardiomyocytes (CMs) located at the wound border, providing a robust hyperplastic response to regenerate the damaged area.

Recently, in our laboratory the BMP (Bone Morphogenetic Protein) signalling has been identified as an essential regulator of zebrafish cardiomyocyte regeneration. BMP/SMAD signaling is upregulated in border zone CMs as an injury-induced specific signal. Its activation triggers CM de-differentiation and cell cycle re-entry, sustaining myocardial regeneration.

In order to identify the BMP ligands responsible for the pro-regenerative BMP signalling activity in heart regeneration, we are currently investigating the function of Bmp2b, Bmp4 and Bmp7a. In SHAM operated hearts, the expression of all three genes is barely detectable. At 7 days post-injury, *bmp2b* and *bmp7a* are slightly upregulated in CMs, while *bmp4* is more strongly induced. Interestingly, while in *bmp2b* or *bmp4* mutants CM proliferation is not affected during zebrafish heart regeneration, *bmp7a* mutants show reduced CM proliferation.

Therefore, our preliminary data suggest that Bmp7a might represent one of the prominent BMP ligands responsible for the BMP signaling role in zebrafish myocardial regeneration. Intriguingly, while the BMP signaling positively regulates zebrafish heart regeneration, *in vivo* down-regulation of BMP signaling in adult mice has been shown to reduce scar size and improve functional recovery after myocardial infarction. Therefore, we have also initiated experiments aimed at elucidating how the activity of this pathway is differentially regulated between fish and mammals, and what role it plays during neonatal mouse heart regeneration.

Cardio-oncology: Design of PKR1 agonists – new agents for treating cardiotoxicity

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Anticancer chemotherapeutics cause cardiotoxicity and heart failure with significant morbidity and mortality. Prokineticin is an angiogenic hormone that exerts biological effects via two trans-membrane G-protein coupled receptors: namely PKR1 and PKR2. PKR1 is an important target to activate cardio-protective signals in cardiac cells. PKR1 gene therapy [1] or PKR1 agonist [2] leads to angiogenesis, prevents apoptosis in cardiomyocytes, and mobilises cardiac progenitor cells, thereby protecting the heart against heart failure in mice models.

In this study, we performed a molecular design of potent PKR1 agonists based on a homology model and a set of IS20 compounds by utilising molecular docking and QSAR modeling [3].

To improve virtual docking scoring we implemented a convolution neural network model and then applied it to select the most promising structures during de-novo design by a special generative Recurrent Neural Network model.

As a result, we found promising compounds with two different scaffolds for which their biological activity was confirmed in experimental studies. We also clarified the possible binding pose of small PKR1 agonist IS20 by performing molecular dynamics (10 ns) simulations on a system comprised of a solvated cell membrane, the protein embedded, and the ligand. This protocol allowed us to calculate the enthalpy of binding and propose new modifications of ligands with better activity to be further investigated.

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CLARIFY: Characterisation of intercellular communication in ischemic heart disease by single-cell RNA sequencing

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Ischemic heart disease (IHD) is a leading cause of death worldwide, initiated by insufficient blood supply to the myocardium resulting in ischemic injury. This injury results in substantial cardiomyocytes death, followed by replacement by non-contractile fibrotic scar tissue and ultimately heart failure. The healing phase between the initial ischemic insult and the fully healed scar is characterised by a series of dynamic processes that involve multiple cell types including cardiomyocytes, fibroblasts and different inflammatory cells. At the damaged myocardium, cell type composition and their respective functions change over time. Correct temporal regulation of the activity and function of these cell types is crucial, as their deregulation exacerbates wound formation and loss of cardiac function following the ischemic insult. This suggests extensive intercellular communication between the cell types within the myocardium to coordinate and synchronise their function relative to each other during the healing phase.

Here, we use single-cell transcriptomics to elucidate intercellular communication between cardiomyocytes, inflammatory cells, endothelial cells and fibroblasts at different stages during the healing phase following ischemic injury. Our data suggests extensive communication between various cell types by endocrine and paracrine signalling, potentially allowing one cell type to regulate activity and function of other cell types over time. Focusing on cardiomyocytes, we identified a set of ligands highly expressed after ischemic injury.

Further analysis revealed receptor expression for these ligands in various cell types, suggesting a regulatory role of injured cardiomyocytes on the activity of these cell types. Current studies on the function of these ligands are ongoing. We hope that this study will contribute towards a better understanding of intercellular communication, and thus regulation, of the various cardiac cell types involved in healing of ischemic injuries.

MINOTAUR: Spermidine improves diastolic heart failure in rats with cardiometabolic syndrome

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Introduction: Due to an ever-increasing prevalence of metabolic syndrome and population ageing, diastolic heart failure (DHF) has been a rapidly growing disease, which so far lacks life-saving therapies. In this regard, caloric restriction has emerged as a promising intervention; however, it has not been adopted as a standard medical practice due to potential safety concerns and poor compliance. Therefore, we examined the cardiometabolic effects of the caloric restriction mimetic spermidine in ZSF1 obese rats, a clinically relevant model of DHF induced by metabolic syndrome, thereby considering both genders. We also quantified naturally occurring spermidine levels in human failing myocardia.

Results: ZSF1 obese rats developed obesity, dyslipidemia, diabetes, hypertension and DHF phenotype as compared to ZSF1 leans. However, spermidine-treated ZSF1 obese rats displayed attenuated hypertrophy and improved diastolic function as assessed by echocardiography and hemodynamics, respectively. In line with this, spermidine-fed rats had reduced lung congestion and tended to have higher exercise tolerance than controls. Spermidine also exerted a small, but significant, anti-hypertensive effect, which was mediated by improved vascular endothelial function as measured ex vivo in isolated aortic rings. Of note, spermidine had no effect on food/water consumption, body weight, glucose homeostasis or systemic inflammation, suggesting a cardiovascular-specific effect. Strikingly, we found spermidine levels to be higher in human failing hearts than in non-failing biopsies, implying a compensatory role of endogenous spermidine in HF.

Conclusion: Administration of exogenous spermidine protects from metabolic syndrome-driven DHF in both genders, at least in preclinical testing, and holds a great promise for DHF patients.

MINOTAUR: Therapeutic effect of the caloric restriction mimetic spermidine on diastolic heart failure: the contribution of titin oxidative posttranslational modifications

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Titin is an important contributor to the passive elasticity of the myocardium. The mechanical properties of titin are determined by unfolding and refolding equilibria of its immunoglobulin-like (Ig) domains. Recent studies have shown that redox post-translational modifications (PTMs) of cysteines located in these domains modulate titin elasticity: disulfide bonds exert a stiffening effect, whereas S-glutathionylation leads to protein softening. Thus, an imbalanced redox status could contribute to the pathological effects and changes in elasticity associated with heart failure.

Here we describe a method for in-gel determination of reversible redox PTMs of titin, based on fluorescent labelling of oxidised thiols with monobromobimane. Using this method, we detected a trend towards increased titin oxidation in human failing hearts. We will also apply our method to myocardial samples of ZSF1 obese rats, a clinically relevant model of cardiometabolic syndrome, which were protected from the development of heart failure by spermidine, a natural caloric restriction mimetic (CRM). The compatibility of our method with mass spectrometry techniques will allow to identify and localize the specific redox PTMs targeting titin, which could shed light on the mechanisms underlying the beneficial effects of spermidine and possibly other CRMs.

MINOTAUR: Unbiased screen of the cardiac proteome and effect of metabolic therapy on titin properties in a rat model of heart failure with preserved ejection fraction (HFpEF)

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Background: Since consumption of polyamines lowers cardiovascular risk, spermidine may have beneficial effects on the hearts of ZSF1 obese rats, a metabolic syndrome-induced HFpEF model.

Objective: To test whether suggested pathomechanistic pathways of HFpEF are altered in hearts of ZSF1 obese rats, and whether proposed regulators of titin phosphorylation/stiffness in HFpEF are affected by spermidine treatment.

Results: Gene ontology enrichment analysis of the cardiac proteome, phosphoproteome and acetylome of obese vs. ZSF1 lean rats by quantitative mass spectrometry revealed proteins associated with metabolic terms to be regulated in male ZSF1 obese rats, whereas postulated key features of HFpEF, such as ‘inflammation’ and ‘oxidative stress’, were not altered. Immunoblot-based quantitation of expression/activity of titin-targeting kinases (ERK1/2, PKG, PKCα) and phosphatase PP5 demonstrated reduced PP5 expression in male spermidine-treated vs. non-treated ZSF1 obese hearts. Kinase properties, total-titin, and site-specific titin phosphorylation were unaltered.

Conclusions: HFpEF pathophysiology and preventive effects of spermidine treatment may relate to metabolic alterations independent of inflammation and oxidative stress.

DETECTIN-HF: Identifying dilated cardiomyopathy-affected individuals in a population-based biobank based on high-coverage sequencing data and electronic health records

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Population-based biobank-contained resources together with electronic health records (EHR) provide a platform for assessing the genetic and phenotypic characteristics of individuals affected by dilated cardiomyopathy (DCM).

