

2 RESEARCH AT THE CENTER

The CNIC is organized into two departments, one focused on Basis Research and the other on Clinical Research. Research in these fields is fully interconnected through three multidisciplinary Research Areas.

VASCULAR PATHOPHYSIOLOGY

Coordinator: **Almudena R. Ramiro**

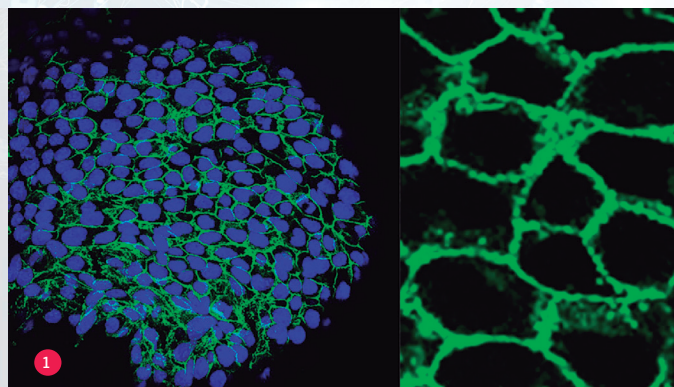
Scientists in the Vascular Pathophysiology Area (VPA) investigate the biology of the vascular system in health and disease through multidisciplinary approaches that include molecular and cellular biology, animal models of disease, and translational and clinical studies. This research takes advantage of high-throughput genomics, proteomics, and metabolomics coupled to bioinformatics analysis, as well as state-of-the-art imaging technologies. The area hosts 12 research groups and 3 core technical units. The VPA has a particular interest in vascular biology and atherosclerosis, the underlying cause of heart attack and stroke.

Scientific highlights in 2020 include the following:

- By using mouse models of aortic disease and various complementary approaches, including non-invasive imaging, lentivirus-mediated transduction of the aorta, transcriptomics, and proteomics, we have identified novel pathogenic mediators and signaling pathways in diseases that involve vascular wall remodeling, including syndromic and non-syndromic aortic diseases and hypertension. Some of the identified mediators in mice have been validated in the human disease and are potential candidate therapeutic targets and disease biomarkers.
- Using several animal models that recapitulate vascular cognitive and mixed Alzheimer's disease/vascular dementia pathology, we are unraveling mechanisms through which cardiovascular risk factors affect cognitive function, and have identified novel prognostic and therapeutic targets related to the role of adult hippocampal neurogenesis in this setting.
- Through a single-cell proteomics analysis of the atherosclerosis antibody repertoire, we have identified a novel atherosclerosis antigen that has potential as a biomarker and a therapeutic target.
- High-throughput proteomics revealed that activation of the complement system is a major alteration in early atherosclerotic plaques and that elevated plasma C5 is a promising biomarker of subclinical atherosclerosis.

TECHNICAL UNITS

- Genomics **Ana Dopazo**
- Proteomics **Juan Antonio López**
- Bioinformatics **Fátima Sánchez cabo**



RESEARCH GROUPS

● Molecular and Genetic Cardiovascular Pathophysiology

Vicente Andrés

● Experimental Pathology of Atherosclerosis

Jacob Fog Bentzon

● Intercellular Signaling in Cardiovascular Development and Disease

José Luis de la Pompa

● Cardiovascular Imaging and Population Studies

Valentín Fuster

● Hematovascular Pathophysiology

José Javier Fuster

● Regulatory Molecules of Inflammatory Processes

Pilar Martín

● Neurovascular Pathophysiology

María Angeles Moro

● Tissue Regeneration

Pura Muñoz

● B Lymphocyte Biology

Almudena R. Ramiro

● Gene regulation in Cardiovascular Remodelling and Inflammation

Juan Miguel Redondo

● Intercellular Communication in the Inflammatory Response

Francisco Sánchez Madrid

● Cardiovascular Proteomics

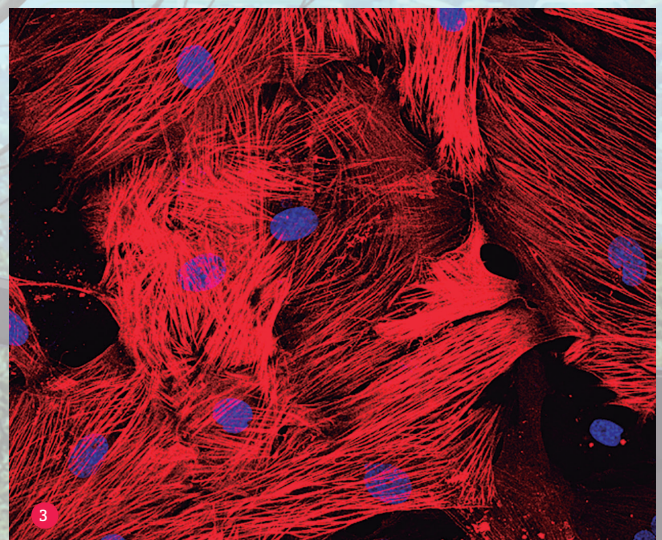
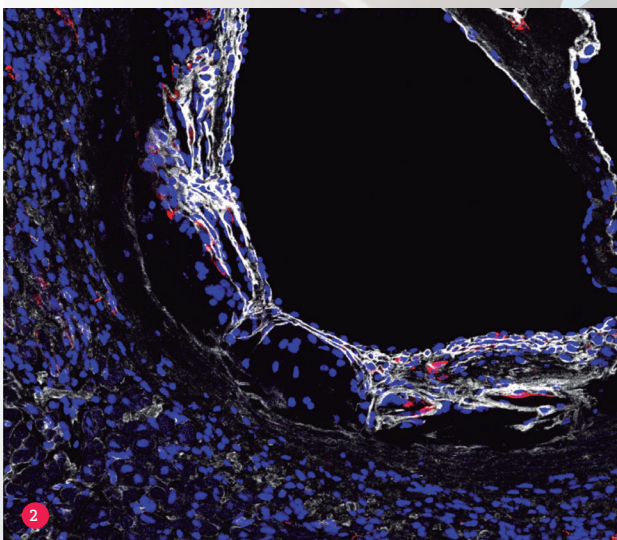
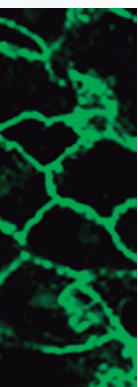
Jesús Vázquez

