ACS 2020

JULY 2020

Personal grant laureates

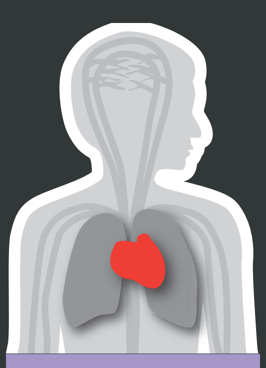
Meet our new professors

Animal-free innovations at ACS

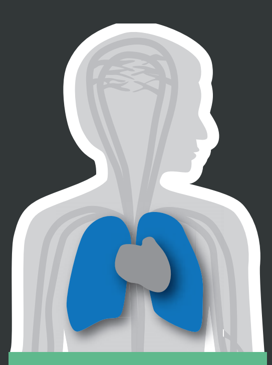
Life after ACS

Mission To design novel treatment strategies to prevent and cure cardiovascular disease.

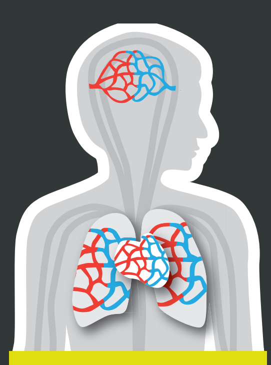
Vision To strengthen our top European Cardiovascular Research Institute by organizing education, research and clinical activities within the 5 Research Programs.



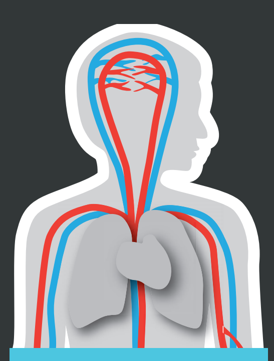
heart failure & arrhythmias



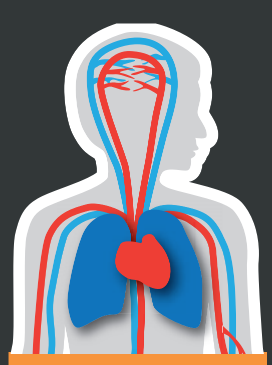
pulmonary hypertension & thrombosis



microcirculation



atherosclerosis & ischemic syndromes



diabetes & metabolism

Science now and in the future

Arthur Wilde & Jolanda van der Velden
Directors of ACS

As our joint research institute will celebrate its 5th anniversary, teaming up in the clinical setting has already become the norm for many departments that are active in ACS. Nephrology was part of Wave 2, and the Cardiothoracic department is part of Wave 3. The two new chairs of the Cardiology and Cardiothoracic departments are Prof. Steven Chamuleau and Prof. Robert Klautz, respectively. It is to be expected that uniting these clinical departments will benefit the cardiovascular research at Amsterdam UMC, which will be further strengthened once all basic cardiovascular science is concentrated at the Meibergdreef.

We are very pleased to welcome Prof. Arthur Wilde, the former chair of the AMC Cardiology department, who is taking over the co-directorship from Prof. Bert Groen. To Bert we say a big thank you for taking care of ACS for almost two years. Arthur aims to optimize and strengthen the clinical research at ACS and to connect it as directly as is possible to preclinical research. A central theme in these efforts will be a new clinical research office organized at ACS. To this end, we hope to motivate our clinical PIs to use their capacity in research support to create a central body, leaving optimal independence for individual PIs. We strongly believe that the ever-increasing regulations around clinical research mandate such an overarching solution.

While patients represent the center of our research, as depicted in the ACS graphic, studies using disease models are essential for our translational research. In this issue several scientists highlight the need for an extensive set of models ranging from animal studies to stem cell-derived models. There is societal pressure to reduce the number of animals used for research, and we have already put a lot of effort into reducing the number of animals needed for our studies. This is exemplified by imaging studies using advanced tools, which enable follow-up *in vivo* studies in animals and thereby reducing the number of animals per study. Another example that illustrates technological innovations which reduce the number of animals for research is high throughput measurements in cardiomyocytes, presented in this issue. Within ACS, we aim to invest in new innovations and collaborations that prepare us for future challenges in cardiovascular research.

Arthur Wilde & Jolanda van der Velden

CONTENTS

Colophon:

Design and layout: Karen Folkertsma
Photographs: DigiDaan
Drawings: Josine de Winter & Pleuni Hooijman (Sketch University)
English editing: Lisa Kohn

ACS Directors: Arthur Wilde & Jolanda van der Velden

Coordinators:
Anne-Lieke van Deijk
Isabelle Vergroesen
Jolanda van der Velden

Website:
www.amsterdam-cardiovascular-sciences.org

STOP THE BLEED, SAVE A LIFE

ADDRESSED ISSUE

3500 deaths from trauma injuries
40% of these due to hemorrhage
Lack of courses on how to stop a bleed
Lack of emergency kits in public spaces

MEDICAL TREATMENT

The three pillars of treatment of life-threatening hemorrhage are: Stop, Supply, Correct
Care is provided from incident to hospital
Time is a lifesaver

AWARDS

1st prize
International IATSIC Conference
2nd prize
Amsterdam UMC Societal Impact Award '19

ACHIEVEMENTS

Lobby Orange & Red Cross
National standard
Courses for citizens, firemen, police & first-aid instructors
Website & webshop
Improved awareness
Lectures & workshops
First-aid courses

Towards a balanced view on animal research

Within our institute, there is ongoing debate on the use of animals in biomedical research. As an *in vitro* cell biologist, member of an Animal Experiment Committee and director of an animal research center, I feel that these discussions should be well balanced and address the following:

WHAT IS THE PROBLEM WE ARE TRYING TO SOLVE?

For some people eliminating animal experiments is an emotional and/or political problem to solve, but these are hardly scientific motives. The problem we really need to solve is to develop and validate the best possible models for human disease. This makes reducing the use of animals in research not a goal in itself, but a potential outcome of the choice for the best model.

Peter Hordijk, dept. Physiology, Scientific director Amsterdam Animal Research Centre

The problem that we really need to solve is to develop and validate the best possible models for human disease

INNOVATION IS EVERYWHERE

Increasingly sophisticated *in vitro* models will address increasingly complex biological processes. Consequently, they will reduce or replace part of the animal-based experiments. At the same time, animal experimentation and the associated high-end technology is also rapidly evolving, resulting in further refinement and reduction. However, it is realistic to say that for a significant portion of biomedical or pre-clinical research, the use of an animal model will remain the best available option.

INTEGRATION AND VALIDATION OF APPROACHES

Ideally, biomedical research integrates *in vitro, in vivo* and *in silico* approaches. If we value equally the experiments that do and those that do not rely on animals, we will create a more balanced view on modeling human disease. It will then be equally valid to critically question experiments on animals as it is to question the use of an organ-on-a-chip or an organoid.

An academic institute such as ours must engage in this debate using scientific arguments and remain optimistic as well as realistic regarding the potential and validity of animal-free new technology.

Animal-free innovations: is it possible to go 100% animal-free and if so, when?

While some people believe that science can do without animal studies, and indicate that the 'valley of death' is largely due to studies in rodents not being translatable to humans, it is my personal belief that we should be very careful and not make the same mistake by thinking that current human-like models better resemble humans than animals do. Of course, stem cell-derived human models have the potential to become key in testing toxicity and effectiveness of new drugs, but we need to be realistic and carefully validate all new human-like disease models. Current stem cell-derived models show fetal-like characteristics, and lack the complex nature of human pathophysiology, in particular for patients with cardiovascular disease caused by multiple co-morbidities.

