2 RESEARCH AT THE CENTER

2.1 SCIENTIFIC PROGRAMS

The CNIC is organized into two departments, one focused on Basic Research and the other on Clinical Research. Research in these fields is fully interconnected through seven focused Programs.

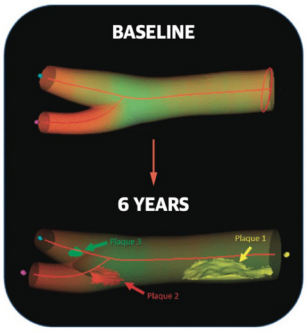
2.1.1 NOVEL MECHANISMS OF ATHEROSCLEROSIS

Coordinator: **José J. Fuster**
Clinical leaders: **Valentín Fuster and Inés García Lunar**

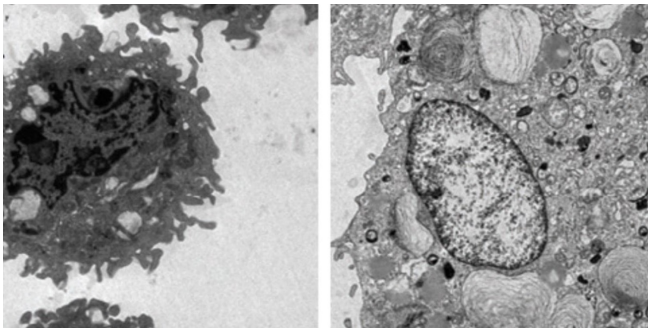
The Novel Mechanisms of Atherosclerosis Program aims to provide key insights into the pathophysiology of atherosclerosis, the underlying cause of the most frequent cardiovascular and cerebrovascular disorders. Despite the efficacy of interventions that target traditional cardiovascular risk factors, a substantial risk of atherosclerotic cardiovascular disease remains, even in individuals who achieve massive reductions in blood cholesterol and are apparently at low cardiovascular risk based on current risk scores. Therefore, while it remains imperative to target well-established cardiovascular risk factors, there is an evident need for a deep understanding of non-conventional risk factors and pathophysiological mechanisms that could lead to new strategies for the prediction, prevention, and treatment of atherosclerotic cardiovascular disease. In this context, the research groups in the Program are working towards the identification and characterization of new inflammatory drivers of atherosclerosis.

Research during the past 30 years has clearly established that atherosclerosis is an inflammatory condition resulting from a maladaptive response of the immune system to the chronic exposure to cardiovascular risk factors. Despite this, targeting inflammation in cardiovascular disease remains an unfulfilled promise, highlighting the need for a comprehensive understanding of the intricacies of inflammatory responses in atherosclerosis. Several research groups in the Program are working towards this goal, building upon prior seminal work and combining human studies (based on existing and novel data from the Progression of Early Subclinical Atherosclerosis [PESA] cohort) and experiments in different models. Ongoing research in this area is focused on the role of acquired mutations in hematopoietic cells (a phenomenon called clonal hematopoiesis), telomere dynamics, autoantibodies, and specific leukocyte subsets in atherosclerosis. Additional projects are related to the biology of vascular smooth muscle cells in atherosclerotic plaques, the sensing of mechanical stress by the vascular wall, the effects of microbiota-derived metabolites, and the identification of circulating and imaging biomarkers of atherosclerosis development.

In 2023, among other **research achievements,** scientists in the Program identified key determinants of human atherosclerosis dynamics during its early, asymptomatic stages. Program scientists also expanded our knowledge of the regulation and function of immune cells at multiple levels. We demonstrated that acquired p53 gene mutations



Standardized 3D vascular ultrasound images illustrating the progression of human atherosclerosis (image from the PESA-HEALTH study, obtained using CM2020 software).



Environmental lipids and cholesterol accumulate in macrophages deficient in TFAM, a mitochondrial transcription factor. Representative transmission electron microscopy images of macrophages sorted by flow cytometry show spiral-like structures in TFAM-deficient macrophages (right) compared to controls (left).