Two different approaches were used at the Estonian Biobank (n=52,000) to specify disease-specific data in these individuals: a phenotype-based approach (PA) by identifying individuals with a DCM diagnosis in EHR for ascertaining carrier status in sequencing data (n=4776), and a genomics-guided approach (GA) by analysing rare (minor allele frequency <0.5%) genetic variants in 144 DCM-related genes in sequencing data for assessing phenotypic information in EHR. PA revealed 274 individuals (60% male) who carried a DCM diagnosis, of whom 22 harbour a deleterious genetic variant in a DCM-related gene. GA unveiled 658 genetic variants linked with cardiomyopathy-related phenotypes in ClinVar in 1096 carriers (53% male), of whom 4% have a cardiomyopathy diagnosis in EHR. Additionally, 155 loss-of-function variants in 261 individuals with 4% carrying a cardiomyopathy diagnosis in EHR were ascertained. Of 1324 and 492 carriers, harbouring a genetic variant previously not associated with cardiomyopathy-related phenotypes in ClinVar or that are completely novel, respectively, 6% and 4% carry a cardiomyopathy diagnosis.

Preliminary results highlight that i) association of genetic variants with DCM is not straight-forward as variants predicted deleterious according to in silico prediction contain additionally those that are not expressed fully or are not causal for the disease, and ii) low EHR-based yield of clinical DCM diagnosis among individuals harbouring deleterious variants implies both under-diagnosis and more complex genetic underpinnings of the disease.

DETECTIN-HF: Genetic testing in Polish dilated cardiomyopathy patients as part of the DETECTIN-HF consortium

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Purposes: To extend the number of dilated cardiomyopathy patients with causative gene mutations, to implement genetic data in the risk model and for validation of other biomarker candidates.

Methods: Next generation sequencing of 174 genes in 78 patients with dilated cardiomyopathy was performed on Illumina MiSeqDx using TruSight Cardio gene panel. In addition to the 174 gene panel whole exome sequencing (WES) was performed in 12 patients using Nextera Rapid Capture Kit and Illumina HiSeq 1500 platform. Variants were classified according to ACMG criteria. Pathogenic/likely pathogenic and some selected variants of uncertain significance were verified by Sanger sequencing in probands and their relatives. *De novo* status of variant was confirmed by paternity and maternity STR genotyping.

Research is a part of Work Package 2 "Molecular targets for disease detection and risk stratification" in DETECTIN-HF project (Determining the role of clinical and epi-genetic risk markers in dilated cardiomyopathies and heart failure).

Results: Pathogenic and likely pathogenic variants in DES, DSP, FLNC, LMNA, MYH7, RBM20, RYR2, TBX20, TNNC1, TNNT2, TNN were found in 22/78 probands (28.2%). Truncating variants in TTN were most common and detected in 11 probands (14.1%). In one proband two likely pathogenic variants were found in TTN and TNNT2. The only pathogenic variant found by WES was truncating FLNC variant NM_001458.4:c.626G>A:p.Trp209Ter detected in one family.

Conclusions: By using commercially available gene panels majority of pathogenic and likely pathogenic variants can be found. Similar as in other populations TTN truncating variants are frequently found in Polish dilated cardiomyopathy patients. Genetic data can be incorporated inthe risk model and used for validation of other biomarkers as candidates for predictors of disease progression. WES can be used for unsolved dilated cardiomyopathy cases.

EXPERT: Dichotomy between the transcriptomic landscape of naturally versus accelerated aged murine hearts

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Objective. Cardiac ageing is a complex and poorly understood phenomenon; trying to shed light on the possible contribution of genomic changes to senescence, we investigated the transcriptome of the murine ageing myocardium. Genome-wide total RNA-sequencing and variant calling was performed on the transcriptome of 1) naturally aged mice; 2) mice with cardiac-specific deficiency of Ercc1, part of the DNA repair machinery; 3) mice with reduced mitochondrial antioxidant capacity; 4) Tert-deficient mice with shorter telomeres and 5) a mouse model of human Hutchinson-Gilford-progeria-syndrome (HGPS).

Methods and Results. Our results demonstrate no dramatic changes in the transcriptomes of naturally senescent murine hearts until two years of age, in contrast to the ones of the accelerated ageing models, which display mostly model-specific alterations in gene expression for protein-coding, long non-coding RNA and circular RNA genes. Genomic instability, the unresolved accumulation of DNA variants, is hypothesised as one of the causes of ageing; our results demonstrated no enrichment in variants in naturally ageing murine hearts, hearts from the HGPS model or Tert-deficient mice. Conversely, a dramatic accumulation of variants was evident in Ercc1 heart-specific knockout mice and in mice with reduced mitochondrial antioxidant capacity.

Conclusions. Our data demonstrate limited overlap in the transcriptomic landscape of all murine ageing models included in this study and question their reliability as models for research on human cardiovascular disease in senescence. Moreover, our data question the contribution of genomic instability to natural ageing of the mouse heart, supporting the contention that the endogenous DNA repair machinery remains active in cardiac cells throughout life.

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EXPERT: Role of microRNAs in cardiac alterations in Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare premature ageing disorder caused by a point mutation in the *LMNA* gene coding for A-type lamins, important functional and structural nuclear proteins. The most clinically relevant feature of HGPS is premature death at an average age of 14.6 years caused by accelerated cardiovascular disease (CVD). MicroRNAs are involved in many pathophysiological processes, including ageing and CVD, however their role in HGPS and the associated cardiovascular alterations remains largely unexplored. Our objective is to identify microRNAs that contribute to the pathogenesis of HGPS. To this end, we use progeroid *Apoe-/-LmnaG609G/G609G* mice, which recapitulate most symptoms of the human disease, including accelerated atherosclerosis and cardiac alterations. We have isolated microRNAs and mRNAs from cardiac tissue of *Apoe-/-LmnaG609G/G609G* (HGPS) and *Apoe-/-Lmna+/+* (control) mice at 8 weeks and 16 weeks of age to examine transcriptome alterations before and after the clinical manifestation of the disease, respectively.

We detected 8 and 135 microRNAs and 1947 and 9231 mRNAs differentially expressed when comparing control and HGPS hearts from 8-week-old and 16-week-old mice, respectively. An integrated analysis of differentially regulated microRNAs and mRNAs identified different biological processes potentially controlled by altered microRNAs. Changes in transcription, translation and splicing in HGPS could be controlled by downregulated microRNAs, whereas actin cytoskeleton, cell-cell and cell-matrix adhesion, protein glycosylation and phosphorylation could be controlled by upregulated microRNAs. We are currently testing RNA-based therapeutic strategies to target altered biological process that might provoke premature CVD and ageing in progeroid mice.

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EXPERT: The novel long non-coding RNA Chheaf-1 regulates early-phase cardiac hypertrophy

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Long non-coding RNAs (lncRNA) are critical regulators of gene expression in several cellular processes. Studies of cardiac-enriched lncRNAs revealed that they are functionally implicated in cardiac pathophysiology and constitute potential therapeutic targets. Since the molecular and epigenetic mechanisms regulating cardiac homeostasis and disease are still not fully understood, elucidating the regulation of lncRNAs is critical in understanding the basis of cardiac disease.

Using RNA-sequencing analysis, we identified a lncRNA enriched in hypertrophic cardiomyocytes: Cardiac Hypertrophy and Heart failure-1 (Chheaf-1). *In vivo* Chheaf-1 silencing improved cardiac functional parameters (%FS and %EF) and reduced hypertrophic gene expression in TAC (transverse aortic constriction) mice in the early hypertrophic phase (1 and 2 weeks post-TAC). Furthermore, GapmeR treatment showed a therapeutic effect in hypertrophic mice, during the late stages of hypertrophy. RNA-seq data from total left ventricle of TAC mice, treated with GapmeR, 1 week after surgery revealed that the GapmeR induces the downregulation of several pathways that are upregulated during hypertrophy. Several of these modulated genes have binding sites for specific cardiac transcription factors such as GATA4, NKX2.5 and MEF2C. Through RNA immunoprecitipation, we found that Chheaf-1 binds GATA4, and chromatin immunoprecipitation experiments revealed that the silencing of Chheaf-1 reduced GATA4 and H3K27Ac on the promoters of hypertrophic genes (Myh7 and NppA), thus promoting inhibition.

Therefore, Chheaf-1 appears to play a critical role in the regulation of the epigenetic landscape of hypertrophic genes, and blocking Chheaf-1 through GapmeR technology could be a potential novel therapeutic avenue in treatment of cardiac hypertrophy and failure.

EXPERT: Influence of ageing on the quality and dynamics of mouse cardiac fibroblasts in culture

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Ageing affects cardiac function in multiple manners. The detrimental effects are initially manifested sub-clinically, at the molecular and cellular levels, and probably affect all cardiac cell types.

Many studies focused on changes of CMC during the ageing process, however, there is not much information about the phenotypic and molecular changes of the cardiac fibroblasts (cFb) in naturally ageing individuals.

Here we analysed several phenotypic and molecular features of heart-derived cFb in culture. Comparative analysis of cells isolated from young (2 mo-old) and old (16-mo old) mice revealed different kinetics of cells with passaging, as well as different metabolic potential. Furthermore, changes in cellular oxidative stress, mitochondrial function and expression of extracellular matrix components (including collagens and fibronectin), alpha-SMA and TGFb have been noticed in cFb derived from aged hearts, as compared to young hearts.

Taken together, our data showed that cFb culture is affected by natural ageing of the organism they are derived from, through important changes both in the quality and dynamics of cells and in cellular molecules, including cytoskeletal proteins and inflammatory cytokines.