Moreover, let's not forget the enormous technological advances that have been made to reduce the number of animals used for our studies, and the efforts to increase measurements in patients, despite all the hurdles related to collecting data from patients. Science is best served by a balanced discussion about the right experimental model for a specific research question and patient problem, as emphasized by Prof. Peter Hordijk, director of the animal facility at the Boelelaan. We have asked several scientists to discuss the pros and cons of current experimental models, and to present new innovations. This discussion is also part of a national initiative, of the Dutch Cardiovascular Alliance, to write a position paper on animal-free innovations within the cardiovascular field for the coming 5-10 years. The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) has asked us to write a position paper with the aim of improving the Replacement, Reduction and Refinement (3Rs) of animal procedures. I would like to invite you to join the discussion, and help think about the strategy for the coming years. At ASC we will support a broad strategy in which we combine *ex vivo* and *in vivo* research in animal models and patients, human-like disease models and *in silico* approaches to model human physiology and study pathomechanisms.

The right experimental model for a specific research question and patient problem

Jolanda van der Velden,
dept. Physiology, director of ACS

Reducing or replacing animal models to find better solutions for heart disease

To understand heart disease and find new therapeutics, there is a strong need for good model systems. As researchers from the Physiology and Experimental Cardiology departments, we highlight different approaches for building translational models that minimize or avoid the use of animals.

DOING MORE WITH FEWER ANIMALS

DIEDERIK KUSTER, DEPT. PHYSIOLOGY

Cardiac diseases often originate in the heart muscle cells themselves. In our group we have specialized in measuring contractile function of these cells. Cardiac muscle cells are beautifully and structurally organized, filled to the brim with contractile machinery and mitochondria. Unfortunately, this comes at a price as they sacrifice their ability to divide. This makes culturing these cells nearly impossible. To be able to study cardiac muscle cell contractility, they are isolated from the hearts of, typically, mice and rats. This results in 1000s of cells, of which only very few can be measured, because of the very low throughput of contractile measurements. Together with the start-up company CytoCypher a system was developed that can measure contractile function of 100s to potentially 1000s of cardiac muscle cells a day. We will use this system to perform the first functional screening in cardiac muscle cells to find compounds that improve relaxation in different disease states. This approach allows us to use fewer animals, and to test many more compounds.

INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES (IPSC-CM)

ANKE TIJSEN, DEPT. EXPERIMENTAL CARDIOLOGY

Because we cannot get cardiac biopsies from all patients and adult cardiomyocytes have a limited survival in culture, we implemented the technique of human iPSC-CM culture. Fibroblasts from skin biopsies of patients with specific mutations are reprogrammed to pluripotent stem cells, which can be cultured as cell-lines. By induction and inhibition of certain pathways we stimulate these stem cells to form cardiomyocytes. Eight to ten days after induction of differentiation this results in human cardiomyocytes in a dish, which is always a magical thing to see. These cells are used

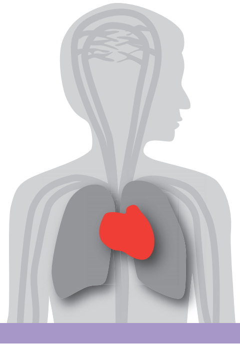
Translational research with fewer animals

for basic molecular studies, and can also be used for functional studies to determine both electrophysiologic and contractility parameters. Furthermore, the development of the CRISPR/Cas9 system has allowed us to correct mutations, change and study common variants or create human cell-lines with deletion of full genes or specific regulatory regions. In the experimental cardiology department, we use these cells to study how mutations in certain genes lead to a specific phenotype, and to study the function of human specific non-coding RNAs or specific regulatory regions. Last but not least these cells also allow us to study possible new therapies based on common human variation, which we would not be able to study in animals.

3D HUMAN HEART MUSCLE MODEL

PAUL WIJNKER, DEPT. PHYSIOLOGY

Our research focuses on the heart muscle disease hypertrophic cardiomyopathy (HCM). HCM is the most common inherited cardiomyopathy and is caused by mutations in genes encoding contractile proteins, the contractile building blocks of the heart. With a 3-dimensional (3D) human heart muscle model we recapitulate the effects of mutations on contractility of the heart. 3D human heart muscles are generated from human iPSC-CM



left to right: **Diederik Kuster, Paul Wijnker, Anke Tijsen**

with specific mutations. This cell culture technique reveals the 'direct' effects of the mutations, since secondary disease changes in, for example, blood-pressure and neuro-humoral signaling are excluded. The 3D human heart muscle model allows us to study contractile function longitudinally, as well as to study and therapeutically intervene with early mutation-induced pathological changes in heart muscle function. This human heart model has an enormous potential for preclinical drug screening and is an important alternative for animal experiments. Only compounds that show promising results in this human heart model will be tested in *in vivo* models, thereby largely reducing the number of animal experiments. The final goal will be to establish mutation-specific therapies by testing clinically available compounds to test the efficacy to prevent and reverse cardiac dysfunction and hypertrophy. We believe that the targeting of the early abnormalities will result in the first HCM therapies that prevent and reverse cardiomyopathy.

Together, we hope that these innovative research tools will give us insight into the mechanisms underlying heart disease and provide an opportunity for highly translational research.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7

Personal Grants

1 **Charissa van den Brom**

2 **Marit Wiersma**

(photo by PicturePeople Fotostudios)

3 **Rik Olde Engberink**

4 **Kak Khee Yeung**

5 **Noam Zelcer**

6 **Esther Lutgens**

(photo by Stefan Heijdendael)

7 **Alexander Vlaar**

8 **Marten Hoeksema**

9 **Hilde Herrema**

10 **Femke Rutters**

Charissa van den Brom, Anesthesiology, Veni 2019: *Inhibition of leaky vessels to prevent kidney damage*

Marit Wiersma, Physiology, NHS Dekker Postdoc 2019: *Unexplored role for desmin and lamin A/C mutations in familial atrial fibrillation: important implications in cardiomyocyte proteostasis derailment*

Rik Olde Engberink, Internal Medicine - Nephrology, NHS Dekker Clinical Scientist 2019: *Nonosmotic sodium storage in heart failure: a new treatment target*
Dutch Kidney Foundation PhD student grant 2019: *Accurate estimation of sodium intake with minimal patient burden*

Kak Khee Yeung, Vascular Surgery, NHS Dekker Senior Clinical Scientist 2019: *The key role of smooth muscle cell function in aortic aneurysms*

Noam Zelcer, Medical Biochemistry, ERC Proof of Concept 2019: *Structure guided inhibition of IDOL to treat atherosclerosis and the metabolic syndrome*

Esther Lutgens, Medical Biochemistry, ERC Proof of Concept 2019: *TRAF-STOP therapy to reduce inflammation in atherosclerosis*

Alexander Vlaar, Intensive Care Medicine, LSBR Fellowship 2019: *Transfusion-associated circulatory overload (TACO) - a breathtaking syndrome.*

Marten Hoeksema, Medical Biochemistry, Marie Curie Individual Fellowship 2019: *Egr1 and Egr2 regulate opposite transcriptional programs in macrophages*

Hilde Herrema, Experimental Vascular Medicine, Diabetes Fonds Senior Fellowship 2019: *Bacteriophages as drivers of gut microbiome composition/function and type 2 diabetes*

Femke Rutters, Epidemiology and Biostatistics, Diabetes Fonds Senior Fellowship 2019: *Effects of irregular sleep timing on glucose metabolism and insulin sensitivity*

Evaluation of awarded ACS grants 2016-2018

At the start of ACS on the 1st of January 2016, ACS provided funds to support talented ACS researchers and to stimulate collaboration between ACS researchers within Amsterdam UMC. Each year several calls have been organized for ACS researchers at different stages of their careers, such as the Postdoc grant (€70,000), MD/PhD grant (€25,000) and the Out of the Box (OOTB) grant (€25,000).