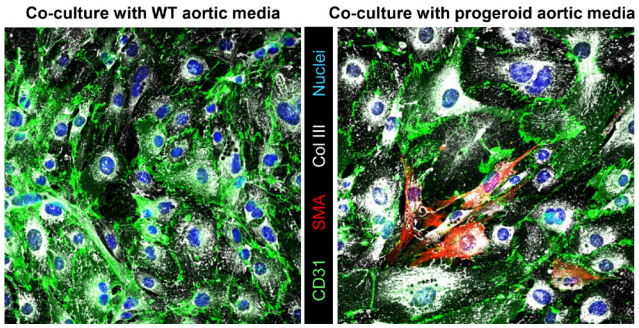
RESEARCH GROUPS

- **Vicente Andrés**
  Molecular and Genetic Cardiovascular Pathophysiology
- **Jacob Fog Bentzon**
  Experimental Pathology of Atherosclerosis
- **Miguel A. del Pozo**
  Mechanoadaptation and Caveolae Biology
- **José J. Fuster**
  Hematovascular Pathophysiology
- **Valentín Fuster**
  Cardiovascular Imaging and Population Studies
- **Inés García Lunar**
  Cardiovascular Prevention through Non-invasive Imaging
- **Carlos Pérez-Medina**
  Nanomedicine and Molecular Imaging
- **Almudena R. Ramiro**
  B Lymphocyte Biology
- **Francisco Sánchez-Madrid**
  Intercellular Communication in the Inflammatory Response
- **David Sancho**
  Immunobiology
- **Jesús Vázquez**
  Cardiovascular Proteomics

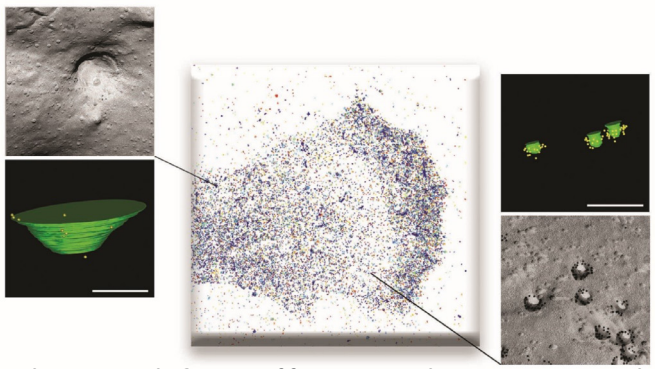
accelerate atherosclerosis development by exacerbating macrophage proliferation within atherosclerotic plaques. We also established that macrophage function is tightly regulated by mitochondrial respiration, which is highly dependent on the organ microenvironment. Furthermore, research into the sensing of mechanical stress by the vascular wall have identified novel plasma membrane invaginations called dolines that respond to weak or medium-strength mechanical forces.

The quality and potential of the research conducted by the Program teams is attested by several successful applications for research funding in 2023, including research grants funded by La Caixa Foundation, La Marató de TV3 Foundation, the ERA4Health CARDINNOV program, the ERC Proof of Concept program, and the EIC Pathfinder program. This new funding will fuel further developments in our scientific project. In addition, among other awards received by Program scientists, Dr David Sancho was garlanded with the 28th Carmen and Severo Ochoa Award for Research in Molecular Biology.

The Program place great emphasis on the effective public communication of our research into atherosclerotic cardiovascular disease. Last year, Program scientists participated in several outreach activities, most notably the Cardioguía: más allá del colesterol alto event, held as part of the XXIII Science and Innovation Week in Madrid.



Conditioned medium from progeroid aortic smooth muscle cells induces wild-type (WT) mouse aortic endothelial cells to undergo endothelial-to-mesenchymal transition



Dolines or caveolae? 2 ways of force sensing. Electron microscopy analysis and 3D reconstruction. Dolines (left) respond to weak or medium-strength forces, whereas the previously known caveolae (right) are essential for the response to large mechanical forces. Program scientists are studying the importance of mechanical forces in atherosclerosis.