Deciphering the mechanisms of ageing at cellular level in the heart may help developing strategies to attenuate or even reverse the cardiac ageing process.

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Fat4Heart: Metabolic regulation of heart failure caused by imbalanced mitochondrial dynamics

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Background: Dilated Cardiomyopathy (DCM) is characterised by an expansion of the left ventricle having as a consequence inefficient oxygenated blood pumping to the body. DCM can result in heart failure and is the most common cause of heart transplantation. The healthy adult heart relies mainly on free fatty acids as its main fuel source and rather than glucose but in the failing heart, a metabolic switch away from fatty acid oxidation and towards glucose uptake has been observed and has been proposed to represent an adaptive bioenergetic mechanism. We developed a mouse model of DCM caused my mitochondrial fragmentation in cardiomyocytes due to the deletion of the mitochondrial protease YME1L. This mouse model recapitulates all the clinical features of DCM as well as the metabolic features, such as cardiac glucose overload, progressing into chronic heart failure and middle-aged death.

Aim: Characterise bioenergetically the cYKO mouse model in order to understand in early stages the molecular triggering mechanism for heart failure. We will focus on the preference of the cardiomyocyte for carbohydrates over fatty acids at the enzymatic level of the complexes of the Oxidative Phosphorilation to try to understand the link between mitochondria respiration and the cell energetic substrates.

Conclusion and perspectives: Our studies will allow us to determine the biochemical role of the different fuel sources during the development of heart failure.

Fat4Heart: Metabolic flexibility of the failing myocardium through high fat diet

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Background: Dilated cardiomyopathy (DCM) is a leading cause of heart failure (HF) without specific therapies. Imbalanced mitochondrial dynamics, driven by the incorrect OPA1 processing by knocking-out the protease YME1L (cYKO mice), has been shown to induce DCM in mice from 20 weeks of age onwards. High fat diet (HFD) has been shown to prevent the development of DCM in cYKO mice. Here we study the effect of different fat-diets to better understand why HFD had a positive effect. We further evaluated if HFD was able to prevent DCM onset in a large animal model.

Methods and results: cYKO mice were randomly assigned to low-fat (LFD>10%), mid-fat (MFD: 30%), high-fat (HFD>30%), or chow diets at 10 weeks of age. Only HFD was associated with a better left ventricular ejection fraction (LVEF; %) at 30 weeks: 37.9±8.5 vs. 29.2±8.9 in control diet, p=0.04. This functional improvement was associated with an increase in fatty acids uptake on microPET/CT. Mitochondrial ultrastructure analysis on electron microscopy in animals subjected to HFD revealed an increase in small mitochondria despite no change in the total number or average mitochondria size. In a pig model of DCM, preliminary results show an impressive increase in LVEF (as assessed by magnetic resonance imaging) from 35.8±9.5 to 47.8±8.5% (p=0.0008) after 2 months of HFD.

Conclusion: HFD is the only diet that prevents HF development. cYKO mice fed with HFD reverse the metabolic reprogramming restoring preferential fatty acid oxidation. HFD rescues from HF in a non-genetic large animal model of DCM.

Fat4Heart: Dietary restriction prevents dilated cardiomyopathy progression by enhancing mitophagy

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Background: Dilated cardiomyopathy (DCM) is a leading cause of heart failure, with insufficient therapeutic approaches. Mitochondria quality control (MQC) mechanisms are essential for maintaining mitochondria fitness and prevent cardiomyocyte dysfunction. By ablating the mitochondria protease YME1L gene, we generated a mouse model (cYKO) with cardiomyocyte-specific fragmented mitochondria that recapitulates DCM features from 20 weeks of age. We speculated that metabolic interventions resulting in enhanced MQC could alleviate DCM. Here, we studied cardiac function trajectories in our mouse model of DCM fed with regular chow or under dietary restriction.

Methods and results: cYKO mice were randomly assigned to non-fat diet (<0.5% crude fat), or chow diet (10% crude fat) before the onset of DCM at 10 weeks of age. Cardiac function was measured every 10 weeks by echocardiography. 20 weeks of non-fat diet was associated with a significantly better left ventricular ejection fraction as well as significantly smaller ventricular diameters. Lifespan of mice under non-fat diet was significantly prolonged by 35% (67 vs 44 weeks). Clearance of damaged mitochondria by the PINK1/PARKIN pathway was observed together with an increase in autophagy related proteins. On electron microscopy, a significant reduction in the number of fragmented mitochondria and an increase in mean mitochondria size was observed in animals fed with non-fat diet.

Conclusion: In a mouse model of fragmented mitochondria, dietary restriction increases MQC, which results in protection against the onset of DCM, and ultimately prolonged lifespan. Nutritional approaches might represent a new therapeutic option to increase MQC and preserve cardiomyocyte homeostasis.

LIPCAR-HF: The impact of glucocorticoids on Neuregulin-1/ERBB2 signalling in cardiomyocyte proliferation and heart regeneration

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Myocardial infarction induces massive loss of cardiomyocytes, which are mainly replaced by fibrotic tissue. Stimulating cardiomyocyte dedifferentiation and proliferation by administration of the growth factor Neuregulin 1 (NRG1) is a promising strategy for cardiac repair. However, our findings in mice suggest that NRG1 mitogenic activity is compromised in the adult mammalian heart by low amount of its coreceptor ERBB2. In a variety of tissues and diseases, the abundance and/or activity of ERBB receptors are modulated by glucocorticoids (GCs).

This study aims at investigating the impact of GCs on NRG1/ERBB2 signalling in the context of cardiomyocyte proliferation and cardiac regeneration.

Our data in mice show that cardiac expression levels of the glucocorticoid receptor (GR) increase post-natal, in opposite trend to that of ERBB2. Furthermore, we observed in neonatal cardiomyocytes that GR activation by GCs restrains NRG1/ERBB2 signalling axis by reducing ERBB2 abundance and by upregulating negative ERBB signalling modulators.

Consistent with the ability of GCs in intercepting NRG1/ERBB2 signalling, GCs administration robustly inhibited cardiomyocyte cell cycle re-entry, DNA synthesis and cytokinesis induced by NRG1 administration or overexpression of a constitutively active ERBB2 isoform. In contrast, interfering with GR activation increased cardiomyocyte proliferation.

Our data support a physiological role for GCs/GR axis in restraining NRG1/ERBB2 signalling after birth, concurring to the post-natal cardiomyocyte differentiation and cell cycle exit. We suggest that the modulation of GCs/GR axis may boost NRG1-based therapies for heart regeneration.

LIPCAR-HF: Post-resuscitation treatment with inhaled argon improves outcome after a prolonged untreated cardiac arrest in a porcine model

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Treatment of post cardiac arrest (CA) syndrome represents a clinical priority. Argon represents an attractive therapeutic option. The purpose of this study was to determine efficacy, safety and the most effective dose of Argon in a porcine model of high-severity CA and cardiopulmonary resuscitation (CPR).

Left anterior descending coronary artery was occluded in 36 pigs, and ventricular fibrillation was induced and left untreated for 12 min. CPR was then performed for 5 min prior to defibrillation. Following successful resuscitation, animals were subjected to 4 hr ventilation with (a) 70% argon - 30% O_2 (n=10); (b) 50% argon - 50% O_2 (n=10); (c) 70% N_2 - 30% O_2 (n=10). Hemodynamic parameters were monitored and serial blood samples were obtained for blood gas analysis. Animals were observed up to 96 h for assessment of survival and neurological recovery.

Ten animals of 12 were successfully resuscitated in each group. After resuscitation, animals treated with Argon 70% showed higher systolic, mean and diastolic arterial pressure compared to controls and to the Argon 50% treated animals ($p<0.05$). Ventilation with argon did not have any detrimental effects on respiratory gas exchange during the 4 hr ventilation. There was no statistically significant difference in the number of the resuscitated animals survived up to 96 hrs. However, 6 (60%) and 8 (80%) resuscitated animals in the Argon 50% and Argon 70% groups respectively achieved a complete neurological recovery (Overall Performance Categories, OPC=1 or 2), whereas only 3 (30%) animals survived up to 96 hours with a complete neurological recovery in the control group ($p<0.0001$).

MacroERA: MicroRNA-21 in cardiac resident macrophages regulates pressure overload-induced cardiac remodeling

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Resident macrophages, in contrast to tissue-invading monocytes, are a poorly understood type of immune cells that colonizes the different tissues of the body during embryonic development and persists throughout adulthood by self-renewal. Recently, this cell type was also found in myocardium, yet the function and regulation of these cardiac resident macrophages (crMPs) remained largely enigmatic. Small non-coding RNAs, namely microRNAs (miRs), are important regulators of gene expression, but till date have not been studied in crMPs.

Small RNA library sequencing identified miR-21 as the most abundant miR in this cell type. Cardiac remodelling - induced by left ventricular pressure overload - led to a further upregulation of miR-21 in crMPs. To assess the role of miR-21 in crMPs *in vivo,* we generated crMP-specific miR-21 KO mice by crossing miR-21 floxed mice with a mouse line expressing Cre recombinase driven by the Cx3cr1-promotor. When tested in a model for chronic left ventricular pressure overload, mice deficient in crMP miR-21 displayed significantly reduced cardiac hypertrophy, myocardial fibrosis and preserved LV function.

RNA sequencing of isolated crMPs revealed a decrease in fibrosis- and inflammation-related genes in KO-mice compared to WT-littermate controls.

