Belgian Society of CARDIOLOGY

BWG Basic Research in Cardiology

BELGIAN WORKING GROUP OF BASIC RESEARCH IN CARDIOLOGY

4th annual meeting, 2nd December 2022

Building W, Erasme Campus, Lennik Road 808, Brussels, Belgium

(RE)BUILDING INTERDISCIPLINARY BRIDGES IN CARDIOVASCULAR RESEARCH

Organisers: Nicolas BAEYENS, Antoine BONDUE et Didier COMMUNI (ULB)

Keynote lectures by

Prof. Dr. Didier Stainier,

Max Planck Institute for Heart and Lung research

Prof. Dr. Cédric Blanpain,

Laboratory of stem cells and cancer, ULB

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Welcome Message

Dear participants, Dear friends, and colleagues,

It is my pleasure to welcome you to the 4th BWG-BRC Congress - "(Re)building Interdisciplinary Bridges in Cardiovascular Research".

The meeting aims to provide participants at the event with the current knowledge on the cellular and molecular mechanisms underlying the pathobiology of cardiovascular diseases. The Organizing Committee has been working hard on preparing a program of scientific excellence that will excite and inspire our community. This program includes two plenary lectures from outstanding research leaders. As is traditional at a BWG-BRC Congress, participants will also play an active part in the event by presenting short talks or posters. We especially look forward to welcoming young Belgian investigators to present their work. There will be many networking opportunities, along with awards to the best oral and poster presentations.

Meeting together in person will enable us to share our common scientific interests and passions and learn about discoveries outside our immediate research focus. It will be also an opportunity to make or strengthen our connections and support each other in our research aims.

I hope you will all enjoy this 4th Annual Meeting of the Belgian Working group for Basic Research in Cardiology!

With best regards,

Dr. Sandrine Horman (UCLouvain), President of the BWG-BRC

Scientific committee:

Jeffrey Aalders (UGent)

Julien De Poortere (UCLouvain)

Esteban Diaz Villamil (ULB)

Mandy Grootaert (KULeuven)

Géraldine Hubesch (ULB).

Ilse Luyckx (UAntwerpen)

Organisation:

Amira Khouiled (ULB)

8h30-8h45	**Registration**
8h45-9h00	**Welcome**
9h00-10h00	**1st Keynote lecture** **Didier Stainier – Max Planck Institute (GER)**
10h00-10h30	**1st Oral communications session** 1. Zebrafish as a tool to study cardiovascular effects caused by fibrillin impairment - **Karo De Rycke** – UGent 2. Influence of hemodynamics on the development of vascular abnomalies in RASA1 related Capillary malformation-arteriovenous malformation (CM-AVM) syndrome - **Yao Du** – ULB
10h30-11h00	**Coffee Break**
11h00-11h45	**2nd Oral communications session** 3. Plasmatic myo-inositol elevation in heart failure with preserved ejection: a potential metabolic actor in the disease pathophysiology and clinical outcome - **Julien Cumps** – UCLouvain 4. The discovery of a key actor of cardiac pathological hypertrophy - **Laura Guilbert** – UCLouvain 5. Pericyte loss initiates microvascular dysfunction in the development of diastolic dysfunction - **Mandy Grootaert** – KULeuven
11h45 - 12h05	**Flash poster session**
12h05 - 13h35	**Lunch + Poster session**

13h35 - 14h35	**2nd Keynote lecture** **Cédric Blanpain – ULB (BE)**
14h35 - 15h05	**3rd Oral communication session** 6. Dimethylation of histone 3 lysine 9 (H3K9) suppresses post-natal cardiomyocyte proliferation - **Konstantinos Chatzieleftheriadis** – KULeuven 7. Molecular characterization and investigation of the role of genetic variation in phenotypic variability and response to treatment in a large pediatric Marfan syndrome cohort - **Lotte Van Den Heuvel** – UAntwerpen
15h05 - 15h25	**Coffee Break**
15h25 - 16h10	**4th Oral communication session** 8. ERBB4-Selective and Sustained activation by NRG1 attenuates atrial fibrosis and fibrillation -**Jens Van Fraeyenhoven** -UAntwerpen 9. Feedback loops regulating mechanical homeostasis in cardiac fibrosis - **Yuliia Mykhailovska** – ULB 10. Anti-thrombogenic, Biocompatible, and Hydrodynamic Prosthetic Heart Valve for Life: Creative Polymer Chemistry for a Sustainable Future - **Sofia Melo** – ULG
16h10 - 16h30	**Awards** **End of the meeting**

Talk 1: Zebrafish as a tool to study cardiovascular effects caused by fibrillin impairment

De Rycke Karo[1], Caboor Lisa[1], Horvat Marina[1], Vermassen Petra[1], De Backer Julie[2], Sips Patrick[1]

1Department of Biomolecular Medicine, Ghent University, Ghent, Belgium 2Department of Cardiology and Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

Background: Marfan syndrome (MFS) is the most common type of fibrillinopathy with a high predisposition to develop aneurysms and dissections of the thoracic aorta. While the development of several mouse models of MFS has contributed greatly to our current knowledge, a thorough understanding of the underlying mechanisms is still lacking. There is a particular need for more flexible in vivo models to address this knowledge gap.

Aims: We aimed to generate a relevant zebrafish model to gain insight into the molecular mechanisms relating fibrillin defects to the cardiovascular system.

Methods: The CRISPR/Cas9 system was used to systematically target the three different fibrillin genes (fbn1, fbn2a and fbn2b) in Tg(kdrl:GFP) reporter zebrafish. Time-lapse fluorescent microscopy was used to evaluate the cardiovascular phenotype.

Results: We found that zebrafish lacking fbn1 and/or fbn2a do not show any cardiovascular phenotype during early-stage development. On the other hand, approximately 50% of homozygous fbn2b mutant (fbn2b-/-) zebrafish embryo's show a severe phenotype characterized by endocardial detachment, leading to vascular embolism and premature mortality at 7-9 dpf. Interestingly, the remaining fbn2b-/- zebrafish survive until adulthood, but during larval stages already develop a dilation of the bulbus arteriosus, a structure anatomically related to the aortic root in humans. In addition, the caudal vein of all fbn2b-/- embryos develops abnormally as a cavernous structure lacking vessel integrity. This phenotype is resolved in embryos retaining normal blood flow and aggravated upon pharmacological inhibition of blood flow during development.

Conclusion: These data indicate that our new fbn2b-/- zebrafish model recapitulates cardiovascular complications observed with fibrillin deficiency, and can thus be considered a relevant model to study the mechanisms underlying MFS pathogenesis. Our preliminary data suggest that there is an interplay between fibrillin deficiency and biomechanical signaling in the regulation of cardiovascular development.

Talk 2: Influence of hemodynamics on the development of vascular abnomalies in *RASA1* related Capillary malformation-arteriovenous malformation (CM-AVM) syndrome

Yao DU[1], Edwige MARTIN-VALIENTE[1], Nicolas BAEYENS[1]

[1]Laboratoire de Physiologie et Pharmacologie, Faculté de Médecine, ULB

Background: CM-AVM syndrome is characterized by small capillary malformations sometimes associated with cutaneous, subcutaneous, muscular, and bone arteriovenous malformations, Parkes-Weber syndrome, or malformations of the vein of Galen (VAGM). Around 60% of patients have *RASA1* germline mutations. However, the incomplete penetrance and specificity of the localization of the lesions cannot be solely explained by the genetic mutation.

Aims: To test the contribution of fluid shear stress in developing AVMs in RASA1-deficient cells.

Methodology: Based on the clinical characteristics of CM-AVM, we analyzed several capillary networks from several affected organs in mice using the tissue-clearing protocol iDISCO. Then, we developed a microfluidic model to reproduce the specific flow profile of vascular networks where the malformation develops. To investigate the mechanism, we used RASA1 deficient cells to measure their response to the specific hemodynamic parameters encountered in those vascular trees by measuring calcium responses or activation of signaling pathways by western blotting.