• We identified mechanisms that precipitate cardiovascular disease progression and aging in Hutchinson-Gilford progeria syndrome (HGPS). High-throughput proteomics identified common cardiometabolic alterations and dysregulated pathways in mouse and pig models of aging, and we also established a new mouse model of HGPS exhibiting premature vascular aging and features of atherosclerotic plaque vulnerability.

• The Genomics Unit successfully implemented an in-house method for the sensitive detection of ultra-low variant allele frequency (VAF) mutations. This strategy has allowed us to identify and monitor clonal hematopoiesis events associated with cardiovascular risk. Additionally, last year saw a surge in single-cell transcriptomics experiments, in which tens of thousands of cells are analyzed in a single experiment. scRNA-Seq data can be analyzed and integrated with public data in an ad-hoc tool developed in-house and available at <https://bioinfo.cnio.es/scdavis/>. This tool allows visualization and integrative analysis of single-cell transcriptomics and flow-cytometry data.

• Research into tissue and organismal aging identified a stem cell subpopulation with superior muscle regenerative capacity. We also found that sestrin prevents atrophy of disused and aging muscles by integrating anabolic and catabolic signals, shedding light on the emergence and remodeling of nestin-expressing coronary vessels. Work in 2020 also targeted cellular senescence to reverse regeneration failure in aging and diseases of the heart and skeletal muscle.

• Finally, we studied the molecular mechanisms that control cardiac chamber development and how are they altered in certain cardiomyopathies. We also characterized the interaction between the actin cytoskeleton and the various signals that govern the early steps of chamber formation, and studied how cardiomyocyte stiffness can lead to cardiac functional decline. Moreover, we have identified a gene signature in peripheral blood that predicts aortic valve calcification, and we are currently trying to expand this gene profile.



2 MYOCARDIAL 2 PATHOPHYSIOLOGY

Coordinator: Guadalupe Sabio

The Myocardial Pathophysiology Area (MPA) includes 11 research groups and 5 core technical units (Transgenesis, Pluripotent Cell Technology, Comparative Medicine, Viral Vectors, and Clinical Trials Coordination). MPA groups work on a wide range of topics: inherited cardiomyopathies, arrhythmia mechanisms and therapy, molecular regulation of heart failure, metabolism and its effect on cardiovascular diseases, functional genetics of the oxidative phosphorylation system, translational cardiovascular imaging and therapy, molecular cardiology, immunobiology, cardiovascular health and imaging, cardiac arrhythmias, and nuclear receptor signaling. Research in these areas produced several scientific advances during 2020.

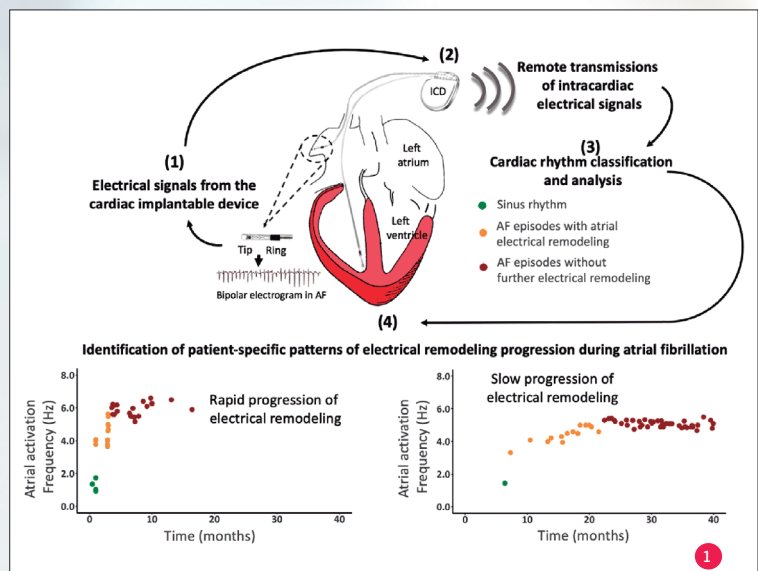
- Results from a CNIC-led multicenter showed that cardiac electrical signals from patients fitted with pacemakers or implantable cardioverter defibrillators can be used to monitor and predict atrial remodeling progression in a personalized and patient-specific manner. This can be achieved with standard data transmission technology installed in the implantable device. (Jose María Lillo-Castellano et al. *Europace* 2020;22:704-715).
- As part of the PESA project, we designed an algorithm that provides a personalized estimate of cardiovascular risk in healthy middle-aged individuals based on a range of variables including age, blood pressure, diet, and blood and urine markers. The EN-PESA algorithm is an affordable tool for estimating the severity of subclinical atherosclerosis—characterized by the deposition of fatty substances in the arterial walls—especially in individuals at higher risk. We believe that EN-PESA will help to personalize the estimation

of cardiovascular risk, leading to tailored treatments and follow-up plans.