In summary, we identified miR-21 as the single highest expressed miR in crMPs. Genetic deletion of miR-21 in these cells prevented the adverse effects of pressure overload with regard to myocardial remodeling and cardiac dysfunction. These results suggest a critical role for cardiac resident macrophages in myocardial remodeling and assign miR-21 a key regulatory role in these cells.

MacroERA: Cardiomyocyte-derived exosomal miR-200c favours a shift towards pro- inflammatory M1 cardiac macrophages and accelerates heart failure

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Although macrophages are the largest fraction of immune cells in native mammalian myocardium, their functional contribution to this organ is insufficiently understood. Resident cardiac macrophages can polarise towards an M1 or M2 phenotype and are hypothesised to contribute to cardiac inflammation. MicroRNAs (miRs) are small non-coding RNA regulators of gene expression that contribute to a variety of cardio-vascular diseases and can be released into extracellular vesicles.

Here, we investigated whether miRNAs in exosomes, secreted by cardiac cells, can act as intercellular signals for resident macrophages to shift towards M1/M2 phenotypes, influencing macrophage gene expression and altering their inflammatory function in the heart.

We detected an enrichment of miR-200c-3p in exosomes released by cardiomyocytes, under stress conditions. In addition, THP-1 cells, a human monocytic cell line, when differentiated towards macrophages displayed a profound induction of M1 markers and impaired efficiency to polarize towards M2 phenotype, when transfected with miR-200c-3p precursor molecules. 2D-coculture experiments with cardiomyocytes and macrophages revealed efficient uptake by macrophages of miR-200c-3p-enriched exosomes secreted by cardiomyocytes and subsequent induction of selected M1 markers.

Finally, in a mouse model of cardiac pressure overload, inhibition of miR-200c-3p in non-myocytes, normalised contractile cardiac function and decreased fibrosis.

Our data demonstrates a direct communication line between cardiomyocytes and macrophages in the heart, by means of miR-200c in secreted exosomes. This direct transfer of miR-200c-3p impacts gene expression, polarisation and function of (resident) cardiac macrophages, highlighting inflammation as a new promising therapeutic tool.

MacroERA: MiR-223-3p inhibition modulates cardiac inflammation in Viral Myocarditis

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Heart failure (HF) is one of the largest contributors to disease burden and healthcare expenditure worldwide and despite significant progress, mortality after diagnosis is still high.We previously studied the importance of non-coding RNAs (1), such as miRNAs, involved in HF and inflammation (2) and detected a significant up-regulation of miR-223-3p in the acute phase of murine Coxsackie Virus B3 (CVB3)-induced Viral Myocarditis (3). VM is a form of cardiac inflammation caused by viruses that in case of an altered immune response provokes chronic cardiac damage, progressing over time to HF. In this project, we investigated the possible therapeutic effect of miR223-3p inhibition and its molecular mechanisms involved in the development of diastolic dysfunction, using VM as our initiating insult.

In vitro, miR-223-3p inhibition prompted an anti-inflammatory response in cultured macrophages at baseline and after IFNγ stimulation, confirmed by reduction in IFNγ-induced transcripts levels of pro-inflammatory markers ICAM and TNFα. *In vivo,* miR-223-3p silencing translated in a reduced cardiac necrosis and immune cells recruitment at four and seven days post infection without affecting viral load. Surprisingly, miR-223-3p inhibition was associated with decreased circulating and cardiac neutrophils.

Our findings suggest that silencing miR-223-3p can have a therapeutic effect on cardiac inflammation. We will further confirm this by analysing its complete immune modulation *in vivo*. Since we observed an association between miR-223-3p inhibition and neutrophils function, we plan to assess further the role of neutrophils in the development of VM-induced diastolic dysfunction and the effects of their absence in the pathophysiology.

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PDE4HEART: Cardiac gene therapy of heart failure with phosphodiesterase PDE4B in mice

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Chronic β-AR activation is detrimental because it promotes cardiac remodeling and ultimately leads to heart failure (HF). Multiple cyclic nucleotide phosphodiesterases (PDEs) finely tune β-AR responses by degrading and compartmentalising cAMP. Since chronic treatment with PDE inhibitors increases mortality in HF, we postulated that decreasing cAMP levels by overexpressing PDE4B in the heart may have therapeutic effects.

To test this hypothesis, we explored whether AAV9-mediated cardiac overexpression of PDE4B (1012 viral particles) could prevent maladaptive hypertrophy in mice subjected either to transverse aortic constriction (TAC) for 6 weeks or 2 weeks isoproterenol (Iso) and phenylephrine (Phe) infusion (30µg/g/day each). Cardiac function was assessed by echocardiography. In control mice injected with a Luciferase-AAV9 (LUC), TAC decreased ejection fraction (EF, -34.2±6%, N=6, $p<0.0001$), increased left ventricular weight/body weight ratio (LVW/BW, +86.7±15.7%, N=6, $p<0.0001$). Although PDE4B overexpression did not ameliorate EF, it delayed the decrease of EF at 2 weeks (N=9, $p<0.05$), prevented LV hypertrophy (N=9, $p<0.001$) and fibrosis (N=9, $p<0.05$). Chronic Iso and Phe treatment induced LV hypertrophy (LVW/BW, +37.8±6.2%, N=7, $p<0.001$) and decreased EF (-25±3.5%, N=7, $p<0.0001$).

While PDE4B overexpression only slightly ameliorated EF (-17±3%, N=9) in this model, LV hypertrophy was significantly diminished (N=9, $p<0.05$). PDE4B protein level was assessed by western blotting and revealed a ~6 to 9-fold increased expression in both HF models. Altogether, our preliminary results suggest that gene therapy with PDE4B is a potential therapeutic approach for cardiac maladaptive hypertrophy.

PDE4HEART: Gene therapy with phosphodiesterase 4B in a murine model of pressure overload-induced heart failure

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Background: In heart failure (HF), chronic activation of the β-adrenergic receptor signaling results in increased production of the second messenger 3′,5′-cyclic adenosine monophosphate (cAMP) to compensate the reduced cardiac function. The protein levels of specific hydrolysing enzymes called phosphodiesterases (PDEs) alter dramatically and their activity to generate functionally relevant cAMP microdomains in the cardiomyocyte is downregulated leading to disease-associated alterations in cAMP compartmentation.

Purpose: To restore nucleotide compartmentation in the sarcoplasmic ryanodine receptor (RyR) microdomain by cardiac overexpression of PDE4B in a mouse model of pressure overload-induced heart failure.

Methods: Transgenic (TG) mice expressing a cAMP biosensor localised in the RyR microdomain were subjected to Transverse Aortic Constriction (TAC) and gene therapy with an adeno-associated virus type 9 (AAV9) expressing the phosphodiesterase PDE4B for eight weeks. Ventricular cardiomyocytes were freshly isolated for Förster Resonance Energy Transfer (FRET)-based cAMP imaging upon β-AR stimulation with the non-selective β-AR agonist isoproterenol (ISO) and subsequent pharmacological inhibition of PDE2, PDE3 or PDE4.

Results & Conclusions: Preliminary data showed that the AAV9-PDE4B treatment leads to PDE4B overexpression at the protein level and may partially improve hypertrophy and contractile function. Eight weeks after TAC, β-adrenergic desensitisation is observed, while PDE4B overexpression leads to reduced cAMP production. Upon submaximal and maximal stimulation, gene therapy increases the PDE4 inhibitor effects in the RYR2 microdomain suggesting that overexpression of PDEs might be a promising strategy to restore altered cAMP compartmentation and cAMP dynamics at the microdomain-specific level.

PDE4HEART: Gene therapy with PDE2A and PDE4B in a mouse model of heart failure

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The β-adrenergic pathway results in an increase in cAMP which plays a key role in the regulation of cardiac contraction. While acute stimulation of β-adrenergic receptors (β-ARs) improves cardiac function, their chronic activation in heart failure (HF) is detrimental to the heart, as it promotes deregulation of intracellular calcium handling and maladaptive remodeling. Multiple phosphodiesterases (PDEs) are responsible for cAMP degradation and compartmentation, and therefore finely tune β-AR responses.

We showed previously that PDE2A is increased in human and animal models of HF and blunts β-AR responses in cardiomyocyte, including the pro-hypertrophic effect of noradrenaline. In contrast, PDE4B is decreased in pathological cardiac hypertrophy and PDE4B ablation in mice exacerbates β-AR stimulation of the L-type Ca^{2+} current and the propensity to cardiac arrhythmias. Since long term treatment with PDE inhibitors increases mortality in HF, we hypothesised that decreasing cAMP levels could have a therapeutic effect in this disease.

PDE4HEART: Development of a rat model of heart failure with preserved ejection fraction (HFpEF) for evaluation of phosphodiesterase gene therapy

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The cyclic nucleotide phosphodiesterases PDE2, PDE3 and PDE4 are important regulators of cardiac myocyte cAMP signaling, with important implications for cardiac physiology and pathologies like heart failure (HF) and arrhythmias. We aim to evaluate the effects of genetically increasing the cardiac expression of PDE2A, PDE3A or PDE4B in the type of HF known as heart failure with preserved ejection fraction (HFpEF).