Summary of results: CM-AVM-related capillary networks display specific characteristics, with elongated collecting vessels and branches entering the collecting vessel at an orthogonal angle. In the collecting blood, we observed the existence of blood streams with distinct hemodynamics. A monolayer of normal endothelial cells subjected in a collecting channel to distinct streams with different shear stress intensities exhibits a uniform propagation of calcium waves. A monolayer of RASA1 KD cells displayed a non-uniform propagation of the calcium waves, indicating a perturbation in cell-cell communication. Further, while the knock-down of RASA1 does not impact some flow responses, we observed an overactivation of ERK activation by flow and a potent inhibition of AKT.

Statement of conclusions: Our observations indicate that vascular network architecture and flow properties might generate the necessary “second hit” to trigger the development of the malformations. RASA1 might play an important role in mitigating flow responses generated by multiple streams within collecting vessels, impairing the fusion process of the vessel during angiogenesis.

Talk 3: Plasmatic *myo*-inositol elevation in heart failure with preserved ejection: a potential metabolic actor in the disease pathophysiology and clinical outcome

J.Cumps[1], Nassiba Menghoum[2], Alice Marino[1], S.Battault[1], Sybille Lejeune[2], Alexandra Furtos[3], M. Badii[2], L. Mahrouche[3], D. Rhainds[3], J-C. Tardiff[3], Julie Thompson[3], Christine Des Rosiers[3], L.Bertrand[1], S.Horman[1], A-C.Pouleur[2], C.Beauloye[1,2].

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[3]Department of Nutrition, Université de Montréal and Montreal Heart Institute, Montreal, Quebec, Canada.

Background: Metabolites availability is crucial to the development of interstitial fibrosis and heart failure with preserved ejection fraction (HFpEF). We recently showed that *myo*-inositol (MYO) is a metabolite that can induce oxidative stress and be harmful to the myocardium. However, plasmatic MYO level has never been measured in the context of heart failure (HF). We hypothesized that MYO level is increased in HFpEF patients and could contribute to its development by promoting cardiac fibrosis.

Aims: Evaluate MYO level in HFpEF patients and investigate its role in cardiac fibrosis.

Methods: 451 patients were prospectively included in a Belgian discovery cohort, containing control, HFrEF and HFpEF patients. MYO plasmatic level was measured by mass spectrometry and its impact on human cardiac fibroblast (HCF) properties was assessed *in vitro*. Our human data have been confirmed in a Canadian confirmatory cohort of 276 patients.

Results: MYO was significantly increased in HF patients compared to controls and even higher in HFpEF population in both Belgian and Canadian cohorts. High MYO level was associated with decreased renal function, HFpEF profile and FGF-23, a fibrotic marker. Interestingly, abnormal MYO level (> 69 µM) in HFpEF population was predictor of poor outcome. We demonstrated that MYO promotes HCF proliferation, migration and myodifferentiation, suggesting that it could favor cardiac fibrosis.

Conclusion: Increased plasmatic MYO level in HFpEF is associated with a decline in renal function and poorer prognosis, probably by favouring fibrosis, a key pathophysiological mechanism in HFpEF.

Talk 4: The discovery of a key actor of cardiac pathological hypertrophy

L. Guilbert[1], J. Dontaine[1], L. Bultot[1], N. Fourny[1], H. Esfahani[2], L. Dumoutier[4], D. Vertommen[5], L. Gatto[6], M. Martin[6], J.-L. Balligand[2], C. Beauloye[1], S. Horman[1], L. Bertrand[1]

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Introduction: Cardiac hypertrophy is initially an adaptative response to maintain cardiac output. However, under chronic stimulus, hypertrophy can become maladaptive and leads to heart failure. We previously showed that protein O-GlcNAcylation level is drastically increase during cardiac hypertrophy development. Using mass spectrometry analysis, we identified several O-GlcNAcylated cardiac proteins that could be involved in the regulation of the disease. Among them, OGP-1* (O-GlcNAcylated protein 1) is becoming a promising candidate.

Aims: We aimed to investigate the role of OGP-1 in cardiac hypertrophy development.

Methods: OGP-1 expression was measured in neonatal rat cardiomyocytes (NRVM) treated with the pro-hypertrophic agent phenylephrine (PE) for 24h. The same was done *in vivo, in a* model of transverse aortic constriction (TAC) for 2 to 6 weeks. OGP-1 expression was also evaluated in human hearts biopsies from different degrees of cardiac remodeling. NRVM size was evaluated using α-actinine staining which marks sarcomere Z-line. The effect of OGP-1 overexpression and silencing was finally analyzed in NRVM.

Results: We showed a significant decrease in OGP-1 expression during cardiac hypertrophy development both *in vitro* and *in vivo*. A similar decrease in OGP-1 expression was observed in human samples of maladaptive cardiac remodeling. To evaluate the putative implication of OGP-1 in the regulation of cardiac hypertrophy, we repressed OGP-1 expression using siRNA in NRVM. We showed that OGP-1 knock-down induced a basal increase in cell size, similar to that found in PE-treated control cardiomyocytes. It suggests that OGP-1 repression may be sufficient to induce cardiomyocyte hypertrophy. Finally, OGP-1 overexpression totally prevented PE-induced NRVM hypertrophy.

Conclusion: Our results underlie OGP-1 as a potential anti-hypertrophic protein and an interesting future target in the prevention of cardiac hypertrophy. Further investigations aim to identify OGP-1 cellular function and the impact of its O-GlcNAcylation in cardiac hypertrophy. *OGP-1 is a fictive name due to patenting issues

Talk 5: Pericyte loss initiates microvascular dysfunction in the development of diastolic dysfunction

Steven J Simmonds[a*], Mandy OJ Grootaert [a*], Ilona Cuijpers[a,b*], Paolo Carai[a], Nadeche Geuens[a], Liene Vertommen[a], Melissa Herwig[c,d], Marc van Bilsen[b] , Pieter Baatsen[e,f], Nazha Hamdani[c,d], Aernout Luttun[a], Stephane Heymans[a,b,*], and Elizabeth A.V. Jones[a,b*].

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Background: More than half of the heart failure (HF) patients suffer from heart failure with preserved ejection fraction (HFpEF), a complex cardiovascular syndrome associated with metabolic comorbidities. Microvascular dysfunction has been proposed to drive HFpEF through inducing chronic inflammation, but the initial molecular and cellular events are largely unknown.

Aims: We aimed to determine the temporal dynamic changes in the microvasculature in relation to the development of key pathological features of HFpEF. Moreover, we aimed to define the role of pericyte dysfunction in the development of diastolic dysfunction.

Methods: The ZSF1 obese rat was used as an established model of HFpEF. We assessed their cardiac function by echocardiography, and cardiac histology at 6, 14 and 21 weeks of age vs. their lean controls. To test the effect of pericyte loss on cardiac function, we used PDGF-Bret/ret mice that carry a mutation resulting in reduced pericyte coverage.

Results: ZSF1 obese rats developed diastolic dysfunction at 21 weeks of age associated with cardiomyocyte hypertrophy and fibrosis. However, ZSF1 rats showed cardiac pericyte loss as of 14 weeks, so prior to capillary density loss and diastolic dysfunction. Human HFpEF biopsies also showed reduced pericyte coverage, indicating clinical relevance. Female PDGF-Bret/ret mice spontaneously developed diastolic dysfunction in the absence of any comorbidities, presenting most histological signs of HFpEF. Exposure of pericytes to oxidative stress or high glucose resulted in downregulation of cell cycle and contraction-associated pathways, and upregulation of inflammatory pathways. *In vivo*, this is mirrored by reduced proliferation of pericytes, increased capillary diameter and endothelial cell (EC) activation. Pericytes exposed to oxidative stress induced inflammation and decreased VE-Cadherin expression in EC when co-cultured.

Conclusion: We show that pericyte dysfunction alone can induce EC inflammation, and propose that this initiates/amplifies the downstream cascade of microvascular dysfunction driving myocardial remodelling in the development of HFpEF.