2.1.2 MYOCARDIAL HOMEOSTASIS AND CARDIAC INJURY

Coordinator: **Enrique Lara-Pezzi**
Clinical leader: **Borja Ibáñez**

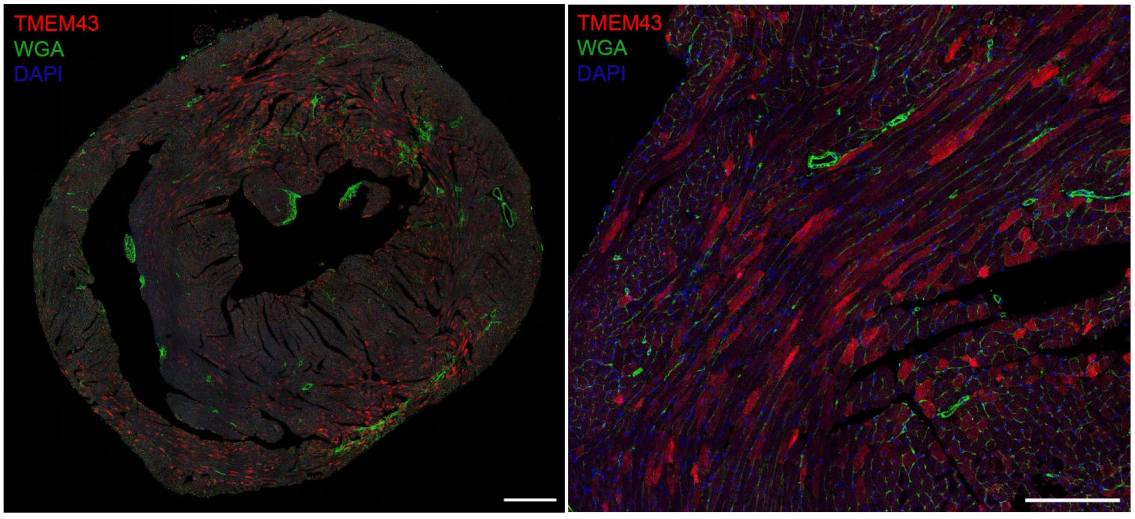
The research groups in the Myocardial Homeostasis and Cardiac Injury (MERCURY) Program investigate the genetic, molecular, and biomechanical mechanisms underlying myocardial injury and the development of inherited cardiomyopathies. MERCURY groups also investigate the development of new therapies based on these mechanisms. Despite the development of diverse cancer therapies, for many cancers the first line treatment remains chemotherapy with anthracyclines. One of the most feared adverse effects of these drugs is irreversible cardiac injury, which affects many patients. Alternative treatments include immune checkpoint inhibitors (ICIs), which are monoclonal antibodies that target host negative immune regulatory receptors. While effective for cancer treatment, this approach can also be cardiotoxic, inducing myocarditis, which is associated with high mortality. Understanding remains limited about the mechanisms through which anthracyclines and ICIs induce cardiac damage, as well as the factors that determine variable interindividual vulnerability to these cytotoxic effects. This knowledge deficit translates into a lack of effective therapies able to prevent or reverse this cardiac pathology.

A major goal of the MERCURY Program is therefore to define the pathways and mechanisms underlying the cardiotoxic effects triggered by anthracyclines and ICIs used to treat cancer. We aim to identify the main determinants of this type of myocardial injury and then to develop new therapeutic approaches based on the inhibition of these pathways.

A second main area of interest of the MERCURY Program investigators is the genetic basis of inherited cardiomyopathies (CMs). Genetic testing has evolved rapidly over the last decade and is now an established element of the clinical management of CM patients and their families. Despite this, the current yield of genetic testing, even in familial cases (with two or more family members affected), is around 40%, and most cases remain unexplained. Furthermore, most genetic heart conditions are treated with drugs developed for generic cardiac pathologies, such as heart failure or cardiac arrhythmias. For most CMs, there are no specific disease-modifying treatments.