Rats with a genetic susceptibility to develop HFpEF will be obtained by crossing Dahl salt-sensitive rats and Zucker rats carrying a missense mutation in the leptin receptor gene (Lepr). After sufficient back-crossings, the resulting homozygous offspring (DahlS.Z-Leprfa/Leprfa or DS-obese) will carry the leptin receptor mutation on a Dahl salt-sensitive background, and will develop metabolic syndrome with obesity and hypertension resulting in cardiac hypertrophy and subsequent HFpEF.

Rats were evaluated after 8 generations. DS-obese rats (Leprfa homozygosity confirmed by genotyping) displayed a body mass about 30% higher than the DS-lean littermate controls (heterozygous DahlS.Z-Leprfa/Lepr+ and homozygous DahlS.Z-Lepr+/Lepr+) at 5 weeks of age, increasing to about 80% at week 10, as well as cardiac ventricular hypertrophy, assessed by transthoracic echocardiography.

Pressure-volume loop analysis will be used to measure the diastolic function. Cardiac PDE overexpression will be achieved after tail vein injection of viral particles (AVV9) to achieve high expression of PDE2A, PDE3A or PDE4B in the heart and the effect on the cardiac function will be evaluated.

VARIATION: Allele-specific shRNAs directed against a common variant to treat cardiomyopathy due to LMNA mutations

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Standard treatment for Heart Failure most often fails to halt disease progression in patients with a mutation in the lamin A/C gene (LMNA). As the majority of the more than 450 mutations identified in LMNA are dominant-negative, we hypothesize that a novel therapy could be based on mutated allele silencing by allele-specific shRNAs (as-shRNAs). The standard as-shRNA approach is to direct them towards a single, discrete mutation. We direct our as-shRNAs against a common variant in LMNA, which enables us to target the mutated allele independent of the causal mutation.

To select and test these as-shRNAs we created an iPSC-line with the Q493X mutation and heterozygosity for SNP rs4641 in LMNA. By CRISPR/Cas9 we corrected the mutation and we confirmed pluripotency, karyotype and differentiation potential towards cardiomyocytes in both lines. Interestingly, we noticed that in both skin fibroblasts and iPSCderived cardiomyocytes the mutated allele was responsible for only 30% of the total LMNA expression, an observation previously suggested to be linked to heterozygosity of rs4641. To test this hypothesis we created a third isogenic line where we mutated rs4641 and also in this line karyotype, pluripotency and differentiation potential were confirmed. We now started comparing the phenotype of these three lines. On the other hand we are selecting the most affective and specific shRNA targeting rs4641 in the original iPSC-line and managed to inhibit the mutated LMNC allele by 60% without inhibition of the normal allele.

In conclusion, we took the first steps towards development of as-shRNAs as the strongly needed molecular therapy for LMNA mutation carriers.

VARIATION: Modelling of LMNA-cardiomyopathy in human iPSC-derived engineered heart tissue for testing novel therapies

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Introduction: Various mutations in the gene *LMNA* encoding for lamin A/C have been associated with dilated cardiomyopathy (DCM). *LMNA* cardiomyopathy accounts for 6 to 8% of inherited DCM and the underlying molecular pathological mechanisms remain to be elucidated. Evidence has been provided both for haploinsufficiency and a dominant negative, toxic effect. Modeling the disease in 3D *in vitro* could give valuable insights with regards to designing novel therapies.

Methods and Results: Human induced pluripotent stem cells (hiPSC) were generated from skin biopsies of two patients heterozygous for either the mutation p.H222P or p.Q493X. HiPSC of these patients and of an unrelated control cell line were differentiated into cardiomyocytes (CM) with an efficiency ranging from 75% to 95%. Engineered heart tissues (EHTs) were generated from dissociated CM and their force development was tracked over time via optical recording. Both patient cell lines showed much lower capacity than control cell lines to remodel in the first two batches. The few EHTs that remodelled showed lower force of contraction than the ones from unrelated control cell line. Currently, we are in the process of generating an isogenic control cell line with CRISPR/Cas9 to verify the observed phenotypes.

Conclusion: Taken together, our preliminary results suggest reduced force of contraction and impaired capability of remodelling as a first phenotype in our model. Identifying a phenotype in the model is the first important step to determine disease mechanisms and test novel therapies.

VARIATION: Optical Mapping of Human Induced Pluripotent Stem Cell Derived Cardiac Cell Sheets

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Introduction: Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) offer unique opportunities for cardiac disease modeling and drug testing. Yet, fulfilling their potential for studying complex electrophysiological phenomena (conduction and reentrant arrhythmias) requires development of multicellular models. We hypothesize that by combining patient/disease-specific and healthy-control hiPSC-CMs cultured as confluent cell-sheets, with a highly-sensitive EM-CCD camera based optical mapping system, will allow to study the afore-mentioned properties.

Methods and Results: We generated confluent hiPSC-derived cardiac cell sheets (hiPSC-CCSs) either from healthy-control hiPSC-CMs or from patient-specific hiPSC-CMs generated from a patient with dilated cardiomyopathy due to a LMNA mutation. Optical mapping was performed using hiPSC-CCSs that were either loaded with voltage-sensitive dyes (DI-4-ANBDQBS) or expressed the genetically-encoded voltage indicator ArcLight. Optical mapping was combined with a custom-written analysis software to generate activation (conduction) and action-potential duration (APD) maps. Optical mapping allowed pharmacologic studies, demonstrating the ability to detect drug-induced tissue conduction slowing (carbenoxolone, lidocaine, and quinidine) and APD prolongation (quinidine and dofetilide). Programmed electrical stimulation allowed the development of reentrant arrhythmias in some tissues and quantification of arrhythmia vulnerability. Phase-mapping analysis of the reentrant arrhythmias allowed biophysical characterisation of spiral waves induced in the hiPSC-CCSs and their termination by electrical cardioversion and overdrive pacing.

Conclusions: We established a large-scale hiPSC-based cardiac tissue model, which allowed to study conduction and arrhythmogenesis. The use of this model provides a reliable and a robust solution for tissue phenotyping and a powerful tool for drug-testing and arrhythmia investigation.

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AtheroMacHete: Spatial mapping of macrophage heterogeneity in murine atherosclerotic plaques

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Atherosclerosis-related CVDs are the leading causes of death worldwide. As the most abundant immune cell type in plaques, macrophages play crucial roles in the atherosclerotic pathogenesis. Due to their versatile and plastic phenotype in response to locally-produced factors, they are very heterogeneous and can exert both pro- and anti-atherogenic activities. As context, and hence macrophage heterogeneity, is highly disease stage-dependent, this considerably complicates the design of effective macrophage-targeting therapies. In recent publications, newly-emerging approaches such as mass cytometry and single-cell RNAseq underpinned this intraplaque phenotypic heterogeneity, but interpreting the classification's functional implications remains difficult since both techniques suffer from the same caveats: not only is all spatial information on the cells' location lost during tissue disruption steps to obtain single-cell suspensions, specific subsets (e.g. the fragile foam cells) will also be lost during this procedure, leading to an underestimation of their contribution to the disease progress.

We therefore propose a multispectral immunofluorescent imaging approach which strongly increases the number of cell subset markers that can be visualised simultaneously on the same tissue section, as compared to classical immunofluorescence microscopy. Applying a 16-colour staining on a series of murine atherosclerotic plaques allowed us to group myeloid subsets into >20 distinct clusters of relevant cell communities according to their marker expression pattern. With this method we can accurately quantify the contribution of the identified macrophage subsets to different disease stages, map their location in the tissue and link this intraplaque heterogeneity to microenvironmental traits identified on adjacent sections.

AtheroMacHete: Deciphering cell-to-cell communication in perturbed whole blood using single-cell RNA sequencing

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Recent single-cell RNA-sequencing (scRNA-seq) studies of samples from healthy and diseased patients have revealed new insights into the communication patterns between different immune cells. The atherosclerotic plaque represents a heterogeneous immune landscape mainly composed of different macrophage subsets. These cells communicate with each other exchanging differentiation signals and chemokines to attract other cell types as shown in scRNA-seq analysis of murine atherosclerotic plaques.

To date we have set-up a portable scRNA-seq technique (Seq-Well) that we will apply to the analysis of human atherosclerotic plaques in Maastricht and Oslo. To illustrate the potential of this fast, cheap and scalable technique we here present a recently generated data set of perturbed whole blood cells. The transcriptional response of single CD45+ immune cells was examined during 32h using Seq-Well.

This time kinetics experiment yielded a data set of about 120.000 single cells over seven different time points and four different conditions. Using state-of-the-art algorithms, we were able to classify the different cell types and mapped their activation states upon different perturbations. We were able to identify transcriptional responses first in the myeloid compartment followed by an activation of lymphoid cells. Clear inter- & intracellular communication patterns were found in the different subsets.

Our results highlight the scalability of Seq-Well and additionally introduce computational approaches that can be easily applied to the scRNA-seq data generated from atherosclerotic plaques.

MICROEXPLORATION: The role of tissue-nonspecific alkaline phosphatase in microcalcification formation

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Background: Cardiovascular calcification has emerged as a predictor of and contributor to cardiovascular morbidity and mortality. Microcalcification associates with reduce atherosclerotic plaque integrity and may trigger rupture. Previously, we demonstrated that calcifying extracellular vesicles (EV) are the smallest nidus to form microcalcification. We aim to identify the molecular mechanism responsible for microcalcification, with particular focus on the specific molecular intra- and extracellular machinery that functionally regulates tissue-nonspecific alkaline phosphatase (TNAP) activation in calcifying smooth muscle cells (SMC) using experimental and computational approaches.