Talk 6: Dimethylation of histone 3 lysine 9 (H3K9) suppresses post-natal cardiomyocyte proliferation

Konstantinos Chatzieleftheriadis, Malina Doynova, Rosa Donate Puertas, Mani Sadredini, Samaneh Ekhteraeitousi, Emma L. Robinson, Patrick Brien, Ivar Sjaastad, H. Llewelyn Roderick[†]

Background/Introduction: The epigenome plays a fundamental role in regulating gene expression. Histone methylation is an important contributor. Cardiomyocytes (CMs) lose gradually their proliferative capacity postnatally, rendering the injured heart unable to repair itself. We hypothesized that the repressive epigenetic mark H3K9me2 which is mainly deposited by the euchromatic histone methyltransferases Ehmt1/2, contributes to CM cell cycle gene silencing. We proposed that loss of H3K9me2 would prolong the window of CM proliferation and augment regeneration.

Purpose: Investigate the role of histone methylation in cardiomyocyte cell cycle and establish whether manipulation of this process can be targeted for therapeutic benefit.

Statement of Methods: FACS sorting of CM nuclei coupled with chromatin immunoprecipation for characterization of histone methyl marks across genome during development, RNA-seq or/and qPCR to identify and validate gene targets, primary cell culture of neonatal cardiac myocytes (NRVMs) for *in vitro* applications, RNA isolation & qPCR for gene expression analysis, immunofluorescence for protein quantitation and visualization, FACS cell cycle analysis, image analysis, histology, MRI.

Summary of Results: Cell cycle activity was found to be inversely correlated with H3K9me2. Consistently, genes associated with cell cycle in CM nuclei flow-sorted based on H3K9me2 abundance showed an inverse H3K9me2/cell cycle relationship. Ehmt inhibition with the highly selective inhibitor A-366, enhanced CM proliferation *in vitro*, and *in vivo*, in rats and neonatal mice respectively, via H3K9me2 reduction. Enhanced cell cycle activity was detected in myocardial infarction-induced infarct border-zone of mice in which Ehmt2 was conditionally knocked-out in CM, as well as improved cardiac function and reduced scar size.

Statement of Conclusions: These data point to a novel role of EHMT-mediated methylation of H3K9, in the post-natal suppression of cell cycle and proliferation in CMs. Targeting this epigenetic pathway provides a potential strategy for recovering cardiomyocytes lost during MI and ageing.

Talk 7: Molecular characterization and investigation of the role of genetic variation in phenotypic variability and response to treatment in a large pediatric Marfan syndrome cohort

Josephina A.N. Meester[1], SilkePeeters[1], **Lotte Van Den Heuvel[1,2]**, Bart L. Loeys[1]

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Background: Marfan syndrome (MFS) is a rare connective tissue disorder caused by mutations in the *FBN1* gene. Its symptomatic triad consists of ectopia lentis, skeletal overgrowth and aortic aneurysms. The latter can lead to aortic dissections and ruptures, a major life-threatening event. Except for the correlation between cysteine variants and ectopia lentis, no convincing genotype-phenotype associations have been reported.

Aims: In a large cohort of 373 pediatric patients with MFS with a severe cardiovascular phenotype, we explored the proportion of patients with MFS with a pathogenic *FBN1* variant and analyzed whether the type/location of *FBN1* variants was associated with specific clinical characteristics and response to treatment. Patients were recruited based on the following criteria: aortic root z-score > 3, age 6 months to 25 years, no prior or planned surgery, and aortic root diameter < 5 cm.

Methods: Targeted resequencing and deletion/duplication testing of *FBN1* and related genes were performed.

Results: We identified (likely) pathogenic *FBN1* variants in 91% of MFS patients. Ectopia lentis was more frequent in patients with dominant-negative (DN) variants (61%) than in those with haploinsufficient variants (27%). For DN *FBN1* variants, the prevalence of ectopia lentis was highest in the N-terminal region (84%) and lowest in the C-terminal region (17%). The association with a more severe cardiovascular phenotype was not restricted to DN variants in the neonatal *FBN1* region (exon 25-33) but expanded to the *FBN1* variants in exons 26 to 49. No difference in the therapeutic response (atenolol versus losartan) was detected between genotypes.

Conclusion: Important novel genotype–phenotype associations involving both cardiovascular and extra-cardiovascular manifestations were identified, and existing ones were confirmed. These findings have implications for prognostic counseling of families with MFS.

Talk 8: ERBB4-Selective and Sustained activation by NRG1 attenuates atrial fibrosis and fibrillation

J. Van fraeyenhove, M. Tubeeckx, E. Feyen, T. Bruyns, G.R. Y. De Meyer, S. Murphy, H. Heidbuchel, V.F.M Segers, G.W. De Keulenaer

Introduction: Atrial fibrillation (AF) results from electrical and structural remodeling of the atria, in which inflammation and fibrosis play a role. Current therapy does not target the structural problem. Recent studies showed that neuregulin-1 (NRG1) has anti-fibrotic and anti-inflammatory effects in the myocardium.

Purpose: To test the effects of JK07, a NRG1 antibody fusion comprising an ERBB3 antagonistic antibody which selectively signals through ERBB4 preferentially over ERBB3, on atrial fibrosis and AF inducibility.

Methods: Rat atrial samples were harvested (male Wistar Han, 10 weeks old), cut into small pieces and treated with or without JK07 (5nM). *Col1a1* and *Col3a1* mRNA was quantified after 24-72 hours. In a first AF mouse model (male C57BL/6N, 12-15 weeks old), mice were treated with angiotensin-II (Ang-II, 4 weeks, osmotic mini-pumps, 3000 ng/kg/min), and in a second AF model, mice received a high fat diet (HFD, 8 weeks, 60% Kcal fat) inducing severe weight gain (56±3% increase compared to 23±4%, control). AF inducibility was tested by 5 runs of programmed electrical stimulation (PES) with a trans-jugular octapolar catheter. AF inducibility (% mice inducible ≥3 PES-runs) and duration of AF were recorded. Mice were randomized for treatment with vehicle or JK07 (2x/week, 1 mg/kg, IV, n=5-7/group).

Results: In cultured atrial samples, *Col1a1* and *Col3a1* mRNA expression gradually increased up to 2-3 fold over 3 days. JK07 attenuated this effect by 59±17% ($p<0.05$). In mice, Ang-II and HFD significantly increased AF inducibility and AF duration. In Ang-II mice, JK07 attenuated AF inducibility (from 57% to 20%) and AF duration (from 33.3 ± 15.1 to 1.5 ± 1 s). In HFD mice, JK07 significantly attenuated AF inducibility (from 57% to 0%) and AF duration (from 10.9±3.2 s to 0.76±0.5 s, $p<0.05$).

Conclusions: These results show anti-fibrotic effects by selective ERBB4 stimulation with JK07 in atrial tissue *in vitro,* together with AF-preventive effects in two unrelated mouse models.

Talk 9: Feedback loops regulating mechanical homeostasis in cardiac fibrosis

Authors: Yuliia MYKHAILOVSKA[1], Charlène JOUY[1], Nicolas BAEYENS[1]
Affiliation: [1]Laboratoy of Physiology and Pharmacology, Faculty of Medicine, ULB

Myocardial infarction is a global problem, contributing to a third of deaths in developed countries. The injury leads to a massive cardiomyocytes loss, swiftly replaced by a fibrin-based clot. Many cells are involved in the healing process, and the most prevalent are cardiac fibroblasts. Their phenoconversion into myofibroblast results in replacing a fibrin-based scar with a collagen-based one. By doing so, the original stiffness and loading are restored, which is essential for proper scar formation. Finally, a negative-feedback mechanism is initiated to reestablish mechanical homeostasis of the tissue. On the other hand, scarification can get out of control, launching a positive-feedback loop and initiating reactive fibrosis by increasing collagen fibers to the point where the extracellular matrix becomes stiff. This event can be observed in the scar border zone and lead to a poor patient prognosis. The decisional switch mechanism between physiological scarification and reactive fibrosis is unknown.

We hypothesize that cell integration of mechanical forces plays a crucial role in regulating scar formation.