- Dysregulation of the circadian clock has been linked to cardiometabolic disorders. We found that neutrophils regulate the liver circadian clock and also affect liver metabolism. Regulation of the liver's molecular clock may provide a new therapeutic approach for metabolic diseases such as diabetes and high blood pressure. We also demonstrated that changes in cholesterol and bile acid metabolism promote cholestasis, bile duct proliferation, and intrahepatic cholangiocarcinoma.
- We found an alarmingly low prevalence of ideal cardiovascular health among early adolescents in Spain. This study demonstrated that adherence to lifestyle interventions can improve health outcomes in people living in diverse and socioeconomically disadvantaged communities.
- To investigate the molecular mechanisms underlying arrhythmias and sudden cardiac death in patients with Andersen-Tawil Syndrome and Short-QT syndrome, we generated mouse models with cardiac-specific expression of mutant Kir2.1 channels, as well as patient-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Our approach is multidisciplinary, involving virus-mediated gene transfer in mice, generation of iPSC-CMs from somatic cells obtained from patients carrying a mutant gene, in-silico modeling of ion channel structure-function relations, transcriptomics, protein chemistry, patch-clamping, ECG recordings, intracardiac stimulation, and optical mapping.
- We have shown how macrophages communicate with endothelial cells to promote cardiac repair and remodeling.

TECHNICAL UNITS

- Comparative Medicina
 - Clinical Trial Coordination
- Antonio J. Quesada**
- Pluripotent Cell Technology
- Gionanna Giovinazzo**
- Transgenesis
- Juan De Dios Hourcade**
- Viral Vectors
- Juan A. Bernal**



RESEARCH GROUPS

- Inherited cardiomyopathies

Juan A. Bernal

- Functional Genetics of the Oxidative Phosphorylation System (GENOPHOS)

José Antonio Enríquez

- Cardiovascular Health and Imaging

Rodrigo Fernández

- Advanced Development in Arrhythmia Mechanisms and Therapy

David Filgueiras

- Translational Laboratory for Cardiovascular Imaging and Therapy

Borja Ibáñez

- Cardiac Arrhythmia

José Jalife

- Molecular Regulation of Heart Failure

Enrique Lara-Pezzi

- Molecular Cardiology

Silvia Priori

- Nuclear Receptor Signaling

Mercedes Ricote

- Stress kinases in Diabetes, Cancer and Cardiovascular Disease

Guadalupe Sabio

- Immunobiology

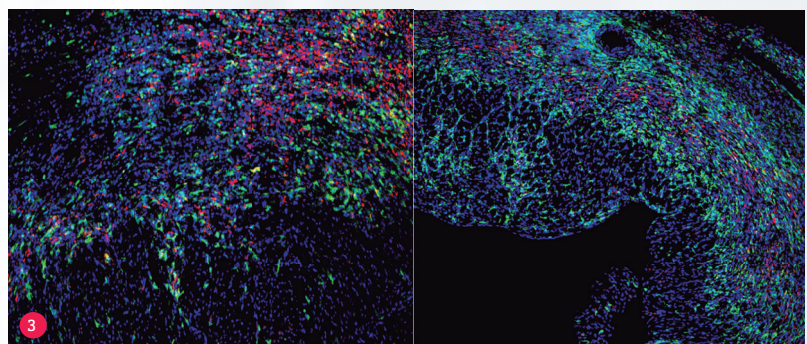
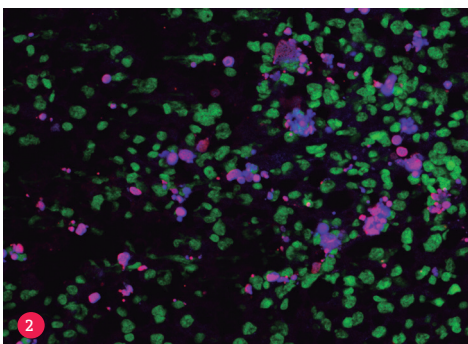
David Sancho

We demonstrated that macrophages promote endothelial-to-mesenchyme transition after myocardial infarction and showed that macrophages and the metalloproteinase MMP14 are key regulators of this process. These findings indicate that patients at risk of developing heart failure after myocardial infarction could benefit from treatments based on controlling dysregulated myocardial MMP14 activity in macrophages with specific MMP14 inhibitors and the implementation of novel nanotechnology-based tools for selective macrophage targeting.