Results: Using an *in vitro* model of SMC calcification, we demonstrated that the TNAP gene/protein expression and the loading of TNAP into EVs is interlinked with a delay of 3 days. Inhibition of TNAP activity prevent TNAP loading into EVs and inhibit SMC calcification. Inhibition of the neutral sphingomyelinase by GW4869 decrease the release of TNAP-containing EVs but increased cellular TNAP activity. Further, we determined the stability of the osteo-chondrogenic SMC phenotype and found that the phenotype adopted by calcifying medium is unstable when assessing gene expression and TNAP activity. Docking analysis revealed most feasible docking pose of TNAP and inhibitor in the active site of TNAP which cause to inactivation of enzyme. According to structural analysis the electrostatic charge distribution on the TNAP surface has more negative distributed charge in TNAP optimum pH. Conclusion: We determined the onset of TNAP activation and demonstrated that TNAP activity is reversible. These findings are of importance for therapeutic targeting of TNAP or its pathway in order to prevent microcalcification formation.

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MICROEXPLORATION: Annexin A6 participates in the tissue nonspecific alkaline phosphatase-induced calcification of human coronary artery smooth muscle cells

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Vascular calcification (VC) is accompanied by inflammation inducing expression of tissue nonspecific alkaline phosphatase (TNAP), a crucial enzyme for calcification in vascular smooth muscle cells (VSMCs). Nevertheless, TNAP activation and interaction with extracellular vesicles (EVs) released by VSMCs, are not fully understood. Recently, it has been shown that membrane-binding annexin A6 (AnxA6) is abundant in sites of calcification and that its depletion reduces VSMC mineralization. Our aim was to identify the molecular mechanisms involved in VC. We used human coronary artery smooth muscle cells (HCASMCs) cultured in an osteogenic medium for 21 days to promote their transition to osteochondrocyte-like cells. The role of AnxA6 was analyzed after AnxA6 overexpression in HCASMCs. We found that TNAP activity in HCASMCs stimulated for 7 days was 5 times higher than in resting cells. After 14 days, stimulated cells produced 2 times more calcium deposits. In both, control and AnxA6 overexpressing cells, mineral was similar to hydroxyapatite. After 21 days overexpressed AnxA6 accumulated in cytoplasm of resting cells, whereas in stimulated cells, it bound to the membrane of EVs. The interaction of AnxA6 with EVs was Ca2+-dependent; in the presence of 2 mM CaCl2 AnxA6 remained in the membrane fraction. However, subsequent treatment with 10 mM EGTA only partially released AnxA6 from EV membranes, suggesting that some AnxA6 molecules may penetrate the EV membrane bilayer. In conclusion, the identification of proteins involved in the regulation of TNAP activity is crucial for generating novel therapeutic strategies to prevent VC during the formation of atherosclerotic plaques.

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PLAQUEFIGHT: Tripartite Cardiovascular Neuroimmune Circuits Regulate Atherosclerosis

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Atherosclerosis is caused by plaques in the inner layer of arteries and accounts for the leading cause of death worldwide. As plaques are not innervated, wiring of atherosclerosis by the nervous system (NS) has not been considered. The peripheral NS (PNS) uses the outer connective tissue coat of arteries, i.e. the adventitia, as conduits to innervate peripheral tissues.

As we had previously observed artery tertiary lymphoid organs (ATLOs) selectively in those adventitia segments that were burdened by atherosclerotic plaques, we speculated that the PNS may sense atherosclerosis *via* ATLOs. We observed in ApoE-deficient mice that adventitial leukocyte aggregates throughout the diseased arterial tree established several types of vascular neuroimmune crosstalks with the PNS ultimately forming atherosclerosis-brain-circuits (ABCs): i) Axon density was higher in adventitia segments located adjacent to atherosclerotic plaques; ii) axons in ATLOs directly interacted with leukocytes expressing adrenergic-β2-receptor or choline-acetyl-transferase; iii) PNS ganglia were surrounded by leukocyte aggregates, while their soma was infiltrated by leukocytes including mast cells; iv) atherosclerotic artery segments were wired to distinct brainstem and hypothalamic nuclei through specific spinal cord segments; v) firing frequencies of the splenic and celiac vagus nerve were amplified during atherogenesis; vi) sympathetic NS ablation disrupted ATLOs and attenuated atherosclerotic plaque burden.

Moreover, atherosclerosis was associated with enhanced adventitia innervation and perivascular neuronal inflammation in diseased human arteries versus healthy controls. Thus, ABCs can sense and affect atherosclerosis.

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PREMED-CAD: Long non-coding RNA RP1-79C4.4 as novel candidate gene for Atrial Fibrillation/Coronary Artery Disease?

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Coronary artery disease (CAD) represents a significant burden on morbidity and mortality worldwide. A major risk factor for CAD is atrial fibrillation (AF), the most common arrhythmia in the general population. The underlying molecular mechanisms of AF in relation to CAD are largely unknown. Here, we aimed to apply a systems medicine approach to identify biomarker for early diagnosis for AF and consequently, CAD.

Expression quantitative trait loci (eQTL) from left-ventricular tissue of the genotype-tissue expression (GTEx) project was analysed with all published AF-related SNPs. Significant eQTLs (P<10-5) were considered for further expression analysis in atrial tissue of AF patients (AF_CCS cohort, n=118) using a duplex TaqMan assay.

Bioinformatical analysis showed long non-coding RNA (lncRNA) RP1-79C4.4 as top candidate gene for AF exhibiting upregulation with the homozygous C allele of AF-related SNP rs629234 in left-ventricular tissue (p-value – 9.9×10^{-7}). This lncRNA (2045 bp) is located within *PRRX1*, which is a member of the paired homeobox transcription factors and has already shown association with AF. Expression analysis of the lncRNA and *PRRX1* in atrial tissue (AF_CCS cohort) showed no regulation between AF-subjects and controls, however, SNP-genotyping revealed that AF-subjects show a significantly higher proportion of the rs629234 heterozygous (C/T) allele. Next, cloning of candidate lncRNA will be performed for over-expression and knockdown analyses. In addition, the phenotypes with alleles of the SNP will be studied for their role in lncRNA-RP1-79C4.4 regulation.

The role of this lncRNA in AF is largely unknown and poses an interesting new candidate for AF for further characterisation.

PREMED-CAD: Molecular assessment of iron deficiency in cardio-vascular disease

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Iron deficiency (ID) is a frequent co-morbidity associated with chronic heart failure (CHF). Cardiovascular medicine distinguishes between absolute ID (serum ferritin < 100µg/l) and functional ID (serum ferritin within the 100-300µg/l range along with a transferrin saturation below 20%). Iron deficiency was historically treated with oral administrated iron supplement, however due to poor absorption, along with negative side effects, guidelines now recommend treatment by intravenous iron. This approach combines rapid absorption and uptake by the reticuloendothelial system with very limited side effect.

When treated with intravenous perfusion of iron, patient with CHF and ID show improved symptoms and reduced inflammation levels. The mechanisms leading to this improvement remain poorly understood. To uncover new mechanisms explaining these improvements, we will determine new candidate genes using GWAS and GWE data in the frame of large-scale population-based studies. After determining these candidate in silico, we will then test these new candidate genes *in vitro,* using ID or iron overload models in human cardiac and white blood cell lines. The most promising candidates will then be tested *in vivo* using ID model in zebrafish, either Wild type or with a cardiac pathological phenotype. In parallel we will also use knock-out models of our candidate genes in mice CHF models with or without ID, to further confirm our observations.

Ultimately, this study aims at determining new therapeutic targets to treat patient with ID and CHF, by translating our results into human setting, using datasets of patients with or without ID.

PROACT: A Novel Urinary Biomarker Predicts 1-Year Mortality after Discharge from Intensive Care

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Rationale: Tools reflecting molecular processes predicting death after discharge from intensive care unit (ICU) are currently unavailable.

Objectives: To construct a urinary proteomic biomarker predicting 1-year post-ICU mortality.

Methods: In 1081 patients, enrolled in the French and European Outcome Registry in Intensive Care Unit (NCT01367093), the urinary proteome was measured at ICU discharge using capillary electrophoresis couple with mass spectrometry along with clinical variables; circulating biomarkers (NT-proBNP, hsTnT, biologically active adrenomedullin, soluble ST2, and NGAL) and urinary albumin were available in 886 patients.

Measurements and Main Results: In the discovery sample (60/256 deaths/survivors), support vector machine modeling identified ACM150, mainly consisting of collagen fragments, yielding an AUC of 0.676 (95% CI, 0.615–0.737). In the replication sample (151/608 deaths/survivors), AUC was 0.704 (0.659–0.750). While accounting for center and clinical risk factors, the hazard ratios in all available patients were 1.27 (1.18–1.37) for ACM150 (+1 SD), 1.20 (1.16–1.33) for the Charlson score (+1 point), and ≥1.30 ($P \leq 0.0071$) for the other biomarkers (+ 1 SD). Model performance assessed by adding ACM150 to a basic model including the aforementioned covariables, the Charlson score or any other biomarker confirmed the prognostic accuracy of ACM15 with significant increases ($P \leq 0.0038$) in integrated discrimination (≥ +0.50) and net reclassification improvement (≥ +53.7) and AUC (≥ +0.037). Interactome mapping (STRING) based on 72 sequenced peptides and 25 parental proteins gravitated around collagen nodes.