We have developed two different 3D matrix models mimicking physiological scarification or reactive fibrosis. With these models, we studied the role of mechanical forces on the feedback loops. We observed that TGFBR1 expression is directly regulated by changes in strain polarity, rather than stress intensity, a key differentiator of the healing scar versus the border zone. Further, we identified let7c, an upregulated miRNA in the fibrotic model, as a potential key regulator of TGFBR1 translation through its 3'UTR. Using antiLet7c oligonucleotides repressed the overexpression of TGFBR1 in response to unpolarized strain, with a drastic reduction in Smad2 activation. On the other hand, using a Let7c mimic in a model of physiological scarification spontaneously triggered TGFBR1 overexpression and reactivation of the TGF beta signaling cascade.
Currently, there does not exist any therapeutic method that targets the switch mechanism in scar formation to overcome and reverse reactive fibrosis development. Characterizing the switch driving the decisional point towards either physiological scarification or pathological fibrosis will uncover druggable targets.

Talk 10: Anti-thrombogenic, Biocompatible, and Hydrodynamic Prosthetic Heart Valve for Life: Creative Polymer Chemistry for a Sustainable Future

Melo S F[1], Pierrard A[2], Aqil A[2], Ditkowski B[1], Jérôme C[2], Lancellotti P[1,3,4], Oury C[1]

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Valvular heart diseases affect about 13% of the elder population (>75), which represents more than 100 million people worldwide. The only available treatment is heart valve replacement, either with mechanical or biological prostheses. The ideal prosthesis does not exist. Mechanical prostheses provide long-term durability, but bring along high risk of thromboembolism. While biological prostheses provide better hemocompatibility, they are prone to degeneration. Our team currently assesses innovative synthetic polymers as potential biomaterials for prosthetic valves with improved hemo/biocompatibility compared to medical grade polyurethane (PU). We developed and tested non-isocyanate polyurethanes (NIPUs) that represent the "green" alternative to PU, since their production is free of toxic diisocyanates. Hemo/biocompatibility was evaluated by incubating PU/NIPUs with different blood components and human cells. Hemolysis tests were performed with washed red blood cells, activation of the coagulation cascade was evaluated using platelet-poor plasma (PPP), and platelet adhesion was quantified upon incubation with platelet-rich plasma. Cytotoxicity was investigated using primary human fibroblasts. After hemo/biocompatibility verification, tri-leaflet valves were produced by injection molding and tested in a pulse duplicator. NIPUs did not show hemolytic effects, inducing less than 2% of hemolysis, similarly to PU. NIPUs did not activate the contact phase of coagulation upon incubation with PPP, in contrast to PU, that shortened the clotting time. Platelet adhesion on NIPUs surfaces was reduced when compared to PU. Altogether, these data confirm low thrombogenicity and improved hemocompatibility of NIPU, which outperforms PU. Upon contact with NIPUs, human fibroblasts kept their normal growth and shape. Synthetic aortic valves made of NIPUs showed hydrodynamic performance comparable to the bioprostheses used in clinics. NIPU valves experienced low regurgitation (<10%), normal transaortic flow, and regulation-compliant effective orifice areas (>0.85 cm2). In conclusion, our team sets up the basis for future development of sustainable, hemocompatible and hydrodynamic-competent prosthetic heart valves made of NIPU.

Poster 1: Creation of two distinct isogenic *TTNtv* hiPSC cell-lines

Hanne Boen[1,2], Bert Vandendriessche[2], Aleksandra Nijak[2], Peter Ponsaerts[3], Emeline Van Craenenbroeck[1], Bart Loeys[2], Maaike Alaerts[2]

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Background: Truncating variants in *TTN (TTNtv)* are common in patients with idiopathic and 'secondary' dilated cardiomyopathy. More proximal I-band *TTNtv (TTNtvI)* harbor less pathogenic potential than distant A-band *TTNtv (TTNtvA).* These location-dependent effects of *TTNtv* need further investigation, especially in the setting of environmental factors.

Aims: To create isogenic human induced pluripotent stem cell lines (hiPSC) for the investigation of *TTNtvI* and *TTNtvA* in hiPSC-derived cardiomyocytes (hiPSC-CMs) in basal conditions and in the presence of an environmental second hit.

Methods: Fibroblasts of a healthy female control individual were reprogrammed to hiPSC. Isogenic *TTNtv* hiPSC lines were created using CRISPR/Cas9 and RNP nucleofection. Exon 48 (E48), located in the I-band, and exon 357 (E357), located in the A-band were targeted. When cells had recovered after nucleofection, a dilution seeding was performed. Single colonies were picked, half of the suspension was reseeded for expansion and half was used for genotyping using Sanger sequencing. Heterozygous and homozygous *TTNtvI* and *TTNtvA* lines were expanded and their genotype was confirmed by NGS. Off-targets were assessed by Sanger sequencing.

Results: A high editing efficiency was obtained. For E357 only 7,5% of all picked clones contained no editing event (=100% wild type), whereas for E48 33,3% of clones were unedited. We created two isogenic lines with a heterozygous *TTNtv* located in the I-band (E48): c.13836_13837insGATACATACAA (p.Leu4612*) and c.13836_13853del (p.Leu4612*) and two with a heterozygous *TTNtv* located in the A-band (E357): c.100373_100374insT (p.Glu33459*) and c.100373delT (p.Val33467*). No off-target editing was present on all predicted sites with ≤ 3 mismatches.

Conclusions:
We created isogenic *TTNtvI* and *TTNtvA* hiPSC lines. These lines will be differentiated into hiPSC-CMs and further characterized using traction-force microscopy to assess contractility, multi-electrode array and western blot of titin. These lines can be exposed to environmental factors to assess the difference in susceptibility.

Poster 2: Characterization of the human dental pulp neurovascular system and its remodeling at various stages of pulpitis

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Background: Dental decays is the world's leading chronic diseases. The dental pulp is a soft tissue that comprises a complex neurovascular system. It has the unique feature to be located within hard, mineralized and not stretchable walls of dentin and enamel. Pulpitis, the pulp inflammation, is a progressive disease that can be reversible at the earliest stages but can progress towards irreversible pulpitis at the latest stages. To date, there is a lack of understanding on how the pulp reacts to the insult, and how the neurovascular network is remodeling at the various stages of pulpitis.

Aims: This study aims to characterize the neurovascular system of the human sound dental pulp and its remodeling at different stages of tooth decay.

Materials and Methods: Sound (n=6) and diseased (n=21) human molar teeth were extracted for medical reasons and divided in 5 groups according to the severity of the disease (stages 0 to 4). Samples of 1mm of thickness were prepared and clarified using a clearing protocol. They were immunomarked using vascular, fibrotic, neuronal, or odontoblastic antibodies and 3D volumes were imaged.

Results: We have mapped the complete neurovascular architecture of the sound tooth, describing the complex architecture of the vascular network. Spatial disorganization and outward remodeling of the vessels (arteriogenesis) were first observed, we then observed some immature angiogenesis then capillary regression and EndMT at later stages of the disease, followed by fibrosis development.

Conclusions: We have developed an innovative methodology to image human dental pulp without removing it from its hard tissue, preserving the fine interface between the pulp and the dentin. Our study demonstrates that vessel remodeling is an important determinant of the response to tooth decay. Controlling vascular remodeling is therefore expected to be a possible target to promote tooth regeneration.

Poster 3: Development of Small-molecule ERBB4 Agonists as a Treatment for Heart Failure

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Introduction: Despite recent therapeutic advances, chronic heart failure (CHF) remains a common and fatal condition. To decrease morbidity and mortality, new therapeutic strategies based on novel targets are needed. The neureguline-1 (NRG1)/ERBB4 signaling pathway represents such a new target due to its pleiotropic profile and key role in cardiac (patho)physiology. Currently, recombinant NRG1 (rNRG1) is being tested in phase II and III clinical trials for CHF. However, the applicability and efficacy of rNRG1 in a chronic disease regimen is limited due to the need for intravenous administration.

Aim: To develop selective small-molecule ERBB4 agonists that circumvent the drawbacks of NRG1 but still exert ERBB4-mediated cardioprotective effects.