- In the cardio-oncology field, it is important to highlight the H2020-HEALTH Project. The CNIC-coordinated project Remote Ischemic Conditioning in Lymphoma Patients Receiving Anthracyclines (RESILIENCE, €6 million) involves 11 partners from 6 European countries. Moreover, through the ongoing ERC-CoG project Novel mitochondria-targeting therapies for chemotherapy-induced cardiotoxicity (MATRIX), we discovered a new mitochondria-targeted therapy for anthracycline-induced cardiotoxicity. Thanks to the use of a large animal model, we identified remote ischemic conditioning as a strong protective intervention (Cardiovasc Res. 2020;117:1132-1143). This therapy is now being tested in a randomized clinical trial (MATRIX).
- In the ischemia-reperfusion field, we identified metoprolol as the only beta blocker able to abrogate infarct-related exacerbated inflammation (Eur Heart J 2020;41:4425-4440). Also in 2020, the REBOOT trial reached half of the anticipated sample size (4250 of 8500).
- The MADRID-COVID trial was conducted in 2020. This trial tested the benefits of metoprolol in critically ill covid19 patients on mechanical ventilation. The results of the trial will be reported soon.

The MPA's core technical units give support to all CNIC scientists:

- 1) The Transgenesis Unit provides services in mouse strain rederivation, production of genetically modified mice, and cryopreservation of mouse strains. Through collaboration with Dr. Jorge Nicolás Domínguez Macías and Dr. Miguel Torres, we are currently developing a microinjection model in post-implantation embryos, which will allow us to obtain interesting results.
- 2) The Pluripotent Cell Technology Unit Pluripotent Cell Technology Unit (PCTUnit) provides technological support in the generation of biological models, both in vivo and in vitro, through the manipulation of mouse embryonic stem cells (mESCs) and human induced pluripotent cells (hiPSCs). In 2020 the Unit collaborated with CNIC researchers on the derivation of hiPSCs from patient-derived dermal fibroblasts, helped fine-tune differentiation protocols for obtaining hiPSC-derived cardiomyocytes, and designed CRISPR/Cas9-based gene editing strategies for generating mouse and pig models and for in vitro CVD modeling in hiPSCs.
- 3) The Clinical Trials Coordination Unit has continued to provide specialized support for clinical trials and studies carried out at the CNIC. Its ultimate goal is to boost Spanish leadership in clinical trials in the cardiovascular area.
- 4) The Viral Vectors Unit provides researchers with access to state-of-the-art viral vector technology for use in preclinical studies and basic research.
- 5) The Comparative Medicine Unit supports in vivo work in the animal facility.



CELL AND DEVELOPMENTAL BIOLOGY

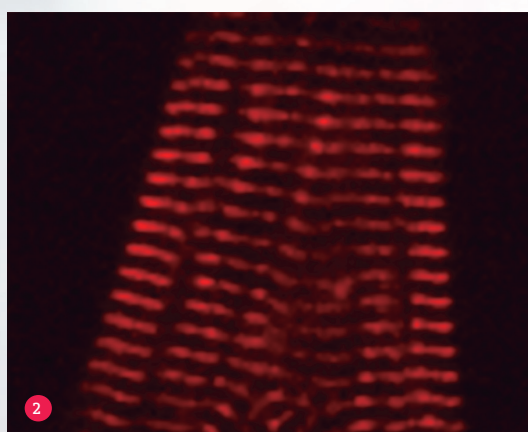
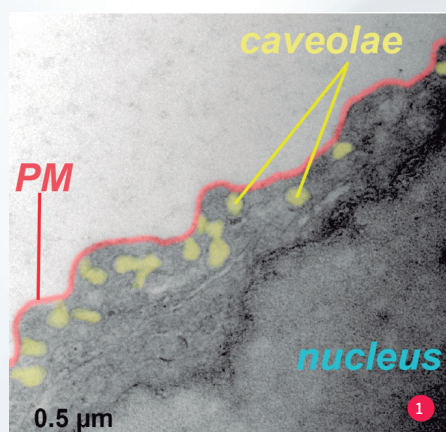
Coordinator: Jorge Alegre-Cebollada

The Cell and Developmental Biology (CDB) area comprises 8 research groups and 3 core technical units (Microscopy, Flow Cytometry, and Advanced Imaging). CDB area members have broad interests in immunology, cell biology, development, biomechanics, nanotechnology, cardiology, and epidemiology, creating a highly nurturing environment for leading-edge interdisciplinary research spanning basic and translational cardiovascular physiology and disease.

Research highlights in 2020 include the discovery that cardiac resident macrophages, a type of immune cell, help cardiomyocytes to eliminate damaged mitochondria. We also found that neutrophils in different tissues adopt unique characteristics that allow them to perform tissue-specific functions, and that the activity of these immune cells fluctuates in a circadian manner. Other work identified mechanisms governed by membrane structures known as caveolae through which cells sense the mechanical properties of their environment and secrete non-collagen ECM proteins to remodel it. Our scientists also uncovered new systems controlling limb and vessel development.

These discoveries include a gradient of transcription factors along the proximo-distal axis of the developing limb that provides essential positional information, the unexpected contribution of cells derived from the second heart field to the coronary lymphatic vasculature, and novel molecular mechanisms sustaining artery development. An emerging research interest in the CDB area is myocardial regeneration, an important process that can limit and revert injury after a myocardial infarction (MI).