Conclusions: ACM150 is a urinary proteomic classifier predicting 1-year post-ICU mortality over and beyond other biomarkers and reflects dysregulation of collagen turnover as underlying pathophysiological process.

Keywords: biomarker, intensive care medicine, heart failure, mortality, urinary proteomics

SCAN: An *in vitro* "circulatory system" to study the mechanical stability of therapeutic nanoparticles

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Introduction: Poly(lactic-co-glycolic acid) (PLGA) nanoparticles with perfluoro-15-crown-5-ether (PFCE) entrapped have been characterised in terms of size, biodistribution, clearance and their application in different imaging modalities. The nanoparticles are used by our group for cell tracking and applications like drug delivery after intraveneous injection, including in humans. (1) However, data is lacking about the mechanical effects of nanoparticles hurtling through the circulatory system (2).

Objective: We aim to develop an *in vitro* model to investigate the effects of flow and shear stress on PLGA-PFCE nanoparticles.

Methods: PLGA PFCE nanoparticles (produced by tip sonication) were run through an Ibidi flow chamber with a continuous flow, collected and again run through the system for 50 runs in total. The chosen shear rate was high (17 dyn/cm2), based on the highest shear rate used in the literature (3). The flow rate was calculated using Ibidi's standard formula flow rate = shear rate / 131.6 resulting in a flow rate of 12.9 ml/min. Before, during and after perfusion samples were collected for analysis with Dynamic Light Scattering (DLS) for diameter and Nuclear Magnetic Resonance (NMR) for fluorine content.

Results: Our preliminary data shows that the average diameter before the first flow was 175 ± 5 nm and after 50 runs 170 ± 6 nm. The fluorine content slightly decreased from 25 ± 2.5% to 18 ± 0.01%, which may suggest that fluorine evaporated from the system. However, further optimisation and a larger sample size are required to draw definite conclusions.

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SCAN: Enzyme-driven multimodal detection of atherosclerosis with iron oxide nanoparticles

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Atherosclerosis and its clinical complications are major constraints to living long and healthy lives. Therefore, a deeper understanding of the pathophysiology of the disease and tools capable of measuring disease activity are necessary. In the project SCAN (Subclinical Atherosclerosis Characterisation: Nanoparticle-Based Molecular and Cellular Imaging), our goal is to develop different types of multimodal nanoparticles (NPs) for the *in vivo* imaging of atherosclerosis. More specifically, our efforts will focus on studying the NPs capability of targeting macrophage-like smooth muscle cells (SMCs), which likely play an important role in plaque progression and stability1.

In this work, the enzyme-driven accumulation of Phosphatidylcholine (PC)2 and Sphingomyelin (SPH) iron oxide nanomicelles (IONM) in the atherosclerotic plaque is studied as a potential contrast agent for MR, fluorescence and PET imaging. PC-IONM and SPH-IONM have been fully characterised and their selective physicochemical change in the presence of Phosphatidylcholine-specific phospholipase C and sphingomyelinase, respectively, enzymes highly expressed in the plaque, has been demonstrated. Furthermore, T2-MR and fluorescence imaging with the NMs have shown the enzyme-selective accumulation of this probe in the atherosclerotic plaque, making it a suitable probe for pathophysiology and activity characterisation.

Future work will focus on further characterisation of the *in vivo* behaviour of the NMs in different atherosclerotic mice models, and different imaging techniques. In addition, together with the other groups involved in SCAN, development of new antibody-functionalised NPs specific for the new targets identified in Tübingen and capable of giving image in additional imaging modalities in collaboration with Nijmegen will be carried out.

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SCAN: Identification of markers associated with transdifferentiated vascular smooth muscle cells to evaluate plaque stability

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Atherosclerosis is a leading cause of death worldwide. Rupture of vulnerable atherosclerotic plaques can lead to unfavourable consequences that are often detrimental to the patients. Thus, understanding the underlying mechanisms that lead to plaque instability is vital to prevent fatal complications associated with plaque rupture. Our group has developed a genetically-encoded Cre/lox-based cell tracking system to investigate the role of vascular smooth muscle cells (VSMCs) in atherosclerotic plaque formation.

Using this model, we have shown clonal expansion of VSMCs and their phenotypic conversion to macrophage-like cells during atherogenesis (Feil et al, 2014). VSMC-to-macrophage transdifferentiation likely plays an important role in plaque progression and stability.

In the project SCAN (Subclinical Atherosclerosis Characterisation: Nanoparticle-Based Molecular and Cellular Imaging), our aim is to identify markers that are differentially expressed between macrophage-like and other VSMC-derived plaque cells in order to identify stable and unstable plaques. Isolated VSMC-derived cell populations from atherosclerotic plaques of our lineage traced mice will be characterised using single cell RNA sequencing. Candidate markers identified from RNA profiling will be further validated by immunostaining of lesions in experimental and human atherosclerosis. As macrophage-like VSMCs appear to be highly selective for atherosclerosis, identification of markers associated with these cells will provide an early diagnostic marker and identify new therapeutic targets associated with plaque vulnerability.

Together with the other groups involved in SCAN, we will use these novel protein targets to develop non-invasive nanoparticle-based imaging to measure plaque progression rate and stability and develop potential therapies towards stabilisation of vulnerable plaque in patients.

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DENIM: Duchenne muscular dystrophy cardiomyopathy – the role of ENdocannabinoids and IMmune regulation.

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Muscular dystrophy (MD) is an X-linked neuromuscular disease manifesting as muscle atrophy and cardiomyopathy in young boys. Carrier females (CF) are also often diagnosed with advanced stage cardiomyopathy. Existing therapy has limited effect and many patients die of heart failure. Therefore discovering new therapies for MD cardiomyopathy (MDC) is a clinical imperative. Altering the endocannabinoid system (ECS) dramatically impacts cardiomyopathy, however its role in MDC remains unexplored.

We have uncovered the involvement of the ECS in MDC. Cardiomyocytes derived from MD patients' induced pluripotent stem cells (iPS-CM) had increased ECS tone, inflammation and membrane-damage which were ameliorated by ECS modulators. Since evidence suggests that immune-regulators contribute to MD, we will incorporate immune-regulatory cells (cardiac stromal cells and macrophages) to our model forming a tri-cell (3C) MDC model. The 3C- MDC model will be used to evaluate the immune-modulatory potential of ECS targeting during the DENIM project.

Clinical data from MD patients and CF will be accrued and correlated with cellular phenotypes. Matched CF and sons, and engineered isogenic iPS-CM will be used to address gender and genetic differences. We expect to provide proof-of-concept data supporting ECS modulation to improve MDC bolstering the pursuit of clinical trials.

DETECT ARRHYTHMIAS: Analysis of the MEIS1 genomic locus in correlation to heart arrhythmias in the Estonian population

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Heart arrhythmias are among the most prevalent healthcare challenges in the European population, while simultaneously remaining elusive in their molecular and cellular mechanisms. The "Investigating DETerminants of atrial Electrical ConducTion underlying ARRHYTHMIAS" *(DETECT ARRHYTHMIAS)* project intends to clarify the role of MEIS1 genomic locus on clinical pathophysiology of arrhythmias. Genome wide association studies have previously correlated the MEIS1 region as a genetic determinant of atrial electrophysiology, while knock-out studies of the Meis1 gene in mice have demonstrated it to be a regulator in cardiac regeneration and metabolism. The *DETECT ARRHYTHMIAS** consortium will concentrate on identifying and describing the biological relevance of the MEIS1 transcription factor through *in silico, in vitro* and *in vivo* methodologies.

The Estonian Biobank consists of blood samples, drug prescriptions data and health records from over 150.000 donors (~17% of nation's adult population), which are being used for metabolomic and genomic analyses. Utilizing this database, we will fine-map the ±1 megabase region around the *MEIS1* gene in subjects who have been previously diagnosed with atrial fibrillation, cardiac arrest or arrhythmias. By identifying single-nucleotide variants, quantitative trait loci and running a phenome-wide analysis, we will determine the genetic modulators, coding and regulatory variants within and close proximity to *MEIS1* gene and genes belonging to downstream signaling cascades.

This rigorous computational analysis will allow us to identify common and rare pathogenic variants of the MEIS1 allele within an European population and grant us to extrapolate the broader functionality of MEIS1 in context of heart arrhythmias.

*The **DETECT ARRHYTHMIAS** consortium consists of a transnational collaboration between the working groups of Dr. Sebastian Clauss (Klinikum der Universität München, Germany), Dr. Davide Rovina (Centro Cardiologico Monzino, Italy), Dr. Fatih Kocabas (Yeditepe University, Turkey) and Dr. Tõnu Esko (University of Tartu, Estonia).

EMPATHY: Differentiating the effects of exercise-induced beta-adrenergic stimulation and stretch on calcium and force dynamics using a novel electromechanical cardiomyocyte model

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Aims: Cardiac arrhythmias are traditionally considered entirely electrical phenomena, but electromechanical interactions may be key in arrhythmogenesis. Physical exercise affects both cardiac electrophysiology and mechanics and can be an important pro-arrhythmic trigger. However, the complex electromechanical dynamic interplay complicates arrhythmia risk prediction. We aim at differentiating the effects of exercise-induced acute β-adrenergic stimulation (β-ARS) from cardiomyocyte stretch (increased sarcomere length, SL) on calcium transient (CaT) dynamics and force generation, using a novel computational model of cardiac electromechanics.