Methods and results: We performed a first high throughput screening (HTS) of 10,240 compounds (cpds) and a second ultraHTS (in collaboration with the European Lead Factory) of 530,000 cpds, resulting in 2 series of structurally unrelated ERBB4 agonists, 15 cpds in total. All 15 hit cpds induced ERBB4/ERBB4 dimerization (E_{max} = 9–56% relative to NRG1, EC_{50} = $6x10^{-6}$ to $1x10^{-9}$M), but not dimerization of the oncogenic ERBB2/ERBB3 receptor pair. *In vitro*, the hit cpds showed no or limited cytotoxicity (adenylate kinase assay), and did not induce cancer cell proliferation. In cultured rat atrial cardiomyocytes, selected cpds (4–32 µM) showed a significant and dose-dependent attenuation ($P<0.05$) of cell death after exposure to 100 µM H_2O_2. Furthermore, the selected cpds (4–32 µM) showed a significant and dose-dependent decrease ($P<0.05$) in collagen expression after exposure to 10 ng/mL TGFβ in human dermal and atrial fibroblasts. Moreover, our lead cpd significantly decreased myocardial fibrosis (by 76±26%) and collagen expression in a mouse model of angiotensin II (1000 ng/kg/min)-induced left ventricular myocardial fibrosis.

Conclusion: We identified 15 selective, potent, and non-toxic small-molecule ERBB4 agonists, with cardioprotective effects *in vitro* and *in vivo*.

Poster 4: Interplay between cytoskeleton and cardiac metabolism: the role of α-tubulin acetylation on Lys40 in glucose transport

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Background: Cardiovascular disease remains the leading cause of death in the diabetic population. Diabetic patients develop a specific heart condition called diabetic cardiomyopathy which is characterized by altered metabolism in the early stages of the disease. Our group previously demonstrated that overfueling the heart with branched-chain amino acids, ketone bodies or fatty acids, substrates that are upregulated in diabetes, inhibits glucose entry in cardiomyocytes through a global rise in protein acetylation that interferes with glucose transporter 4 (GLUT4) translocation to the plasma membrane. However, the acetylated proteins involved in this process were not yet identified.

Aim: We investigated the role of α-tubulin, an important cytoskeleton element responsible for GLUT4 translocation, that is known to be acetylable on Lys40 (K40).

Methods: Acetylation level of a-tubulin on K40 was evaluated in a mouse model of diabetes (high-fat diet, 4 months) and in primary cultured adult rat cardiomyocytes. Its acetylation level was modulated by using a pharmacological inhibitor of its deacetylase (tubacin 10µM, 5hrs) and by overexpressing a non-acetylable form of tubulin (mCherry a-tubulin K40A). Glucose transport was measured by following the detritiation rate of 2-^{3}H-glucose in insulin-sensitive and insulin-resistant cardiomyocytes. GLUT4 translocation was assessed by fluorescent immunostaining in cells expressing the fusion protein HA-GLUT4-GFP.

Results: Acetylation of a-tubulin on K40 was significantly enhanced in the heart of diet-induced diabetic mice. Experimentally increasing its acetylation level in cardiomyocytes with tubacin impaired GLUT4 translocation to the plasma membrane and reduced both basal and insulin-dependent glucose uptake. On the other hand, decreasing α-tubulin K40 acetylation via the overexpression of the K40A form of tubulin stimulated glucose transport. Interestingly, reducing a-tubulin K40 acetylation similarly promoted glucose entry in insulin-resistant conditions.

Conclusion: Cardiac a-tubulin K40 acetylation, which is elevated in diabetes, can modulate glucose entry in the heart. This may lead to new therapeutic strategies preventing the metabolic changes occurring during the development of diabetic cardiomyopathy.

Poster 5: Single-nucleus demultiplexing based on genetic variation supports effective experimental design

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Background: Single-nucleus RNA sequencing (snRNA-seq) has become a powerful tool to identify transcriptional change and cell types. Pooling cells from multiple individuals in a single capture provides several advantages, including reduced batch effects and lower library preparation costs. Bioinformatics demultiplexing tools based on inherent genetic variation between individuals can be used to assign droplets to a specific individual. However, these tools have not been evaluated in the heart. The inaccurate assignment of droplets to individuals might result in erroneous scientific conclusions.

Aims: Here, we aim to evaluate the performance for demultiplexing accuracy of cell assignment by using inherent genetic differences.

Methods: The *in silico* benchmark evaluation was performed by combining raw sequencing reads from 4 single-nucleus ventricle samples. We evaluated a commonly used demultiplexing tool, Vireo, and selected 2.5 million markers obtained from the SNP array as the genotype reference. We also included varying proportions of simulated doublets by merging raw sequencing reads from multiple cell barcodes.

Results: Our results demonstrated that genetic variation-based demultiplexing provides high recall and precision in the heart. Even with 20% simulated doublets, it can still achieve high accuracy. Our results also showed that demultiplexing tool has the ability to assign samples correctly at an accuracy of 95% in real snRNA samples.

Conclusions: Demultiplexing strategy based on genetic variation supports the experimental designs that incorporate sample pooling in multiplexed snRNA-seq experiments

Poster 6: Linking platelet lipidome and inflammatory response in septic patients

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Background: Sepsis is characterized by major endothelial dysfunction, microvascular alterations, and coagulopathy. Platelets are important players in sepsis as they promote thrombo-inflammation and generate lipidic mediators of inflammation. Previously, we observed that platelet acetyl-CoA carboxylase 1 (ACC1), responsible for *de novo* lipid synthesis, is phosphorylated and thus inhibited by AMP-activated protein kinase (AMPK) upon thrombin stimulation, leading to platelet lipidome changes. The role of the AMPK-ACC signaling in the regulation of platelet lipidome during sepsis, and its potential impact on inflammation, has never been investigated.
Aim: To investigate whether changes in ACC phosphorylation can influence the platelet lipidome and the inflammatory response of patients during sepsis.
Methods: Platelets were isolated from 48 septic and 48 control patients. In parallel, mice injected intraperitoneally with lipopolysaccharides were used as a model of endotoxemia. Platelets ACC1 phosphorylation/expression levels were measured by western blot. Lipidomics analysis was carried out by untargeted liquid chromatography–mass spectrometry or on the commercial Lipidyzer platform. Plasma factors were measured by Enzyme-linked immunosorbent assays (ELISA).
Results: Platelet ACC1 expression and phosphorylation were increased both in septic patients and in septic mice. Lipidomics analysis highlighted significant changes of lipids in human and murine platelets during sepsis, with a substantial reduction in phosphatidylcholines and phosphatidylethanolamines containing long polyunsaturated fatty acid chains (ω3 and ω6), which are key phospholipids for the generation of pro- and anti-inflammatory lipid mediators. Our preliminary data obtained in humans and mice, suggest that elevated ACC phosphorylation in sepsis is associated with a higher content of these phospholipids as well as a plasma reduction in myeloid inflammatory parameters

Conclusions: Our data reveal that critical changes in the platelet lipidome occur during sepsis and may contribute to the pathophysiology of the disease. Our results also suggest that ACC can influence the platelet lipid content and/or composition.