CBD scientists have engineered several animal models to decipher molecular and cellular mechanisms underlying heart regeneration, including a mouse model that makes it possible to mechanically phenotype titin, a key protein of the contractile apparatus of cardiomyocytes. From a clinical perspective, we have contributed to the development of quality indicators for the management of acute MI. Looking to the future, in 2020 we launched two coordinated international consortia. REANIMA aims to enable cardiac regeneration after MI, while AtheroConvergence will decipher the role of mechanical forces in atherosclerosis. Additional international funds obtained in 2020 include two ERC Consolidator grants—to develop novel tools to manipulate protein mechanics in living matter and to explore vasculature development—and a Myokardia-Myoseeds grant to uncover molecular mechanisms sustaining development of dilated cardiomyopathy, the most frequent cause of heart transplantation worldwide. The Microscopy technical unit associated with the CDB area has secured funding from the European Regional Development Fund (ERDF) to set up pioneering imaging approaches for complex ultrastructural studies within the National Infrastructure ReDIB network.



TECHNICAL UNITS

- Advanced Imaging

Manuel Desco

- Flow Cytometry

Beatriz Álvarez

- Microscopy

Valeria Caiolfa

RESEARCH GROUPS

- Molecular Mechanics of the Cardiovascular System

Jorge Alegre-Cebollada

- Molecular Genetics of Angiogenesis

Rui Benedito

- Multidisciplinary Translational Cardiovascular Research (MTCR)

Héctor Bueno

- Mechanoadaptation and Caveolae Biology

Miguel Ángel del Pozo

- Imaging the Cardiovascular Inflammation and the Immune Response

Andrés Hidalgo

- Development of the epicardium and its role during regeneration

Nadia Mercader

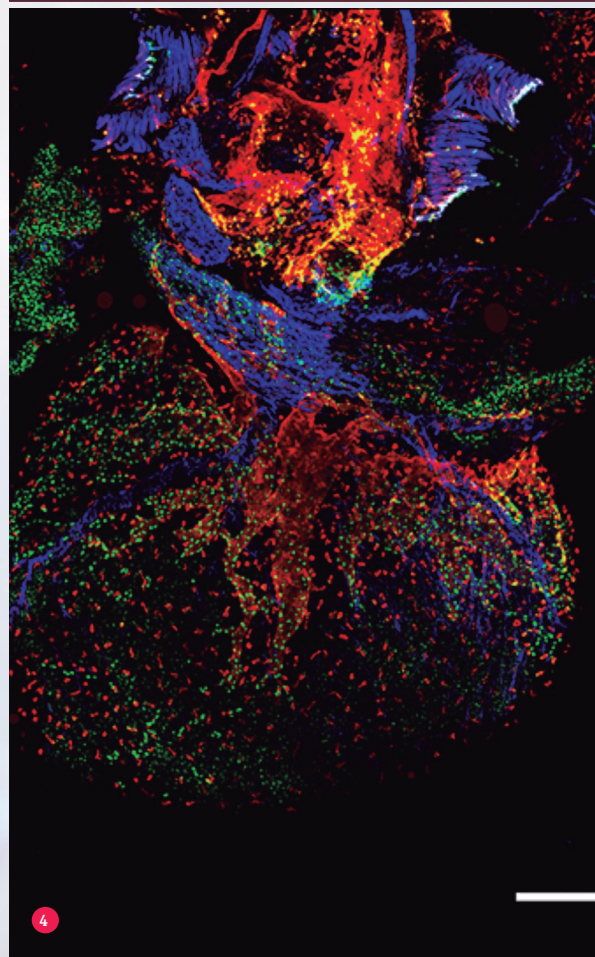
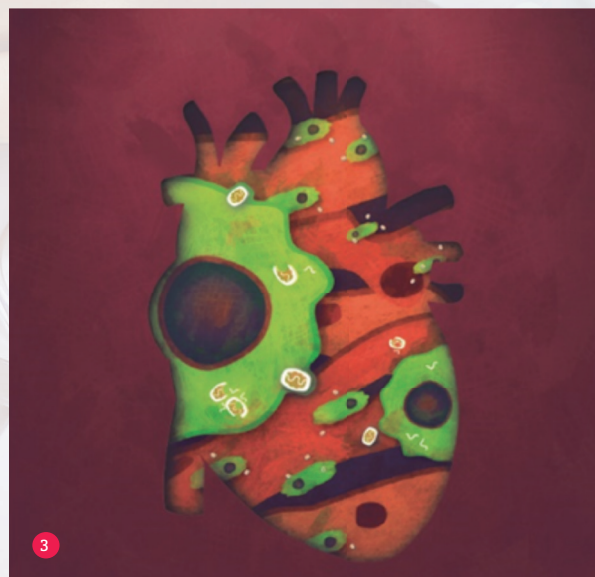
- Nanomedicine and Molecular Imaging

Carlos Pérez Medina

- Genetic Control of Organ Development and Regeneration

Miguel Torres

The CDB area is committed to public outreach and has contributed to the CNIC's efforts to address the challenges posed by the Covid-19 pandemic. In 2020, we established high specificity and sensitivity ELISA assays to detect antibodies to SARS-CoV-2 in human serum samples, and our scientists engage actively in public dissemination events related to the pandemic.



4⁺2i CLINICAL STUDIES



EARLY DETECTION OF SUBCLINICAL ATHEROSCLEROSIS, PROGRESSION AND CARDIOVASCULAR HEALTH

(PESA-HEALTH-CNIC-SANTANDER STUDY)

Principal investigator: **Valentín Fuster**

The PESA-Health-CNIC-Santander study is the natural continuation of the long-term endeavor started in 2010 with the PESA Study, carried out by the CNIC in collaboration with Santander Bank. Within PESA-Health, the PESA participants enrolled in 2010 (4184 asymptomatic individuals between the ages of 40 and 55 years at enrollment) will be actively followed up over an additional 10 years.