Supporting talented ACS researchers

The Postdoc and MD/PhD grants are for excellent young basic and clinical researchers, respectively, and aim to support talented ACS researchers.

ACS grants support talented researchers and stimulate collaboration between ACS researchers

The Postdoc grant provides the opportunity to obtain postdoc experience for a period of one year in a prestigious research center abroad. Between 2016 and 2018, seven Postdoc grants were awarded by the ACS directorate (Figure 1). All seven laureates obtained additional grants (2 to 3 grants on average), for instance a Veni, Rubicon or Dutch Heart Foundation Postdoc Dekker grant.

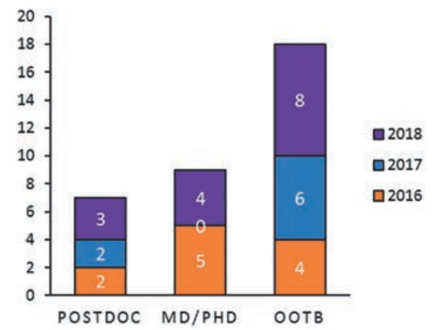
The MD/PhD grant covers one day a week protected research time for a two-year post-doctoral project for medical specialists in training. A total of nine MD/PhD grants were awarded between 2016-2018 (Figure 1). Four laureates (44%) obtained additional grants, of which three laureates received the prestigious Dutch Heart Foundation Senior Clinical Scientist Dekker grant. The ACS Postdoc and MD/PhD grants have led to new data for scientific papers and laid the foundation for future research projects.

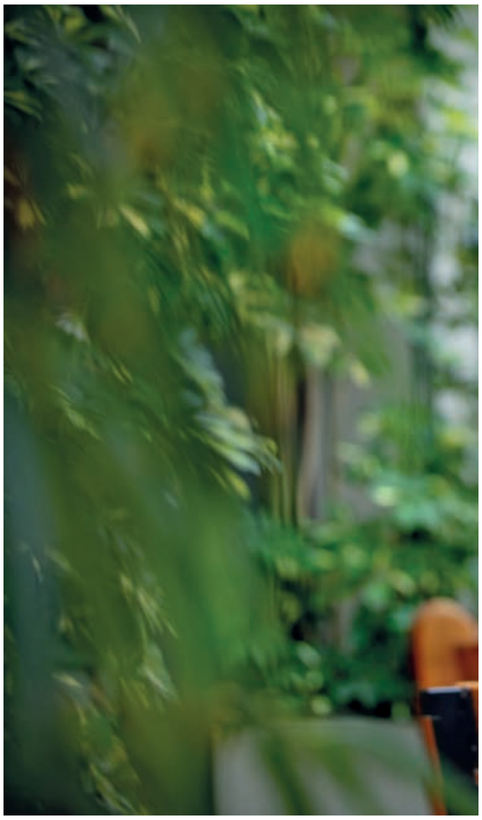
Supporting collaboration between ACS researchers

The 18 awarded OOTB grants aimed at supporting ACS project leaders to set up new collaborations between Amsterdam UMC locations AMC and VUmc and to strengthening joint future grant applications. An impressive number of the awarded project leaders, 17 (94%), indicated that the grants resulted in new collaborations of: newly implemented weekly or monthly meetings, new contacts, shared protocols and materials, and shared PhD students and postdocs. Thirteen newly initiated collaborations (72%) have already published their results, and 10 have resulted in joint grant applications (56%). The recently awarded ZonMw Open Competition grant of Leo Heunks and Aart Nederveen is a good example of the success of the OOTB grants.

This shows that the ACS grants are very effective in supporting talented ACS researchers and in stimulating collaboration between ACS researchers at Amsterdam UMC.

NUMBER OF ACS GRANTS AWARDED 2016-2018





Marten Hoeksema

ACS Postdoc grant 2016

The ACS Postdoc fellowship allowed me to perform my postdoc in the lab of Professor Glass, a world expert in macrophages and gene regulation. As the result of the vibrant and collaborative environment at UC San Diego, I have been able to master novel state-of-the-art genetic techniques and analysis tools, and to expand my scientific network. During my time here, I have identified key transcription factors for activation of both anti-inflammatory and pro-inflammatory gene enhancers in macrophages, fundamental information that we can use for inflammatory and cardiovascular diseases. Based on these findings, I applied for additional research grants and was recently awarded with the American Heart Association Postdoc grant and a Marie Curie Individual Fellowship.

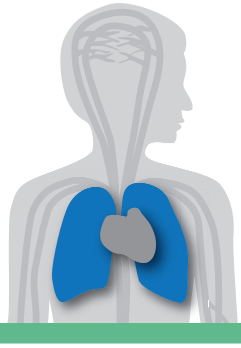
I highly recommend young researchers apply for the ACS postdoc grant to visit a lab abroad. It has been a great life experience for me, as I have learned so much both inside and outside the lab!

Ronak Delewi

ACS MD/PhD grant 2016

During my residency in Cardiology at the AMC, I remained captivated by cardiovascular research in the hope of improving clinical outcomes on a larger scale. Although I am a devoted physician, I missed having dedicated research time. As such, this ACS MD/PhD grant provided the perfect combination for my last years of training. I spent this time performing research in the field of transcatheter aortic valve implantation, supervising PhD students and also set up new research ideas and grants. It truly was the foundation for the future, where I will hopefully be able to further combine the love for research and interventional cardiology.

Combing daily patient care with research is truly inspiring



Thrombosis research at ACS

The World Thrombosis Day, a collaborative movement, has been successfully raising awareness for thrombosis since 2014. At Amsterdam UMC, the tradition of thrombosis research goes back a lot longer. Research areas span all clinical domains of thromboembolism, from prevention to diagnosis and from etiology to treatment. The flip side of the coagulation coin, bleeding disorders, is also a subject of our research interests.

One might say that our research directly stems from clinical questions. Although we have a broad scope, focus is needed, as in any research program, in order to be successful. A key to success is the balance between pharmaceutical company-initiated research with investigator-initiated studies. For instance, our memberships on steering committees of large research programs for novel anticoagulant drugs and antidotes: is academically satisfying, important for networking, provides a means to initiate academic studies and generates pilot studies for grant applications. Being an active recruiting site, with the help of the clinical trial unit of the Department of Vascular Medicine, is a big asset.

A common denominator in our research lines is special populations. For instance, patients with cancer are not only at increased risk of thrombosis, but also have a higher risk of bleeding and need a specific approach for diagnosis and optimal anticoagulation. Furthermore, patients with thrombosis have an increased risk of having an underlying malignancy. We are currently evaluating the applicability of a test that uses tumor-educated platelets in patients with otherwise unexplained thrombosis or pulmonary embolism. This is being done under the supervision of Dr. Nick van Es and in collaboration with Prof. Tom Wurdinger.

1 in 4 people worldwide die from conditions caused by thrombosis

There is a an extremely exciting development in gene therapy for patients with haemophilia. At Amsterdam UMC, the first Dutch patient received this life changing gene therapy for haemophilia B, under the supervision of Dr. Michiel Coppens. Since then, haemophilia A patients have also successfully undergone this experimental therapy.

Pregnant women constitute another special population. Pregnancy is a risk factor for thrombosis, and pulmonary embolism is the number one cause of maternal death in Western societies. For young women who have experienced thrombosis, prevention with injections of the anticoagulant low-molecular-weight heparin (LMWH) during a subsequent pregnancy and the postpartum period is indicated. However, to date there are no adequate data on the optimal prophylactic dose, as research in this population has been largely neglected. Observational cohort studies have shown conflicting results.