Poster 7: Defects in the first hybrid domain of fibrillin-1 affect vascular wall homeostasis in the thoracic aorta

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Background: Aortic dissection and rupture is the main cause of early cardiovascular mortality in patients with Marfan syndrome (MFS). MFS is caused by a fibrillin-1 deficiency, which binds transforming growth factor beta (TGF-beta) via interaction with latent TGF-beta binding proteins (LTBPs). The role of TGF-beta in MFS has been controversial, with earlier studies suggesting that excess release of TGF-beta due to decreased interaction with dysfunctional fibrillin-1 leads to aortic dilation and vascular damage, while other studies have shown an important protective effect.
Aims: We aim to use dedicated mouse models for MFS, with defects interfering with TGF-beta binding, to gain insights into the role of TGF-beta signaling in aneurysm formation and dissection.
Methods: Mice lacking the fibrillin-1 binding site for LTBPs (Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ), mice with a truncated fibrillin-1 (Fbn1GT-8/+), and mice with a combination of both alleles (Fbn1GT-8/H1Δ) were subjected to cardiac ultrasound and ex vivo synchrotron X-ray imaging.
Results: Only Fbn1GT-8/H1Δ mice showed increased mortality due to aortic rupture starting at 4-5 months of age, whereas all other mice had a normal lifespan. Aortic root dilatation occurred in both Fbn1GT-8/+ and Fbn1GT-8/H1Δ mice at 6 months of age, but not in Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ mice. Significant elastic lamellae fragmentation was observed in the thoracic aortic wall of Fbn1GT-8/+ mice, and to a larger extent in Fbn1GT-8/H1Δ mice. Surprisingly, localized elastin fragmentation was also found in the ascending thoracic aorta of Fbn1H1Δ/+ and Fbn1H1Δ/H1Δ mice despite a lack of aneurysm development.
Conclusions: Our data indicate that loss of LTBP binding to fibrillin-1 leads to the development of localized microdissections in the absence of aortic aneurysm, and exacerbates the aortic wall morphology in mice with truncated fibrillin-1. We therefore hypothesize that local TGF-beta sequestration is required to maintain aortic homeostasis.

Poster 8: Right ventricular-pulmonary arterial uncoupling in experimental HFpEF in rats

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Pulmonary hypertension (PH) and right ventricular (RV) failure are common complications of heart failure with preserved ejection fraction (HFpEF) tightly linked to worse morbidity and increased mortality in patients. To date, therapeutic options are limited for PH-HFpEF.

In this context, we sought to decipher mechanisms underlying the pathogenesis of PH, as well as the RV-pulmonary arterial (PA) uncoupling in an experimental model of obesity-associated HFpEF in rats.

Obesity-prone (OP) and -resistant Sprague Dawley rats were respectively fed with high-fat or standard rat chow diet for 12 months (n=10 in each group) and evaluated by echocardiography, left (LV) and RV catheterization, histological and PA vasoactive analyses.

Twelve-month high-fat diet fed OP rats presented with HFpEF, characterized by preserved LV ejection fraction and LV diastolic dysfunction (assessed by increased LV end-diastolic pressure) associated to concentric LV hypertrophy and fibrosis. In HFpEF rats, RV systolic pressure increased (31.6 ± 1.0 *versus* 26.3 ± 0.5 mmHg, $p<0.001$), with no significant changes in PA remodeling and inflammatory cell infiltration. *Ex vivo* PA contraction to phenylephrine and endothelin-1 was similar in both groups. In HFpEF rats, echocardiography showed increases in PA diameter and PA-to-aorta diameter ratio, associated with an increase in RV-to-LV end-diastolic area ratio. RV function was altered, with decreased fractional area change (FAC) and altered RV-PA coupling (assessed by TAPSE/sPAP). Cardiomyocyte apoptotic rate (assessed by TUNEL staining) was increased in the RV of HFpEF rats and correlated to RV-PA uncoupling. Seric levels of sST2, interleukin(IL)-6,-1β -17a and vascular endothelial growth factor (VEGF) increased in HFpEF rats, while NT-proBNP decreased.

Our experimental model of obesity-associated HFpEF naturally develops PH, as it occurs in patients. This PH was not associated with PA remodeling, nor sustained PA vasoreactive response. However, RV structure and function, as well as RV-PA coupling were altered in HFpEF rats.

Poster 9: Comparison of two obesogenic models on the systemic appearance of post-translational modifications involved in diabetic cardiomyopathy

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Background: Recent studies show an important role of protein acetylation and O-GlcNAcylation, two post-translational modifications (PTMs) in the development of type 2 diabetes (T2D) and associated cardiomyopathy (DCM). However, the mechanisms underlying these events remain unclear and their role in other tissues participating in the development of T2D such as skeletal muscle has never been explored.

Aims: We aim to characterize O-GlcNAcylation and acetylation profile in the skeletal muscle and their role in the development of glucose intolerance, insulin resistance and DCM.

Methods: PTMs and the expression of their regulatory enzymes were assessed by Western blot. Protein lysates were obtained from gastrocnemius (glycolytic) and soleus (oxidative) muscles of mice under control diet, high-fat diet (HFD) or high-fat high-sucrose diet (WD) for 1, 2 or 4 months. Obesity, glucose intolerance and insulin resistance were assessed in parallel.

Results: Obesity and T2D develop over months in both HFD and WD mice. However, HFD promotes more rapid metabolic alterations than WD mice, with a higher body weight, amount of adipose tissue, glucose intolerance and insulin resistance. Protein O-GlcNAcylation mainly increases in WD gastrocnemius from the first month of diet while it is delayed in WD soleus. At 4 months, this increase is associated with a decrease level of O-GlcNAcase protein, which is responsible for O-GlcNAcylation removal. On the other hand, protein acetylation increases mainly with HFD in both type of skeletal muscle. The deacetylase SIRT2 protein is decreased in gastrocnemius while it is increased in soleus.

Conclusion: Consistent with what we previously observed in the heart, HFD preferentially induces acetylation while WD mainly promotes O-GlcNAcylation. Interestingly, those PTMs seems to appear and to be regulated differently depending on tissue metabolism. SIRT2 is particularly noteworthy and will be further investigated since it has been previously linked to both acetylation and O-GlcNAcylation.

Poster 10: Role of platelet GARP in post-myocardial infarction fibrosis

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Introduction. After myocardial infarction (MI), cardiac fibroblasts differentiate into myofibroblasts. Myodifferentiation leads to excessive extracellular matrix proteins secretion within the myocardium, resulting in fibrosis which holds a prominent role in adverse left ventricular (LV) remodeling. TGFβ1 is the main mediator of myodifferentiation and is abundantly produced by platelets. Moreover, platelets are involved in TGFβ1 activation via the Glycoprotein A Repetitions Predominant (GARP) present on their surface. Although platelets are known to infiltrate the infarcted myocardium, their role as a source of active TGFβ1 in post-MI cardiac remodeling has never been investigated.

Aims. The current proposal sought to determine the contribution of platelet GARP in the regulation of fibrosis and LV remodeling after MI.

Methods. We generated a new Cre transgenic mouse strain that allows megakaryocyte/platelet specific invalidation of GARP (GpIba-Cre x GARPfl/fl). Platelet function and serum production of active TGFβ1 was assessed by FACS and ELISA, respectively. WT and pGARP KO mice underwent MI via permanent ligation of left anterior descending coronary artery. Immune cells infiltration and cardiac fibrosis were evaluated by immunohistochemistry or RT-qPCR. Cardiac function was measured by echocardiography.

Results. GARP exposure at platelet surface increased upon thrombin and CRP stimulation. Interestingly, GARP deficiency does not impair platelet function but dramatically reduces serum production of active TGFβ. Following MI, platelets infiltrate the myocardium between 1 and 3 days, compared to the sham-operated mice. As expected, a decrease in collagen I, and III mRNA content were measured in pGARP KO infarcts, at 7 days post-MI. We revealed an increased content in M2 macrophages at 3 days post-infarction. Moreover, preliminary echocardiography data highlights the presence of LV pseudoaneurysm and increased LV dilation in the pGARP KO infarcted hearts.

Conclusions. Our data confirms that GARP protein is crucial for TGFβ1 activation. The early platelet infiltration into the infarcted myocardium seems to be associated with a modulation of the inflammatory process, which might impact the healing process.