The original aim of the study was to identify the presence of subclinical atherosclerosis (SA) long before symptoms appear and to understand the cues leading to its development and progression. PESA-Health will expand these objectives to new areas, such as the correlation of SA with Alzheimer's and cognitive diseases, the acquisition of somatic mutations during aging, and the correlation of these mutations with increasing cardiovascular event rates and SA progression. PESA-Health will continue to take

advantage of state-of-the-art imaging technologies, including 3D vascular ultrasound of the carotid arteries and aorta, coronary artery calcium quantification by computed tomography, cardiac magnetic resonance, AngioTC, NaF PET, PET-amyloid analysis, and biosampling for omics analysis.

PESA-Health is the CNIC's flagship study, and several CNIC clinical and basic research groups participate in it. The PESA study is already making seminal contributions to our understanding of the origin and progression of atherosclerosis.

The PESA-Health-CNIC-Santander study welcomed its first participant in February 2020, taking advantage of the follow-up of the PESA cohort to continue and expand the scientific approaches performed.



SECONDARY PREVENTION OF CARDIOVASCULAR DISEASE IN THE ELDERLY POPULATION

(SECURE)

Principal investigator: **Valentín Fuster**

Co- Principal investigator: **José M^o Castellano**

Adherence to treatment after acute myocardial infarction (MI) is essential for efficient secondary prevention. Despite this, many post-MI patients abandon prescribed medication. To address this issue, CNIC researchers and FERRER laboratories developed a "polypill" including three key drugs prescribed to post-MI patients (aspirin, an ACE-inhibitor, and a statin). Having demonstrated that prescription of the CNIC Polypill significantly increases treatment adherence among post-MI patients (J Am Coll Cardiol. 2014; 64:2071-82), CNIC researchers are now leading a multinational randomized clinical trial supported by the H2020 program. The

ongoing SECURE trial (trial identifier NCT02596126) has enrolled around 2500 patients soon after an MI and randomized them to standard treatment or a CNIC Polypill-based strategy. Patients will be followed-up for a minimum of 2 years, and the incidence of major cardiovascular events will be assessed. Trial enrollment was completed by the end of 2019, and the trial is expected to complete its follow-up phase by the end of October 2021.

This trial grant has been extended until December 2021, and publication of the results is expected by mid 2022.



TREATMENT WITH BETA-BLOCKERS AFTER MYOCARDIAL INFARCTION WITHOUT REDUCED EJECTION FRACTION (REBOOT)

Principal investigator:
Borja Ibáñez

The prescription of beta-blockers to patients after an MI is based on evidence from trials performed in the pre-reperfusion era. While there is solid evidence for the benefit of these drugs in post-MI patients with reduced ejection fraction, evidence is lacking for patients with preserved ejection fraction. Despite this, more than 80% of post-MI patients in this category are prescribed beta-blockers for the rest of their lives. REBOOT is a multinational trial that will enroll 8468 post-MI patients with a left ventricular ejection fraction >40%. Patients will be randomized to beta-blocker therapy (type and dose decided by the attending physician) or to no treatment. The primary endpoint

is the composite of all-cause death, reinfarction, or heart failure admission during 3-year follow-up. This trial is coordinated by the CNIC Clinical Trials Coordination Unit and is run in close collaboration with the Mario Negri Institute of Research in Milan. More than 75 hospitals in Spain and more than 25 in Italy are participating in this large-scale project, which will have a major impact on clinical practice.

The first patients were enrolled in October 2018, and 4070 had been recruited by the end of 2020. The first follow-up assessment has been completed in 80% of patients, and the second follow-up in 45%.



THE TANSNIP-PESA RANDOMIZED CONTROL TRIAL: A 30-MONTH WORKSITE-BASED LIFESTYLE PROGRAM TO PROMOTE CARDIOVASCULAR HEALTH IN MIDDLE-AGED BANK EMPLOYEES (TANSNIP)

Principal investigator:
Valentín Fuster

Existing tools for characterizing atherosclerosis and determining the risk of its complications are inadequate. These deficiencies limit effective management across the spectrum of this disease, and therefore opportunities are lost for early, cost-effective interventions in sub-clinical disease, while high-risk populations with manifest disease are administered treatments almost indiscriminately. This leads to high 'numbers needed-to-treat' (NNT), unnecessary patient risk, wasted resources, and unsustainable costs for health care purchasers. In a relatively low-risk population (the PESA-CNIC cohort), we are studying whether a personalized worksite based lifestyle intervention driven by imaging data (2D and 3D-ultrasound of the carotid and iliofemoral arteries, and coronary artery calcification) results in changes in behavior, improved control of risk factors, and reduced progression of subclinical atherosclerosis plaque burden (SAPB). TANSNIP is a randomized control trial (RCT) including middle-aged bank employees from the PESA cohort stratified by SAPB (high SAPB n=260; low SAPB n= 590). Within each stratum, participants are randomized 1:1 to join a lifestyle program or receive standard care. The program consists of three elements: (1) 12 personalized lifestyle counseling sessions using motivational

interviewing (MI) over a 30-month period; (2) a wrist-worn physical activity tracker, and (3) a sit-stand workstation. The primary outcome measure is a composite score of blood pressure (BP), physical activity, sedentary time, body weight, diet, and smoking (the adapted FUSTER-BEWAT score) measured at baseline and at 1-, 2-, and 3-year follow-up. Secondary outcomes are individual changes in lifestyle behaviors and specific changes in anthropometric measures, blood biomarkers, self-rated health, work-related outcomes (including work productivity and absenteeism), health care consumption, program process measures, and cost measures at different measurement points.