This was the motivation to initiate a large randomized controlled trial that simply compares two doses of LMWH, the Highlow Study (www.highlowstudy.org). The trial started recruiting in 2013, and has subsequently been expanded to eight countries and has recruited almost 1000 patients to date. Although the study design is simple and pragmatic, funding has been extremely challenging. The success story of this randomized trial is a kind of "wiki-funding" with a modest startup grant from a pharmaceutical company, to Ministry of Health grants in France and Ireland.

Another landmark trial that is ongoing is the

ALIFE2 trial (www.alife2study.org), a study that investigates whether anticoagulant treatment with LMWH improves the chance of a successful pregnancy in women with an inherited tendency to form blood clots (thrombophilia) and who have had two or more miscarriages. This trial was funded by my VIDI trial and an NHS Research for Patient Benefit Grant in the UK. Both trials will complete recruitment within one year from now, and I can't wait until the results can be analyzed, made public and have an impact on guidelines and patient care.

Finally, the importance of translational research is well acknowledged. We have a biobank of antiphospholipid syndrome patients, and are currently doing an intervention trial (ROMAS) in women with obstetric antiphospholipid syndrome, to assess how manipulating the microbiome with the antibiotic vancomycin affects antibodies. This is being done under the supervision of Dr. Thijs van Mens, in collaboration with Prof. Jan Voorberg and Prof. Joost Meijers.

About one thing I am quite confident: thrombosis and haemostasis research at Amsterdam UMC is alive and kicking!

EYES OPEN TO THROMBOSIS

Saskia Middeldorp, dept. Vascular Medicine
(photo by Jeroen van Kooten)

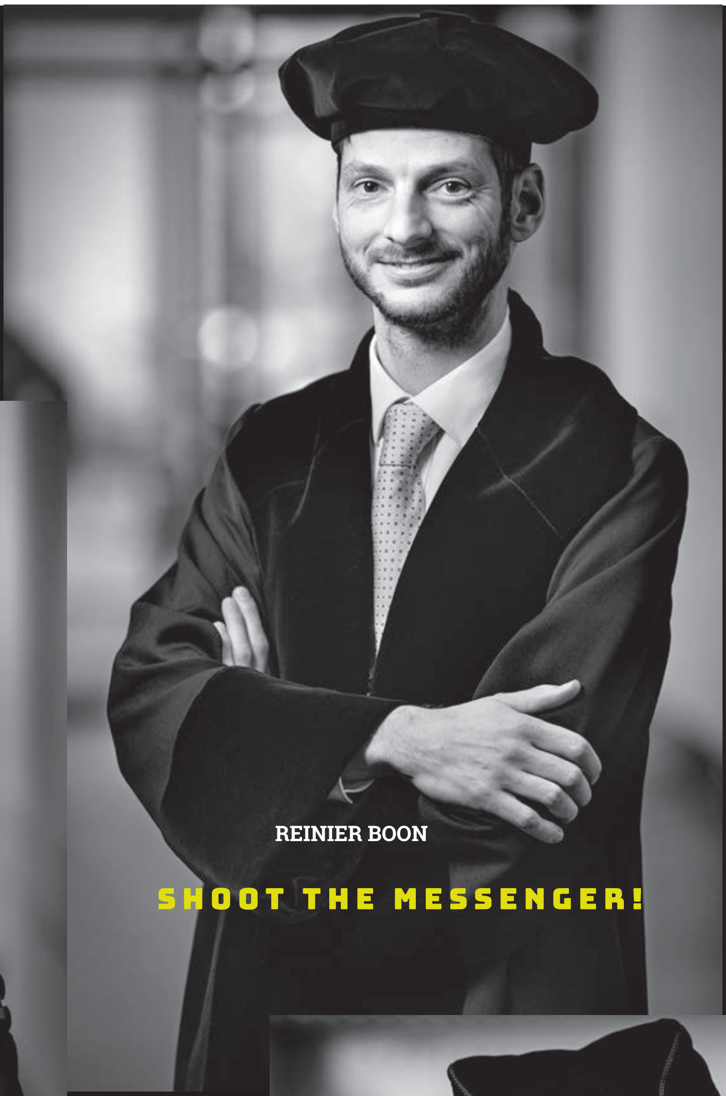
MEET OUR NEW PROFESSORS

CHARLES AGYEMANG

HEALTHY MIGRANTS
HEALTHY SOCIETY

STEVEN CHAMULEAU

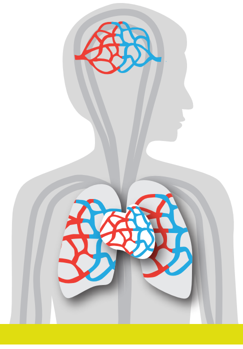
CARDIOLOGY


REINIER BOON
SHOOT THE MESSENGER!


AI AND MEDICAL IMAGING
IVANA IŠGUM


WAVES IN RESPIRATION
JOOST VAN DEN AARDWEG

Endothelial dynamics in blood vessels



STEPHAN HUVENEERS, DEPT. MEDICAL BIOCHEMISTRY

The integrity of healthy blood vessels is secured by endothelial cells that form a barrier layer between the blood and tissue. Blood vessels are part of a fascinating dynamic environment. The endothelium is capable of adapting rapidly to the mechanical forces that are derived from changes in blood pressure, blood flow, or stiffening of the vessel wall. This implies that the endothelium contains unique mechanisms to protect the blood vessels and prevent vascular leakage. Indeed, it turns out that the endothelial cells have various adaptive interactions that are crucial for sensing forces from their tissue environment.

Stiffness-related vascular disease

Age is one of the most important risk factors for the development of cardiovascular disease and is strongly associated with vascular stiffening. Pathological stiffening disturbs endothelial integrity, increases vascular permeability and inflammation, as well as the development of atherosclerosis and hypertension. We envision that by understanding the endothelial pathways that sense vascular stiffening, trigger permeability and inflammatory processes, we will be able to lay the groundwork for new treatment options in cardiovascular disease. In light of this research goal, we are very pleased to be the recent recipients of a grant from the Rembrandt Institute for investigating the physical interactions between endothelial cells and T-cell subsets during the development of atherosclerosis. This will be a collaboration with the labs of Margreet de Vries (LUMC) and Jaap van Buul (Sanquin).

Dynamic endothelial contacts

A large part of our group is investigating the impact of mechanical forces on endothelial interactions with the extracellular surroundings and the contacts between endothelial cells.

We are studying the making and breaking of those interactions in the context of broad fundamental questions:

- How do contacts form between neighboring endothelial cells?
- How do endothelial cells attach to the extracellular matrix and move?
- How do endothelial cells form new blood vessels?
- How does the endothelium repair vascular injuries?
- Can we target the endothelial contacts to normalize the vasculature in disease and improve therapeutic treatments?

By using advanced microscopy approaches we can visualize the active machineries within the endothelial cells during those processes. We are developing tools to fluorescently label molecular complexes, which will allow us to see those dynamic responses live and to pinpoint when and where force-dependent events take place. Typical experiments in the lab include: genetic modulations in primary human endothelial cells, culturing vascular sprouts in 3D, applying flow, imaging the development of vessels in fluorescent zebrafish, and mapping the organization of the endothelium within blood vessels from patient tissues.