Poster 11: Dielectric barrier discharge-treated aligned electrospun chitosan/polycaprolactone nanofibers for cardiac tissue engineering

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Keywords: Electrospinning, nanofibers, plasma treatment, stem cell-derived cardiomyocytes, maturation

The potential of stem-cell derived cardiomyocytes in heart failure therapy and disease modelling is enormous, but still hampered by the immaturity displayed by these cells. Several strategies aiming to improve the maturity and clinical applicability are being investigated, including patterning of the cardiomyocytes on substrates mimicking the *in vivo* ECM environment. Material surface properties, both topographical and chemical are paramount for optimal cell-substrate binding and cell function. In this work, the potential of Argon and Nitrogen plasma-treated chitosan/polycaprolactone nanofibers (NFs) in cardiac tissue engineering is explored. Random and aligned oriented NFs were electrospun, plasma-treated and subsequently characterized by contact angle goniometry, scanning electron microscopy, X-ray photoelectron spectroscopy and tensile testing. A plasma treatment time of 15s at medium pressure showed an oxygen surface content of 30% and 28% in Ar and N2 discharges, respectively. The surface nitrogen content was elevated to 3% in the N2 condition. Seeding human embryonic stem cell-derived cardiomyocytes on untreated and plasma-treated NFs revealed increased attachment and spreading after one week of culture. The aligned NFs also induce alignment of the cardiomyocytes, which was less pronounced in the untreated and geltrex-coated (control) aligned NFs. Cardiomyocytes attached to the aligned plasma-treated NFs showed an increase in sarcomere distance irrespective of gas environment, suggesting increased sarcomere organization and maturation.

Poster 12: Dual antiplatelet therapy is associated with high α-tubulin acetylation in circulating platelets from coronary artery disease patients

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Background: Platelet inhibition is the main treatment strategy to prevent atherothrombosis. Despite dual antiplatelet therapy (DAPT) combining aspirin and a P2Y12 receptor inhibitor, high on-treatment platelet reactivity (HPR) persists in some patients due to poor response to treatment and is associated with ischemic risk. It remains unknown if circulating platelets in high-risk patients have distinct morphological characteristics which could contribute in their pro-thrombotic potential.

Aims: Knowing the key role of α-tubulin acetylation in regulating platelet shape change, we investigated whether this post-translational modification differs according to the antiplatelet therapy and the response to it.

Methods: Platelets were isolated from arterial blood samples of 240 patients admitted for coronary angiography and acetyl α-tubulin levels were evaluated by immunoblotting. Platelet reactivity was assessed using multiplate analysis. 141 (59%) patients were taking aspirin among which 32 (13%) were treated with an additional P2Y12 inhibitor. HPR was detected in 8 out of 32 DAPT-treated patients. Participants provided written informed consent and the study was approved by the institutional **ethics committee.**

Results: Platelet α-tubulin acetylation was significantly increased in DAPT-treated patients ($p<0.001$). A minority of non-treated patients (11.4%) exhibited high α-tubulin acetylation level (third acetyl α-tubulin tertile). In contrast, most patients on DAPT without high residual platelet reactivity (83.3%) belonged to this high acetyl α-tubulin tertile. Interestingly, the proportion of patients with high α-tubulin acetylation level (37.5%) was drastically decreased among DAPT-treated patients with HPR. Multivariate logistic regression further supported that efficient DAPT was associated with a 27-fold increase in the chance of having high α-tubulin acetylation ($p<0.001$).

Conclusions: We revealed high platelet α-tubulin acetylation as a potential marker of adequate platelet inhibition in response to DAPT. Since α-tubulin acetylation is a hallmark of stable microtubules, its increase could contribute to maintaining resting morphology of circulating platelets.

Poster 13: Contribution of mechanical forces from blood flow in neurovascular aneurysmal malformations

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Vein of Galen aneurysmal malformations are anomalies of the cerebral vasculature representing up to 30% of pediatric vascular malformations. Unfortunately, it is associated with a poor prognosis for young patients. This malformation is associated with a germinal mutation within *EPHB4* or *RASA1* genes. Interestingly, not all carriers of *RASA1* or *EPHB4* germline mutations develop this high-flow aneurysm which only occurs at that precise location.

We hypothesized that abnormal vascular remodeling events occur at this precise location because it is subjected to specific, disease-enabling blood flow profiles.

We used a zebrafish embryo model in which the dorsal longitudinal vein (DLV), the homolog of the vein of Galen, develops between 50 hpf and 72 hpf. This allows for the complete visualization of both hemodynamics and angiogenesis. Morpholino knockdown of rasa1a or Rasa1a mutants generated by CRISPR fully recapitulated the phenotype in human patients. During the development of the DLV, we observed a formation with two or three lumenized vessels on the posterior segment and independent connections of anterior vessels as observed in choroidal malformations. Hemodynamics analysis explained the formation of multiple perfused vessels: the DLV is a collecting vessel with multiple pulsatile streams which do not mix. Rasa1a mutants and morphants fail to develop a fused collecting vessel and, instead, generate independent vessels, without increasing cell numbers by proliferation.

This study highlights the role of RASA1 in forming collecting vessels and capillaries networks during angiogenesis and the contribution of specific hemodynamics as a second hit to trigger malformations. It might play a major role in endothelial cell specification.

Poster 14: Optimization of seeding conditions of induced pluripotent stem cell-derived cardiomyocytes on multi electrode array (MEA) devices

Dogan Akdeniz, Eline Simons, Dorien Schepers, Bart Loeys, Maaike Alaerts

Background: Induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) are an innovative useful tool for the study of inherited cardiac disorders. They recapitulate the disease phenotype of the patient *in vitro* allowing the study of disease mechanisms through the use of multielectrode arrays (MEA). As MEA offers high-throughput and repetitive analysis of electrically active cells, it's an interesting substitute for the patch-clamp technique. As this technology is rather new, there are still some challenges in its application for analysis of iPSC-CM. An important hurdle is the passaging of iPSC-CM onto the MEA surface.

Aim: The aim of this experiment is to find the optimal seeding conditions of iPSC-CM on MEA devices for electrophysiological analysis.

Methodology: To find the most optimal conditions to passage in-house created iPSC-CM, they were passaged on different timepoints (day 20 or 30 of differentiation), density (30 000 or 60 000 cells/6µL) and surface coatings (Matrigel, Fibronectin or Gelatin). These cells were passaged on dummy single-well MEAs and onto glass coverslips, which form a substitute to the MEA surface due to the likeliness between both surfaces.

Results: The success of passaging was evaluated based on the number of cells that survived the passaging and their capacity to form a monolayer consisting of beating iPSC-CM, cell morphology parameters (cell circularity and aspect ratio) and cytoskeletal structures such as sarcomeric alpha actinin (SAA), cardiac troponin I (TNNI), connexin 43 (Cx43) and myosin light chain 2 (MYL2) by immunocytochemistry.

Conclusion: Our experiments showed that in-house differentiated iPSC-CM are better passaged at a later timepoint of differentiation and need to be seeded in a high density on a Matrigel coated surface. These parameters delivered a more mature cell morphology. We found an optimal timeframe for subsequent electrophysiological analysis of five days (between day 5 and day 10 after passaging).

Poster 15: A NGS-based and marker-free CRISPR pipeline speeds up the creation of knock-in zebrafish and hiPSC disease models

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The rearing and selection process of correctly CRISPRed zebrafish and human induced pluripotent stem cell (hiPSC) clones remains time-consuming, especially if you want to introduce the genetic variant in a marker-free way. In general, the average *knock-in* (KI) efficiency is low (<5%). Hence, many fish need to be reared at random while only a fraction potentially passes the edit on to their offspring. Analogously, many hiPSC clones need to be individually screened to increase the chance of finding the KI clone. To functionally assess genetic variants found in Brugada syndrome (BrS) patients we aimed to develop a marker-free, Next Generation Sequencing (NGS) based CRISPR workflow to facilitate fast and efficient CRISPR editing, potentially applicable in a diagnostic setting. To create a BrS KI zebrafish and hiPSC line, different CRISPR component combinations were tested. In zebrafish the components were injected in fertilized eggs at the one cell stage and after targeted NGS (MiSeq), only the embryos with ≥2% KI reads were reared. In the hiPSCs after nucleofection with the components, a dilution seeding was performed and only the wells with the highest KI%, confirmed by NGS, were kept in culture for further validation. The best CRISPR component combination had an average KI% of 2.99 (± 5.09, N=122) in the zebrafish. Currently, already one fish passes on the mutation. For the hiPSC, the best component combination achieved a KI% of 64.2 (± 27.1, N=21 wells). In 51% of the wells (N=35), a pure KI haplotype cell population was found. Hence, homo- and heterozygous KI BrS hiPSC clones were successfully obtained. We showed that with our customised NGS workflow, we can successfully create KI lines in a marker-free way, irrespective of the study model. Due to parallel NGS screening, the overall time needed to obtain the desired KI line is reduced.