The expectation is that individual awareness of CVD risk stratification in the intervention group will lead to a reduction in the prevalence of CV risk factors related to lifestyle and an increase in physical activity compared with the control group. A second rationale is that the level of compliance with the comprehensive 3-year worksite-based lifestyle intervention will be higher among participants with a high imaging defined CV risk. Follow-up is now complete, and the TANSNIP-PESA Study is now in the analysis phase.



ATHERO-BRAIN. THE HEART TO HEAD STUDY

(H2H)

Principal investigator: **Valentín Fuster**

Co- Principal Investigator: **Héctor Bueno**

There is increasing awareness of the association between atherosclerosis and cognitive function, but the mechanisms linking these processes are not fully understood. The Heart-to-Head (H2H) study is testing the hypothesis that extensive subclinical atherosclerosis is associated with subtle cognitive decline and beta-amyloid deposition in the brain. This transatlantic collaboration is framed within an agreement between the CNIC and Mount Sinai Hospital in New York and is led by CNIC General Director Valentín Fuster. In Spain, the H2H project is coordinated between the CNIC and 12 de Octubre Hospital. Other university hospitals (Fundación

Jiménez Díaz, Clínico San Carlos, and Gregorio Marañón) participate in the project, which receives funding from the Carlos III Institute of Health through the Proyecto Integrado de Excelencia program. A total of 300 participants are undergoing extensive atherosclerosis phenotyping (multi-territory 3D vascular ultrasound and cardiac computed tomography) and thorough brain imaging (anatomical and functional magnetic resonance imaging and positron emission tomography (PET)-amyloid scan), as well as cognitive function testing. Follow-up visits finished in 2020, and the study is now in the analysis phase.



MULTIMODALITY MYOCARDIAL TISSUE CHARACTERIZATION IN PATIENTS WITH SIGNIFICANT VALVULAR DISEASE

(MRVALVE)

Principal investigator: **Borja Ibáñez**

The consequences of valvular heart disease (VHD) on left ventricular (LV) dimensions, function, and tissue composition are important determinants in clinical decision-making. Current practice guidelines recommend surgical treatment for patients with significant valvular heart disease when symptoms develop or when LV remodeling or dysfunction occur. The most prevalent valvulopathies are aortic valve stenosis (AS) and mitral regurgitation (MR). Transition from asymptomatic to symptomatic disease or from normal LV dimensions and function to LV dilatation/hypertrophy (LVH) and dysfunction is determined by changes in tissue composition (predominantly cardiomyocyte death, extracellular volume expansion, and fibrosis). The current therapies for severe VHD are surgery or percutaneous valve repair or replacement, and the decision to intervene is based on the presence of symptoms and/or gross anatomical and functional LV involvement, evident as significant chamber dilatation or reduced ejection fraction. When these features appear, it is often too late for interventions to fully restore heart function. There is therefore a need for tools for the early detection of myocardial involvement in patients with asymptomatic VHD, thus enabling appropriate intervention before overt deterioration of heart function. Cardiac magnetic resonance (CMR) is the gold standard for anatomical and functional cardiac assessment, including the detection of focal areas of fibrosis by late gadolinium enhancement (LGE) after contrast-gadolinium administration. Moreover, highly accurate tissue characterization is available with recent CMR advances

such as parametric T1/T2 mapping, absolute myocardial perfusion quantification, extracellular volume calculation (a surrogate of diffuse fibrosis), and tagging. The assessment of focal and diffuse fibrosis requires endovenous contrast. We will use gadolinium contrast agent, which has the maximal safety profile and is in routine clinical use. Assessment of diffuse fibrosis also requires a blood sample for determination of the hematocrit. For the study of active deformation of the LV myocardium, the best imaging modality is strain echocardiography, which can detect impaired multidirectional strain (active deformation) even when overall LV function is preserved. We will correlate the imaging data with functional data from the 6-minute walking test, which provides an objective assessment of functional exercise capacity. The amount and extent of calcium deposition in the coronary arteries and heart valves will be assessed by cardiac computed tomography, a noninvasive method that gives the calcium score, a diagnostic and prognostic tool in AS patients. This project will use a multimodality imaging approach (CMR plus strain echocardiography) to better characterize LV status in patients with significant VHD, whether AS (a paradigm of LV pressure overload) or MR (a paradigm of LV volume overload).

So far 57 patients has been recruited, and 30 have completed the one-year follow-up visit.



TREATMENT WITH β 3 AGONISTS IN CHRONIC PULMONARY HYPERTENSION SECONDARY TO HEART FAILURE

(SPHERE- HF)

Principal investigator: **Ana García-Alvarez**

Co-Principal Investigators: **Valentín Fuster and Borja Ibáñez**

Pulmonary hypertension (PH) secondary to left heart disease (group 2 PH) is the most common form of PH and currently lacks effective therapy. CNIC researchers have identified the β 3 adrenergic receptor as a novel therapeutic target for this disease in a large animal model of PH (Basic Res Cardiol. 2016;111:49). The CNIC is currently leading a phase 2 clinical trial in which group 2 PH patients are randomized to standard therapy vs standard therapy plus a β 3-selective agonist

(trial identifier NCT02775539 and N° EudraCT: 2016-002949-32). A total of 80 patients are being recruited in four Spanish hospitals and will be followed under treatment for 4 months. The study endpoints are pulmonary artery hemodynamics and the CMR profile.