Based on the research lines that were funded by a Vidi grant and supported by ACS, we have now discovered several important molecular players that protect the endothelium on-demand in response to mechanical forces, such as vessel wall stiffening. We are currently investigating their role in angiogenesis and their importance in the development of stiffness-related vascular diseases. An exciting next step is to validate whether those molecular processes can be targeted to restore barrier function in vascular disease.

www.huveneerslab.eu

Stephan Huveneers (r) and **Aukie Hooglugt** (l)

left to right: **Annett de Haan, Stephan Huveneers, Ana Angulo Urarte, Reimer Janssen, Beau Neep, Vanessa Meyn, Aukie Hooglugt**

Seeing is believing: using advanced microscopy approaches to visualize the active machineries within endothelial cells

ACS SYMPOSIA AND EVENTS 2020 - 2021

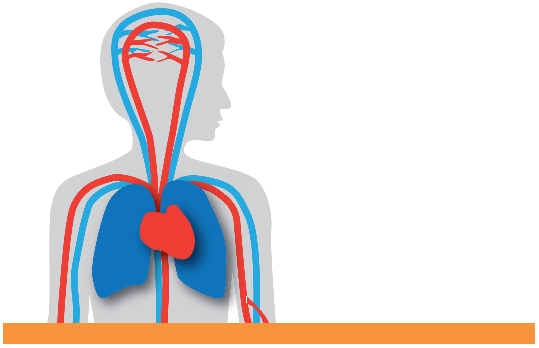
Amsterdam Cardiovascular Sciences Activities 2020

FEBRUARY 3	Atherosclerosis & Ischemic Syndromes
MARCH 2	Microcirculation
APRIL 6	Diabetes & Metabolism
MAY 11	Pulmonary Hypertension & Thrombosis
JUNE 8	Heart Failure & Arrhythmias
JULY 2	6th ACS conference
SEPTEMBER 7	Atherosclerosis & Ischemic Syndromes
OCTOBER 5	Pulmonary Hypertension & Thrombosis
NOVEMBER 2	Diabetes & Metabolism
DECEMBER 7	Microcirculation

Amsterdam Cardiovascular Sciences Activities 2021

FEBRUARY 1	Atherosclerosis & Ischemic Syndromes
MARCH 1	Heart Failure & Arrhythmias
MARCH 11-12	ACS PhD retreat
APRIL 12	Pulmonary Hypertension & Thrombosis
MAY 3	Diabetes & Metabolism
JUNE 7	Microcirculation
JULY 1	7th ACS conference
SEPTEMBER 6	Atherosclerosis & Ischemic Syndromes
OCTOBER 4	Heart Failure & Arrhythmias
NOVEMBER 1	Pulmonary Hypertension & Thrombosis
DECEMBER 6	Diabetes & Metabolism

Bacteriophages as drivers of gut microbiome composition and function and type 2 diabetes



HILDE HERREMA, DEPT. EXPERIMENTAL VASCULAR MEDICINE

The gut microbiome has now been embraced as an endocrine organ with critical functions for human metabolism, digestion and immunomodulation. It has been linked to a plethora of diseases not classically associated with microbes, including type 2 diabetes. Importantly, interventions aiming to modulate the gut microbiome (*e.g.*, by fecal microbiota transplantation) indicate that targeting the gut microbiome holds merit to serve as a preventive measure for the development of type 2 diabetes or to lower the burden on those already affected. However, the development of strategies to favorably and durably alter the gut microbiome is halted, because there is a lack of knowledge about the factors that drive gut microbiota composition and function (*e.g.*, metabolite production).

It is important to realize that during fecal microbiota transplantations, we transplant large numbers of phages

Phages are viruses that specifically target and eliminate bacteria. Phages make up a large part of the gut microbial community with estimated ratios of bacteria:phages ranging from 1:1 to 10:1. It is important to realize that during fecal microbiota transplantations, we transplant large numbers of phages. This is of importance since phages exert selective pressure on bacteria by either eliminating their bacterial host (lytic phages) or by providing additive traits to their bacterial host upon integration into their genome (lysogenic phages). Moreover, although phages are unable to infect human cells, they have recently been shown to be recognized by the eukaryotic immune system and to evoke an immune response.

Despite the ubiquitous abundance of phages in the human gut, their potential to control microbial communities and their suggested role in immunomodulation, there is limited attention on the potential of phages to alter human health and type 2 diabetes development.

Using the Diabetes Fund Senior Fellowship, I aim to create deeper understanding of the role of phages in gut microbiome composition and function in the context of type 2 diabetes. Population cohorts will be used to better characterize the human gut phage population, its relation with microbiota (co-abundance) and type 2 diabetes. Fecal microbiota transplantation and fecal phage transplantation in prediabetic humans (the latter study is currently ongoing, funded by a Diabetes II Breakthrough grant) will be used to link phages with the success of these interventions to modulate glucose homeostasis. Mouse models for type 2 diabetes will be used to address the extent of mammalian immune activation by free fecal phages derived from healthy and type 2 diabetes donors. Together, the studies proposed will unravel the contribution of the 'forgotten' phage community to gut microbiome composition and function in type 2 diabetes.

left to right: **Hilde Herrema, Torsten Scheithauer, Koen Wortelboer**

Activities of Carol Ann Remme at the ESC

Carol Ann Remme, Associated Professor at the Department of Experimental Cardiology, is an active member of the European Society of Cardiology (ESC). She is Chair of the ESC Working Group of Cardiac Cellular Electrophysiology (EWGCCE) and is member of the ESC Media Committee.

Carol Ann Remme explains: "The EWGCCE is a tight-knit community with a unique character. Our annual meeting, which we are organizing this year in Amsterdam, is well-known for its high-quality science and informal friendly setting. Cellular electrophysiology is a relatively small and highly specialized discipline, and I think the EWGCCE is vital for ensuring the future of our research field by training the next generation of electrophysiological scientists. As Chair, I have started some new initiatives including: The Scientific Exchange Grants, a webinar and a joint position paper written by the Nucleus members. Through these initiatives, I aim to increase the visibility and impact of basic electrophysiological research, and basic science in general. At times, it is a lot of work but it is very rewarding to see this wonderful community thrive and to actively contribute to its success."

In 2018, Carol Ann Remme was also appointed member of the ESC Media Committee. "Throughout the year, Media Committee members provide feedback on ESC press releases, and assist in disseminating news from scientific meetings and publications. The majority of our activities are, however, centered around the annual ESC Congress", Remme explains. "Each Media Committee member goes through the submitted abstracts of her/his specialty and together we select the most relevant abstracts for ESC Congress press conferences, press releases, and news. During the Congress itself, Committee members attend press conferences to provide additional background information and act as official ESC Spokespersons to the press. In preparation for this, we received special media training, which was in itself a very interesting and useful experience. I am particularly looking forward to the upcoming ESC Congress in Amsterdam in 2020, where I will be mainly liaising with the Dutch press. Being a member of the ESC Media Committee has given me unique insight into the ESC and the cardiology field, and I hope to use this experience to further strengthen the position of basic and translational cardiovascular science."

It is rewarding to see this wonderful community thrive and to actively contribute to its success

Carol Ann Remme, dept. Experimental Cardiology

Three ZonMw Open Competition grants for ACS research

Leo Heunks, Menno de Winther, Gustav Strijkers and partners received a ZonMw Open Competition grant. This grant, the former ZonMw TOP grant, offers excellent research groups the opportunity to collaborate and to perform studies of exceptional quality in the field of health.

left to right: **Leo Heunks** (dept. Intensive Care), **Tim Marcus and Aart Nederveen** (dept. Radiology)

Menno de Winther, dept. Medical Biochemistry

Gustav Strijkers, dept. Biomedical Engineering and Physics

DIAPHRAGM PROTECTIVE MECHANICAL VENTILATION IN CRITICALLY ILL PATIENTS: THE ROLE OF POSITIVE END-EXPIRATORY PRESSURE

The diaphragm is the most prominent muscle for respiration. Mechanically ventilated critically ill patients rapidly develop diaphragm weakness, which is associated with prolonged mechanical ventilation. With mechanical ventilation positive end-expiratory pressure (PEEP) is applied to improve oxygenation, but on the other hand PEEP may affect diaphragm geometry and structure. In this project, we apply sophisticated functional imaging (Magnetic Resonance Imaging) and molecular techniques to assess the impact of PEEP on the diaphragm in healthy subjects and critically ill patients. Ultimately, these studies should result in the development of new drugs and/or mechanical ventilator strategies that limit the impact of mechanical ventilation on the diaphragm and improve clinical outcomes.