Poster 16: JK07 is unable to reduce atrial fibrosis and atrial fibrillation inducibility in a porcine sterile pericarditis model

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Background: Atrial fibrosis forms a substrate for re-entry in atrial fibrillation (AF), and the degree of fibrosis predicts prognosis and therapy refractoriness. Currently, no therapies exist that target atrial fibrosis.

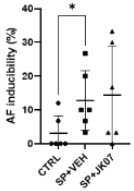
JK07 is a fusion protein that, through selective stimulation of the ErbB4 receptor, has proven its anti-fibrotic properties in animal models of heart failure.

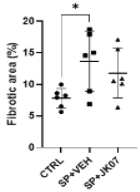
Aims: To test the ability of JK07 to suppress atrial fibrosis and consequently, to reduce the AF inducibility in a sterile pericarditis model of Aachener minipigs.

Methods: 18 pigs were divided into 3 groups (n=6): control (CTRL), sterile pericarditis + Vehicle (SP+VEH) and Sterile pericarditis + JK07 (SP+JK07). All animals underwent sternotomy and implantation of two epicardial pacing leads for electrophysiologic testing. The CTRL group did not receive any other intervention. In the SP+VEH and SP+JK07 group, 3 grams of strile talcum was sprayed on the atrial surface, and a layer of sterile gauze was left behind to induce an inflammatory and fibrotic reaction. The SP+JK07 group was treated weekly with 0,3 mg/kg JK07 intravenously, while the SP+VEH group only received vehicle. After 28 days, AF inducibility was tested by performing 30 burst pacing episodes. Atrial fibrosis was quantified using ImageJ software on Masson trichrome staining of atrial specimens.

Results: AF inducibility in the SP+VEH group was significantly higher compared to the CTRL group (12.78 ± 8.81% vs. 3.12 ± 5.11%, P=0.0424), whereas no significant difference was observed between SP+VEH and SP+JK07 (12.78 ± 8.81% vs. 14.43 ± 14.56%, P=0.8171). Likewise, atrial fibrosis in the SP+VEH group was significantly higher compared to the CTRL group (13.66 ± 4.78% vs. 7.81 ± 1.54%, P=0.0172) while there was no significant difference between SP+VEH and SP+JK07 (13.66 ± 4.78% vs. 11.78 ± 3.92%, P=0.4723).

Conclusions: In the sterile pericarditis model, JK07 was unable to reduce atrial fibrosis and AF inducibility.





Poster 17: Sodium myo-inositol cotransporter 1 affects cardiac hypertrophy in pressure-overloaded mouse hearts

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Background: Sodium myo-inositol cotransporter 1 (SMIT1) belongs to the sodium-glucose cotransporter family and accounts for intracellular accumulation of myo-inositol. SMIT1 is expressed in the heart, where its function still remains unknown.

Aim: Our aim is to establish the contribution of SMIT1 in pathological hypertrophic cardiac remodeling and the progression to heart failure.

Methods: Cardiac hypertrophy and heart failure were induced by transverse aortic constriction (TAC) in SMIT1 WT and deficient (Smit1-/-) mice.

Results: During hemodynamic stress, deletion of SMIT1 reduces cardiac fibrosis and hypertrophy and preserves cardiac function in TAC-operated mice. In isolated cardiomyocytes the lack of SMIT1 prevents cardiac hypertrophy induced by phenylephrine, a pro-hypertrophic agent. This effect on cardiomyocytes size is associated with a reduction in total protein O-GlcNAcylation, a post-translational modification resulting from the hexosamine biosynthetic pathway and controlling cardiac hypertrophy.

Conclusion: Lack of SMIT1 protects the heart from pressure overload-induced hypertrophy by reducing protein O-GlcNAcylation. This work provides important insights into the role of SMIT1 at the onset of heart failure, opening new avenues for potential therapeutic strategy to prevent or treat pathological hypertrophy and heart failure.

Poster 18: Ticagrelor prevents Staphylococcus aureus infective endocarditis by mitigating bacterial virulence

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Background: Infective endocarditis is a deadly disease mainly caused by gram-positive and highly virulent Staphylococcus aureus. Current therapy for these patients consists of antimicrobial therapy, having a low efficacy. Therefore, there is an urgent need to discover new strategies that could prevent this disease.

Aim: Assessing the ability of the antiplatelet drug ticagrelor, having antibacterial activity against gram-positive bacteria, to prevent Staphylococcus aureus Infective endocarditis.

Methods: We used a mouse model of infective endocarditis induced by a Staphylococcus aureus infective endocarditis clinical isolate. Ticagrelor and clopidogrel were administered prior to local histamine infusion on the aortic valve, and infection. The presence of infected vegetations was determined by a Gram staining on heart sections after three days. The antibacterial effect of ticagrelor on key mechanisms of infective endocarditis was assessed in vitro.

Results: A single administration of ticagrelor at conventional antiplatelet dosage (3mg/kg) prior to infection significantly prevented the formation of infected vegetations, with infective endocarditis in 14.3% of ticagrelor-treated mice, compared to 55 % of vehicle-treated mice. Interestingly, clopidogrel treatment failed to prevent disease development with infective endocarditis in 61.1% of mice which made it unlikely that solely the antiplatelet effect would explain infective endocarditis prevention. Ticagrelor dosed at plasma levels achieved in patients (0.75ug/ml-1.25ug/ml) did not affect Staphylococcus aureus growth in liquid cultures but caused drastic adherence defects on activated endothelial cells and extracellular components. We found that growing Staphylococcus aureus in the presence of ticagrelor altered the Staphylococcus aureus accessory gene regulator system, leading to reduced toxin production and toxin-induced platelet aggregation while preserving the ability of platelets to kill bacteria.

Conclusion: Our study demonstrates unprecedented ability of ticagrelor to prevent infective endocarditis by directly mitigating bacterial virulence. Hence, clinical trials using ticagrelor as adjunct therapy to antibiotics in patients at risk for infective endocarditis are warranted

Poster 19: Living myocardial slices as a novel tool to explore cellular mechanisms underlying arrhythmogenesis in the post-myocardial infarction border zone

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Background: Ventricular tachyarrhythmias are the leading cause of sudden cardiac death after myocardial infarction (MI). The post-MI border zone (BZ) has been implicated as a source of premature ventricular complexes (PVCs). Although underlying mechanisms that underlie PVCs have been extensively studied on isolated cells and whole heart preparations, little is known about mechanisms at the tissue level. Established methodologies such as single cell and whole heart electrophysiology are limited in providing in-depth insights into complex cell-cell interactions and tissue microarchitecture. Here, we used living myocardial slices (LMS) that maintain in vivo multicellular structure and interactions.

Methods & Results: We used an established pig model of ischemia /reperfusion. After 4 weeks, LMS were prepared from different areas from MI (BZ and remote) and Sham animals. Using a high precision vibratome, 300 µm thin slices were obtained from a LV tissue block of approximately 1.5 cm2, which was glued to a specimen holder with the epicardium facing down. The slices were then trimmed according to their fiber orientation and mechanically stretched to mimic diastolic preload. Tissue handling was carried out at 4 °C with constant O2 supply. Cardiac slices were then incubated simultaneously with cell permeable calcium dye Fluo8-AM (10 µM) and voltage sensitive dye di-4-ANBDQPQ (10 mM). Tissue contraction was inhibited with blebbistatin (10 µM). 2D images were acquired at 200 Hz. We successfully performed dual recording of action potentials and calcium transients from single cells within a tissue slice under field stimulation at 1 Hz. Following a fast pacing protocol at 2 Hz in the presence of adrenergic stimulation, we successfully captured spontaneous calcium waves and accompanied changes in membrane voltages.

Conclusion: Living cardiac slices will serve as a novel tool to bridge the gap between aberrant behavior of single cardiac myocytes and manifestation of arrhythmia at the whole heart level. Our approach will contribute to a better understanding of cardiac disease in future studies.

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