Methods: We implemented a bidirectional coupling between the O'Hara-Rudy model of human ventricular electrophysiology and the MechChem model of sarcomere mechanics. The coupled model was validated using a range of experimental data from large mammals or human samples. CaT and force were simulated with varying degrees of acute β-ARS and various SL.

Results: The model reproduced experimental data including force-frequency and quick-release experiments, validating the bidirectional electromechanical interaction. Increasing β-ARS and SL both increased CaT and force amplitude (positive inotropy), but the influence of β-ARS on force amplitude was smaller than that of SL. Increasing β-ARS and SL had opposite effects on CaT and force duration (reflecting lusitropy), with increased β-ARS decreasing CaT and force duration whereas increased SL prolonged CaT and force duration.

Conclusions: Using a novel coupled human electromechanical model, we identified differential effects of acute β-ARS and stretch on CaT and force dynamics. We showed that their combination, rather than individual contributions, is key to ensure force generation, rapid relaxation for diastolic filling, and low diastolic calcium levels during exercise.

HyperDiP: Alterations in Cardiac Structure and Function caused by Preeclampsia

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Preeclampsia is a major cause of maternal and fetal mortality and morbidity. Since 2011, the American Heart Association mentioned the history of preeclampsia as a risk factor for later cardiovascular disease.

To investigate the cardiovascular impact of preeclampsia, we use a transgenic rat model with females harbouring the gene for human angiotensinogen and males for human renin. After mating, the female rat shows typical symptoms of preeclampsia at the end of the second trimester, like high blood pressure, proteinuria or growth restriction of the fetus. With echocardiography, a mild but significant reduced systolic ejection fraction indicates functional changes. Speckle trackle analysis allows tracing the myocardium over the whole cardiac cycle and gives more sensitive results. A significant decrease in longitudinal, radial and circumferential strain and all global strain rates was measured and describes a reduced myocardial deformation in relation to its original shape and within the speed at which this occurs.

These data goes closely together with echocardiography of a human cohort and underline the translational approach. In the animal model, persistent alterations concerning hypertrophy, fibrosis and capillary rarefication were investigated. This demonstrates extensive alterations in structure and function of maternal hearts caused by preeclamptic pregnancy persisting even postpartum.

HyperDiP: Maternal hemodynamics in hypertensive disorders of pregnancy – a human and animal model under antihypertensive therapy

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Introduction: Hypertensive disorders of pregnancy (HDP) are still the leading cause of maternal and neonatal morbidity and mortality worldwide. HDP, in particular preeclampsia (PE), have been determined as risk factors for future cardiovascular disease (CVD). Recently, the common hypothesis of PE being a placental disorder, was challenged as numerous studies show evidence for short- and long-term cardiovascular changes in pregnancies affected by HDP, suggesting a cardiovascular origin of the disease. Despite new insights into the pathophysiology of HDP, concepts of therapy remain unchanged and evidence for improved maternal and neonatal outcome by using antihypertensive agents is lacking.

Objectives: This project aims to evaluate the cardiovascular risk in women affected by HDP by assessing maternal hemodynamics and cardiovascular markers (sFlt-1/PlGF-ratio, NT- proBNP) in women with HDP compared to a healthy control group and a preeclamptic rat model under antihypertensive therapy (AT).

Methods: This study incorporates three work-packages:
A prospective observational case control study over a 36-month period in Vienna, covering a follow-up one year post-partum, including women with HDP and a healthy control group, will assess maternal hemodynamics using USCOM©1A Monitor along with cardiovascular markers in women with HDP under AT.
A prospective, longitudinal study of cardiovascular surrogate markers conducted in Oslo will serve as a comparative cohort for the Vienna cohort of hemodynamics in pregnancy.
Furthermore, we aim to obtain new insights into the pathophysiology of PE as well as treatment mechanisms by using invasive procedures and assessing the structural morphology of the heart in our rat model in Berlin.

INNOVATION: Investigating long non-coding RNA-regulated pathways driving cardiac regeneration

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The interdisciplinary three-years program INNOVATION brings together five European nations aiming to exploit RNA biology for the stimulation of cardiac regeneration and thus, ultimately combating heart failure. While neonatal hearts fully regenerate without scarring after myocardial infarction (MI), the adult mammalian heart is void of significant regenerative capacities. INNOVATION will identify unknown pathways distinguishing regenerative neonatal mouse hearts from non-regenerative adult hearts. In contrast to the traditional focus which lies on protein-coding genes, INNOVATION will explore long non-coding RNA (lncRNA)-mediated regulatory pathways. To do so, this project will use state-of-the-art molecular tools paired with innovative *in vitro* and *in vivo* models which will lead to the discovery of novel regeneration-associated lncRNAs.

The specific objectives of INNOVATION are the generation of a gender and cell-type specific atlas of conserved lncRNAs, the validation of regeneration-related cardiovascular pathways by testing lncRNA interventions in a neonatal mouse model of MI and testing of pro-regenerative lncRNAs in human cardiac cells and engineered heart tissue.

Furthermore, therapeutic lncRNA-based strategies to boost cardiac regeneration after MI in adult mice will be developed, aimed at a potential translation into patients.

INNOVATION: Exploiting the role of long non-coding RNAs in the direct conversion of fibroblasts into functional cardiomyocytes

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Direct conversion of fibroblasts into a desired cell type is an alternative to the use of induced pluripotent stem cells (iPSCs). This method has been used to generate cardiomyocytes, although it remains somewhat inefficient. Long non-coding RNAs (lncRNAs), key regulators of gene expression, have been shown to mediate reprogramming of fibroblasts into iPSCs and are differentially expressed during different stages of cardiac development. Likewise, lncRNAs might be novel modulators of direct conversion and may contribute to the efficient generation of cells for regenerative purposes.

Therefore, we set out to sequence the non-coding genome and identify lncRNA signatures in mature cardiomyocytes. Mice and human fibroblasts will be directly converted into cardiomyocytes using transcription factors, and identified lncRNAs will be modulated, in order to assess whether they improve the direct conversion efficiency of fibroblasts into induced cardiomyocytes (iCMs).

MEND-AGE: Identifying Cellular and Molecular Mechanisms of Vascular Sclerosis Development

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Vascular sclerosis contributes tremendously to cardiovascular morbidity and mortality. The paradigm to date is that in cardiovascular disease (CVD) smooth muscle cells (VSMCs) become activated and secrete extracellular matrix to remodel the vascular wall and later transdifferentiate into osteoblast like cells, ultimately resulting in vascular calcification.

We have recently identified an adventitial progenitor population and our work indicates that these Gli1+ cells are progenitors of VSMCs being the actual drivers of vascular sclerosis. As a proof of concept we were able to show that genetic ablation of Gli1+ cells before onset of atherosclerosis in mice resulted in complete inhibition of vascular calcification. Therefore, Gli1+ cells represent a promising therapeutic target for the treatment of CVD. Based on our preliminary data we hypothesize that Gli1+ cells are quite heterogeneous with a subset of cells, being a smooth muscle cell (VSMC) progenitor while others are more committed to the osteoblast lineage. We performed combined genetic fate tracing and single cell RNA-sequencing of Gli1+ cells in a mouse model of severe atherosclerosis using AAV mediated PCSK9 gain of function mutation in Gli1CreER;tdTomato mice.

Our data indicates that Gli1+ cells are indeed heterogeneous with several subsets that represent distinct lineages. We are currently performing *in vitro* experiments and single cell DNA-accessibility measurements from Gli1+ cells to further validate our findings.

PICASSO: Involvement of proprotein convertase subtilisin/kexin type 9 (PCSK9) in aortic valvular calcification

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Background: Calcific aortic valve stenosis (CAVS) is the most common form of valve heart degeneration, affecting almost 10% of the population aged 65 years and above. It has been hypothesised that PCSK9 might accelerate CAVS. Whether PCSK9 is expressed in the aortic valve and if it could play a role in calcification is still unknown.

Methods: We used whole protein extracts from human aortic valves and PCSK9-/- and wild type mice. Calcification assays were performed on aortic valve interstitial cells (VIC) by β-glycerophosphate and ascorbic acid (βGAA) treatment. RNA-sequencing was used to evaluate differentially expressed genes in mouse VICs.

Results: PCSK9 was significantly increased in both fibrotic and stenotic aortic valves than controls (ANOVA $p<0.05$). Aortic valve total calcium content from PCSK9-/- mice was significantly lower than WT mice ($p=0.0002$). Calcium quantification revealed that PCSK9-/- VICs (untreated) were able to calcify at a less extent than WT VICs ($p=0.0001$). The βGAA treatment induced calcification in both cell types; however, a significant lower calcification rate was noted in PCSK9-/- VICs compared to WT VICs ($p=0.01$). RNA-Seq revealed that 363 genes were differentially expressed, regulating p38 cascade, cytoskeleton modifications, cell proliferation and adhesion, and oxidative stress.

Conclusion: These results show for the first time that PCSK9 is expressed in human aortic valve cells and high levels are found in CAVS. Mice lacking PCSK9 are protected from aortic valve calcification. Thus, pharmacological intervention aimed to reducing PCSK9 plasma levels may be beneficial to patients affected by CAVS.

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