FINDING THE REGULATORS

We have recently identified an epigenetic enzyme that is important in controlling macrophage activation and atherosclerosis development. In this new project, the labs of Menno de Winther of Medical Biochemistry, Amsterdam UMC and Michiel Vermeulen of Molecular Biology, Radboud University, will join forces to investigate the mechanisms that control this epigenetic enzyme. We will apply state-of-the-art molecular biology and proteomic tools to investigate the enzyme's interaction partners and define protein complexes in which it operates. Our aim is to identify new pathways that regulate macrophages in inflammatory disease.

TRIPLET: TARGETED THERAPY AND IMAGING IN EXPERIMENTAL PLACENTA INSUFFICIENCY

Fetal growth restriction and preeclampsia are caused by placenta insufficiency, which affects the mother and unborn child in ~10% of pregnancies. Approximately 50% of stillbirths are associated with fetal growth restriction and survivors often suffer from neurodevelopmental disorders. In the TRIPLET project, experts in the field of nanomedicines (Raymond Schiffelers, UMC Utrecht), imaging (Gustav Strijkers, Amsterdam UMC) and translational animal and *in vitro* models (Titia Lely, UMC Utrecht) will develop a new approach for the treatment and monitoring of fetal growth restriction and preeclampsia. Meeting both the urgent need for an effective and safe therapy as well as the need for an effective monitoring technique for placental and fetal oxygenation.

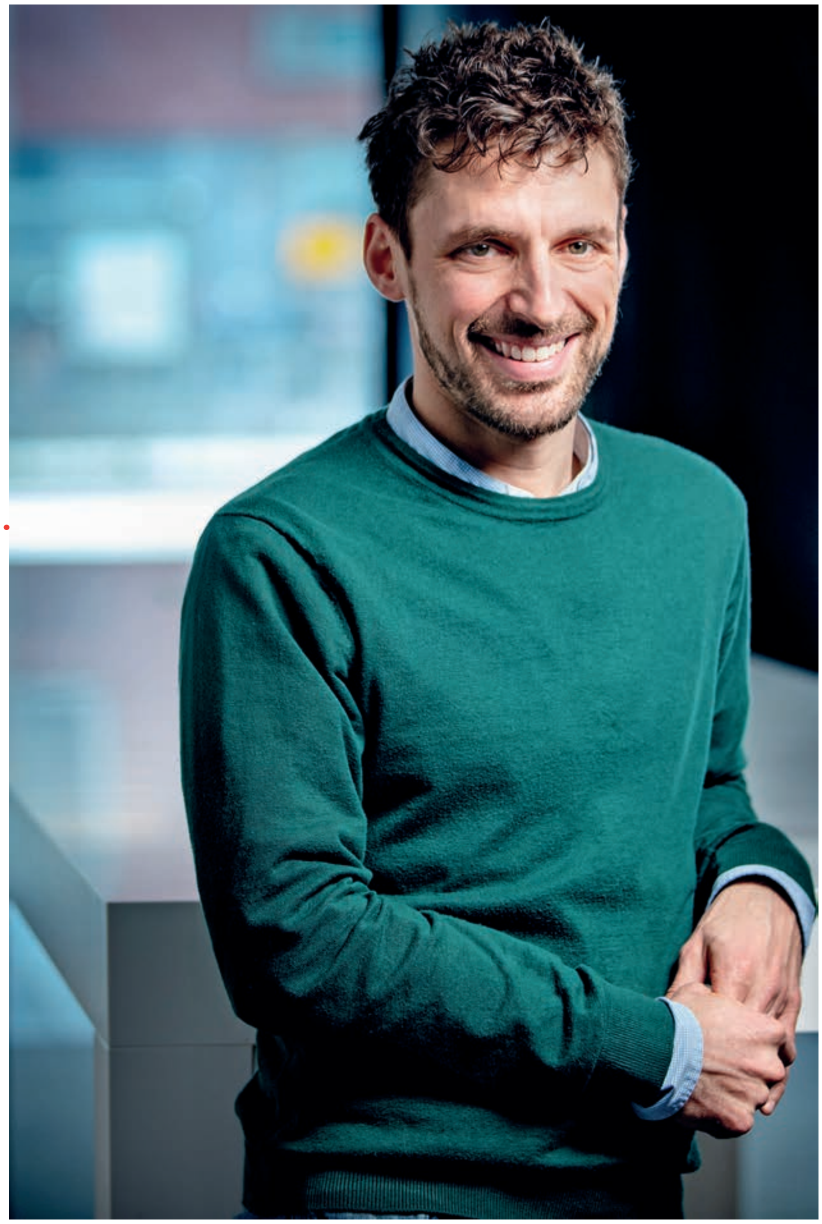
Tackling immune cell metabolism to dampen inflammation and atherosclerosis progression

Jan Van den Bossche, dept. Molecular Cell Biology and Immunology, has received 250,000 euros as part of a European ERA-CVD consortium of 1 million euros.

Atherosclerosis is the main cause of myocardial infarction and stroke, contributing to the global burden of cardiovascular diseases (CVD). Immune cell dysregulation and chronic inflammation are major causes of atherosclerotic plaque development. Despite the important proof-of-concept that inflammation is a clinically relevant contributor to CVD, there is a clear need to better define and understand the inflammatory components that drive CVD.

Importantly, myeloid cell activation in CVD is paralleled by an increase in glucose consumption in these immune cells. Metabolic syndrome is hallmarked by chronic inflammation and the dysfunction of key processes that regulate glucose metabolism, escalating the risk of atherosclerosis. As such, nutrient overload and a dysregulated metabolic state within myeloid cells could have major implications in metabolic syndrome and associated complications such as atherosclerosis, insulin resistance and type 2 diabetes.

Bringing together human cohorts and expertise to delineate the impact of metabolic dysfunction on inflammation and CVD development in the clinical context



Jan Van den Bossche, dept. Molecular Cell Biology and Immunology

For this joint transnational European Research Area Network on Cardiovascular Diseases (ERA-CVD) research project, we hypothesize that metabolic dysfunction in atherosclerosis reprograms glucose utilization in immune cells, leading to their expansion and activation in atherosclerotic plaques.

Our European consortium is coordinated by Stoyan Ivanov (INSERM, Nice, France). His group will focus on the use of pre-clinical models to link myeloid cell glucose utilization to cellular function and atherosclerosis development. Arvand Haschemi (Medical University of Vienna, Austria) will tackle the pentose phosphate pathway (PPP) branch and will apply a unique tool to map the metabolism of myeloid cells within atherosclerotic plaques at single-cell resolution. Florian Kahles (University of Aachen, Germany) is a medical doctor who is responsible for the translation of our findings to human cells and the clinic. At Amsterdam UMC, our group aims to unravel the molecular mechanisms by which modulations in the PPP and glycolysis translate into altered macrophage functions and disease outcome.

Together, myPenPath (tackling myeloid cell Pentose Phosphate Pathway activation in atherosclerosis) connects interdisciplinary and key expertise to investigate the contribution of glucose metabolism. In particular, the balance between glycolysis and PPP in myeloid cell functions during atherosclerosis. We will define the contribution of glycolysis and the PPP to immune cell migration, proliferation and activation in atherosclerosis using genetic mouse models and pharmacological inhibitors. Moreover, in a translational approach, our consortium will bring together human cohorts and expertise to delineate the impact of metabolic dysfunction on inflammation and CVD development in the clinical context.