Enrollment was extended up to 2020. Of the 80 recruited patients, 75 had completed the 4-month follow-up by the end of 2020.



PULMONARY VASCULOPATHY IN PATIENTS WITH ADVANCED HEART FAILURE

(HPIC)

Principal Investigator Hospital 12 de Octubre: **Juan F. Delgado Jiménez**

Co-Principal Investigator CNIC: **Borja Ibáñez**

Pulmonary hypertension due to left heart disease is a pathophysiological and hemodynamic state found in a wide range of clinical conditions that affect left heart structures. Although the pulmonary circulation has traditionally received little attention, it is today a fundamental part of cardiological evaluation. The most important clinical factors in heart-failure patients are the presence of pulmonary hypertension and right ventricular function. These factors are also essential prognostic determinants and must be taken into account when making important therapeutic decisions. The pathophysiological process starts passively but later transforms into a reactive process. This reactive process has a reversible component,

which can be treated with vasodilators, and fixed component, in which the underlying mechanism is congestive vasculopathy (essentially medial hypertrophy and pulmonary arterial intimal fibrosis). There is currently no specific therapy available for this type of pulmonary hypertension, and treatment is the same as for heart failure itself. Drugs known to be effective in pulmonary arterial hypertension have generally shown a neutral effect in clinical trials. This project is focused on the clinical development of a number of groups of pharmacological compounds that will enable us to make progress in the near future.

So far we have recruited 45 patients and recruitment is continuing.



S. Pocock (Left) and B. Ibáñez (Right)



3D ULTRA-FAST COMPREHENSIVE CARDIAC MAGNETIC RESONANCE STUDY (CARDIO-RESONANCIA MAGNÉTICA INTEGRAL 3D ULTRA-RAPIDA)

(DASH – CMR)

Principal investigator:

Borja Ibáñez

FIS-DTS (Proyecto de Investigación en Salud modalidad Proyectos de Desarrollo Tecnológico en Salud Ref DTS17/00136.)

This study is divided into 2 separate substudy protocols:

- 1) Myocardial study, which uses gadolinium contrast to study post-infarction patients, cardiomyopathy, and myocarditis.
- 2) Study of coronary angiography with MRI without gadolinium contrast, for the study of patients undergoing coronary angio-CT.

MRI exams in this study have been conducted at Hospital Universitario de Salamanca (1.5 Tesla MRI) and with the new MRI equipment located at the CNIC. This project was funded by a grant from the ISCIII (FIS-Technological Development (FIS-DTS). During the first year, we validated and improved the MRI technology for the pig model and worked on making study reconstruction achievable in real time in the MRI console itself.

Enrolment was completed in 2020, and a total of 147 participants were recruited.



NOVEL MITOCHONDRIA-TARGETED THERAPIES FOR CANCER TREATMENT-INDUCED CARDIOTOXICITY

(MATRIX)

Principal investigator: **Borja Ibáñez**

ERC Consolidator Grant#819775

The MATRIX Project aims to develop new and innovative treatments for cardiac toxicity associated with some cancer treatments. MATRIX will be jointly run at the CNIC and Fundación Jiménez Díaz (FJD) University Hospital within a collaborative framework established in 2015 to study myocardial diseases.

Great advances in the treatment of cancer—a disease with 4 million new diagnoses every year in Europe—sometimes come with a 'toll' to pay in the form of major adverse effects. One of the most common adverse effects is myocardial toxicity, which affects up to 25% of patients treated with the common anticancer drugs anthracyclines or trastuzumab. The cardiotoxic effects of these drugs can be very serious and condemn the cancer survivor to chronic heart failure or even death from this complication.

Cancer treatment-induced cardiotoxicity (CTiCT) can result in severe heart failure. The trade-off between cancer and chronic heart failure places an immense personal burden on patients, with physical and psychological consequences. Current therapies for CTiCT are suboptimal, featuring poor early detection algorithms and nonspecific heart failure treatments. Our recently published results and additional preliminary data indicate that CTiCT is associated with altered mitochondrial dynamics,

triggering cardiomyocyte metabolic reprogramming. MATRIX adopts a holistic approach to tackling mitochondrial dysfunction in CTiCT. We propose that early-stage CTiCT could be reverted by metabolic reprogramming to shift mitochondrial substrate utilization. By refining a novel imaging-based algorithm recently developed by our group, we will achieve very early detection of myocardial damage in patients treated with commonly prescribed cancer therapies, long before clinically used parameters become abnormal. Such early detection, not available currently, is crucial for early therapeutic intervention. We also hypothesize that in end-stage CTiCT, mitochondrial dysfunction has passed a no-return point, and the failing heart will only be rescued by a strategy to replenish the myocardium with fresh healthy mitochondria. This can be achieved with the radical new therapeutic option of in-vivo mitochondrial transplant. The MATRIX project has broad translational potential, including a new therapeutic approach to a clinically relevant condition, the development of technology for early diagnosis, and advances in knowledge of basic disease mechanisms.

Patient recruitment began in 2020, and by the end of the year we had already hosted 12 participants.