Numbers & Facts

Symposia organized last year

2 Annual meetings: 5th Annual ACS Meeting and 10th Rembrandt Symposium
8 Monthly ACS symposia
447 Scientist and students registered and attended the monthly symposia
30 Educational lectures and discussions at these symposia

ACS grants awarded last year

2 ACS PhD grants
2 ACS Postdoc grants of €70,000
6 ACS MD/PhD grants of €25,000 awarded in 2 rounds
11 ACS Out of the Box grants of €25,000 awarded in 2 rounds
4 ACS Equipment grants

ACS published in 2019

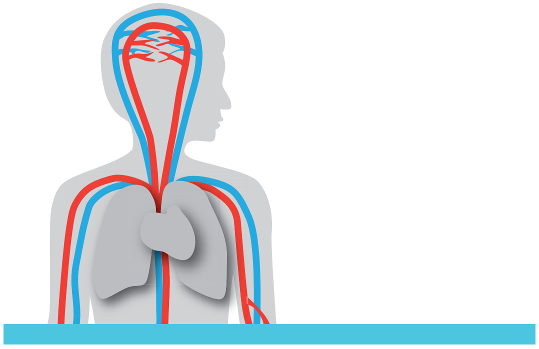
1 ACS glossy
19 ACS newsletters

ACS PhD defenses and inaugural lectures in 2019

91 PhD defenses
9 Inaugural lectures

ACS members in 2020

366 PhD students
66 Postdocs
31 Specialists in training
154 Principal Investigators
107 Staff members



Developing immunotherapy for atherosclerosis: a challenging adventure

ESTHER LUTGENS, DEPT. MEDICAL BIOCHEMISTRY

Immunotherapy has revolutionized the way cancer patients can be treated. Patients with solid tumors are now being successfully treated with antibodies against inhibitory immune checkpoints, underlining the widespread therapeutic potential of this class of immunomodulators. Immune checkpoint proteins are master regulators of inflammation and can propagate or halt immune-cell activation. Although immune checkpoint targeting has been rapidly integrated in oncology treatment, the exploitation in cardiovascular disease is in its infancy.

The underlying cause of the majority of cardiovascular diseases is atherosclerosis. Due to the formation of atherosclerotic plaques, life-threatening atherothrombotic events, including myocardial infarction or stroke, may arise when these plaques rupture or suffer from erosion. Recent clinical trials in patients have revealed that lowering inflammation is an important strategy for combating cardiovascular disease. However, these anti-inflammatory immunotherapies were not developed specifically for cardiovascular disease and therefore exhibited suboptimal efficacy and induced unwanted side effects.

Immune checkpoint proteins to propagate or halt immune-cell activation in atherosclerosis

The team of Esther Lutgens has discovered that co-stimulatory immune checkpoints play a central role in the pathogenesis of atherosclerosis. Her team has developed novel small molecule-based therapies based on inhibiting immune checkpoint signaling intermediates, in this case the interaction between the immune checkpoint CD40 and the signaling intermediate TRAF6. In experimental proof of concept studies, her team has shown that these are efficient and safe immune-therapeutics for the treatment of atherosclerosis.

Current projects in the lab

Identifying the immune checkpoint landscape in atherosclerosis

We are currently answering the question of which cell types express which immune checkpoints in which stage of atherosclerosis. The lab uses single cell technologies such as mass-cytometry and single cell RNA-sequencing to generate the immune checkpoint atlas of cardiovascular disease.

The Experimental Vascular Biology team

Bottom, left to right: Oliver Chen, Marion Gijbels, Kikkie Poels, Laura Bosmans, Esther Lutgens, Menno de Winther, Annette Neele, Suzanne Aarts, Linda Beckers, Lisa Willemsen, Saskia van der Velden
Top, left to right: Myrthe Reiche, Cindy van Roomen, Claudia van Tiel, Myrthe den Toom, Bram van Os, Thijs Beldman, Guillermo Griffith, Koen Prange, Jeroen Baardman

Unraveling the role of recently identified immune checkpoint proteins in atherosclerosis

The family of immune checkpoints is expanding. We are currently identifying the immunogenic properties of these novel members, as well as their effects on atherosclerosis.

From bench to bedside: bringing our CD40-TRAF6 small molecule inhibitor to the clinic

Our proof of concept studies on blocking CD40-TRAF6 interactions in atherosclerosis showed a spectacular decrease in atherosclerosis without inducing immune suppressive side effects. We are currently optimizing the chemical and pharmacokinetic properties to be added to the translational pipeline.

Use of animal models in atherosclerosis research

Atherosclerosis is a complex, multifactorial disease which cannot be mimicked using a 'lab on a chip' approach. As the interactions between many different immune cell types, flow, shear stress, hyperlipidemia and endocrine factors all affect its pathogenesis, we make use of living organisms, especially mice. However, in the Lutgens' lab, we aim to reduce the number of laboratory animals that we use by carefully designing our experiments, and by testing aspects of the disease (i.e. production of inflammatory mediators, or cell migration) as much as possible in *in vitro* systems. Moreover, we use a virtual ligand screening and computer-based modeling approach to design the best small molecule inhibitors possible. We are convinced that more and more 'lab on a chip' methods will be developed, thereby reducing the numbers of laboratory animals needed. However, as atherosclerosis is a complex, multifactorial disease, it is likely that we will always have to rely on laboratory animals to properly study this disease and design treatments for humans.

LIFE AFTER ACS

Since the start of ACS in 2016, more than 300 ACS PhD students have successfully obtained their doctorate. Some PhD students have remained active in ACS whereas others have spread their wings and work elsewhere, either within or outside of academia. Three former ACS PhD students were interviewed about their career paths.

Susan van den Berg

PhD: Experimental Vascular Biology department
Promotors: Esther Lutgens & Menno de Winther
Thesis defense: June 2017
Current position: Project manager Netherlands Heart Institute (since September 2017)

What did you do when you were at ACS and what are you doing now?

I did my PhD in the experimental vascular biology group of Prof. Esther Lutgens and Prof. Menno de Winther. After writing my thesis 'A hot interaction between immune cells and adipose tissue', I started working as a project manager for the Netherlands Heart Institute.

How did you find this position?

I was told about the vacancy via my 'cardiovascular network'. Quite a typical way of finding a new job. It is a cliché, but your network is often key in finding new opportunities in your career. ACS can be really important for that.

What do you do as a project manager?

My main activity is to start the 'Hartenbank', a Cardiac Tissue Bank. This will be a biobank where we collect cardiac tissue from donors which can be requested by cardiovascular researchers from all around the world.

Setting up the Cardiac Tissue Bank has been widely supported and I am happy that I get to work with many people across the country: cardiologists, pathologists, fundamental researchers, patient organizations and the Netherlands Brain Bank. Starting this project is challenging and I have a wide variety of daily activities that include taking care of all the: logistics, ethics, legal requirements, finances, and keeping all the stakeholders happy and involved. Of course, it's not always sunshine and roses. Like academic research, it wasn't easy getting the financial means but we have it now and this means we can soon start registering donors.

Wino Wijnen

PhD: Heart Failure Research Center (Experimental Cardiology department)
Promotors: Yigal Pinto & Esther Creemers (copromotor)
Defense: May 2015
Current position: Founder & Innovation Manager at WiWright

My PhD at ACS

I trained as a molecular biologist with a thirst for knowledge. I pursued my PhD in cardiovascular medicine by investigating the molecular mechanisms underlying heart failure. In the research group of Prof. Yigal Pinto, I found a stimulating academic environment that combined excellent basic research with clinically relevant applications. Moreover, the strong team-spirit was echoed in the quality of the research.

More than academic training

Besides sharpening my academic skills, I learned (admittedly with some hindsight) the importance of interdisciplinary translational research, the value of a supportive and encouraging environment, to always stay focused on the final objective, and to overcome my flaws while developing my strengths.

From being empowered to empowering others

After my PhD, I founded a company that offers academic writing services, career coaching, fundraising strategies, and innovation counselling to scientists and entrepreneurs in medical research. My work exposes me to state-of-the-art science in diverse research fields while working with internationally renowned innovators. It allows me to contribute to scientific excellence, meet interesting people, and satisfy my personal thirst for knowledge. Moreover, my experiences help to coach young scientists on their journey through academia.

The value of ACS

Being trained in the stimulating atmosphere of ACS, in one of the world's most innovative countries, has enabled me to face the challenges of entrepreneurship with persistence, creativity, virtue, and an open mind. My PhD at ACS was, therefore, more than an academic degree, it served as an accelerator for personal and professional growth.

Vasco Sequeira

PhD: Physiology department
Promotor: Jolanda van der Velden
Thesis defense: February 2016
Current position: Junior Research Scientist at the University Klinik - DZHI Würzburg, Germany (since January 2018)

What did you do when you were at ACS and what are you doing now?

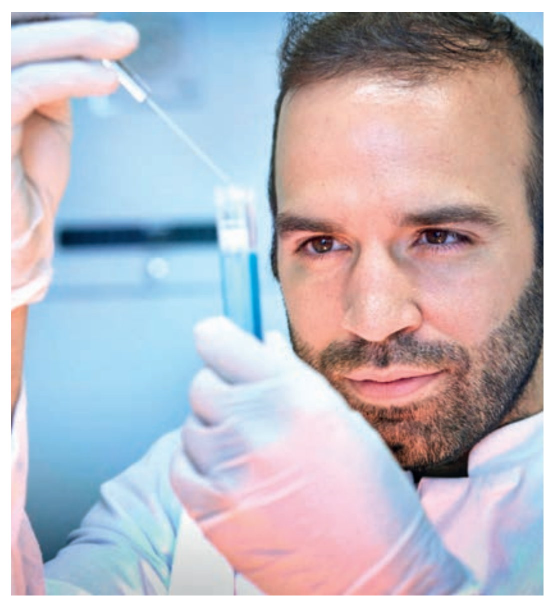
My past, and current research, is focused on the "energy starvation", a hypothesis that suggests the failing heart is energy-starved. The dogma is decades-old and dictates that, because the heart´s function depends on the chemical energy in the form of ATP, there is low [ATP] available during heart failure. However, my research sets aside this hypothesis. First of all, it is unclear whether [ATP] decreases in disease. Secondly, even when assuming ATP reductions occur during stress-conditions, the remaining ATP is high enough to supply the myocardium. ACS provided me with the support to demonstrate that it is actually cellular ADP that mainly increases and causes heart failure. Additionally, I coincidently showed that ADP increases because the ADP-to-ATP regeneration systems, *e.g.*, creatine kinase and mitochondria, are hypoactive in disease.

What is your current job and how did you get there?

The foundations set during the ACS fellowship were instrumental in determining my current situation. I was offered a Junior Research Scientist position at the University Klinik in Würzburg Germany, in the group of Prof. Christoph Maack. This partnership is instrumental in my investigation of the myocardial energy-buffering systems in heart failure, including how redox alterations, e.g. oxidative stress, play a causal role in predisposing the heart for life-threatening arrhythmias and cardiac arrest.

Where do you see yourself in five years?

I hope to become an independent scientist. I anticipate that in the next 5-years I will further strengthen my research line, which will increase the chances of obtaining future funding to secure a group of my own. It is my ambition to develop a research group focused on myocardial energetics and redox alterations in heart failure.



ACS awards 2019-2020

ACS awarded: PhD grants (€200,000), Postdoc grants (€70,000), MD/PhD grants (€25,000), Out of the Box grants (€25,000) and Equipment grants to stimulate innovative collaborative research

2019 JULY-DECEMBER

Marco Götte & Aart Nederveen	MRI guided cardiac ablation in explanted beating pig hearts	PhD
Vivian de Waard & Dimitra Micha	Genetic characteristics of Marfan patients dictate aneurysm severity and treatment	PhD
Pranav Bhagirath	Defining atrial cardiomyopathy using serial evaluation with cardiac magnetic resonance imaging and biomarkers in patients with atrial fibrillation (DESCRIBE-AF trial)	MD/PhD
Jouke Bokma	To optimize timing of pulmonary valve replacement in adults with tetralogy of Fallot	MD/PhD
Camiel de Roij van Zuijdewijn	Peri and intradialytic hemodynamic stability in patients treated with various dialysis modalities	MD/PhD
Fleur Tjong	Deep learning models for development of novel tools for identification of patients at risk of complications and shock therapy following pacemaker and ICD implantation	MD/PhD
Bianca Brundel & Riekelt Houtkooper	Staging of clinical atrial fibrillation: metabolomics as diagnostic tool?	OOTB
Elga de Vries & Carlie de Vries	Four and a half lim domain 2 (fhl2) regulates vascular barrier function in aorta and brain microvasculature	OOTB
Ed Eringa & Jeffrey Kroon	Get to the heart of the matter: mapping metabolic reprogramming in valvular interstitial cells in order to combat aortic valve stenosis	OOTB
Peter Hordijk & Ed van Bavel	Endothelial-cardiomyocyte communication through electrical coupling	OOTB
Arjan Houweling & Vivian de Waard	Unravelling modifier genes responsible for variable clinical presentation of aortic complications in Marfan syndrome	OOTB
Mathilde Rivaud, Veronique Meijborg & Paul Wijnker	Effects of modifying electrical diastole on diastolic function	OOTB
Eliane Wenstedt, Liffert Vogt, Niels Heemskerk & Jan Van den Bossche	Does salt consumption drive leukocyte infiltration in humans?	OOTB

2020 JANUARY-JUNE

Najim Lahrouchi	Personalized medicine for patients with the long QT syndrome	MD/PhD
Nick van Es	Combining clinical and genetic risk factors to identify cancer patients at high risk of arterial and venous thromboembolism: a new challenge in cardio-oncology	MD/PhD
Fleur Tjong	Deep learning for identification of ICD patients at risk of lethal cardiac arrhythmias: The deep risk ICD Study	Postdoc
Annette Neele	Targeting the Polycomb Repressive Complex 2 (PRC2) in atherosclerosis by small molecule drugs	Postdoc
Menno de Winther, Jan Van den Bossche, Max Nieuwdorp & Maarten Soeters	Promoting immune health by intermittent fasting: taming the monocytes?	OOTB
Liffert Vogt, Majon de Boer & Marie van Dijk	Investigating placental non-osmotic sodium buffering across pregnancy	OOTB
Meeike Kusters & Barbara Hutten	The search for new biomarkers in risk stratification: towards a tailor made treatment for young patients with familial hypercholesterolemia	OOTB
Harm Jan Bogaard, Harry Buller, Josien Smits & Azar Kianzad	Detecting CTEPH with educated platelets: a case-control study	OOTB
Muriel Grooteman & Frans van Ittersum	Sodium receiver coil for body imaging on the 7 Tesla MRI scanner	Equipment grant
Daniel van Raalte & Max Nieuwdorp	High Performance Liquid Chromatography (uHPLC) system	Equipment grant
Erik Serné & Ed Eringa	PeriCam PSI NR	Equipment grant
Jan Van den Bossche & Elga de Vries	Cytation 1 cell imaging device	Equipment grant

Amsterdam Cardiovascular Sciences