Our researchers are working to identify new disease-causing mutations in noncoding regions, chiefly introns, and are investigating the molecular basis of genetic cardiomyopathies. We are also developing large animal models of hypertrophic and arrhythmogenic cardiomyopathies, based on the success of our previous mouse models, and we aim to use these models to develop new gene therapy tools and identify small molecules with the potential to improve cardiac function.

The MERCURY Program relies on the combination of our research teams' strong complementary expertise in biomechanics, molecular biology, physiology, immunology, and genetics. This complementary approach provides each task with additional granularity. The program has a clear translational orientation, aimed at developing new diagnostic and therapeutic tools.



Mouse heart infected with adeno-associated virus (AAV) containing S358L-TMEM43. TMEM43 immunofluorescence analysis in a myocardial section (left) and magnification (right) of a 16 weeks old mouse after neonatal injection of AAV-S358L-TMEM43 though the temporal vein. Bar, 500 µm (left) and 150 µm (right).

RESEARCH GROUPS

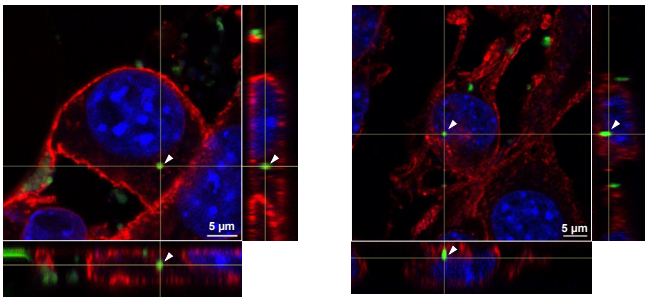
Jorge Alegre-Cebollada
Molecular Mechanics of the Cardiovascular System

Ana García Álvarez
Heart Failure and Pulmonary Hypertension Translational Research

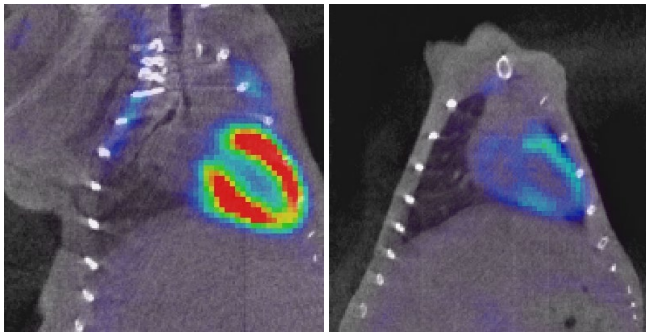
Pablo García-Pavía
Inherited Cardiomyopathies

Borja Ibáñez
Translational Laboratory for Cardiovascular Imaging and Therapy

Enrique Lara-Pezzi
Molecular Regulation of Heart Failure



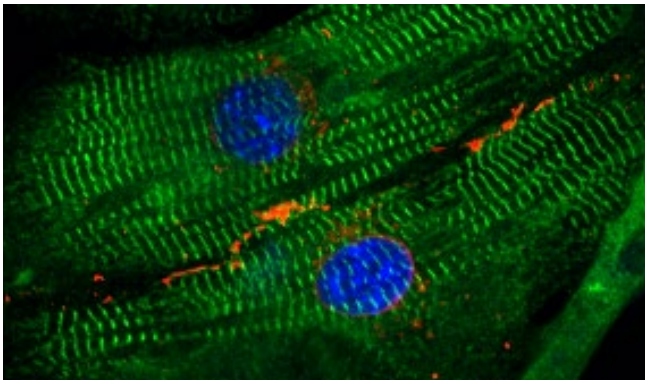
Confocal fluorescence images of cultured MEFs co-incubated with isolated mitochondria from Dendra2 mice for 6 hours or 24 hours. Pseudo colors: blue (nuclei–DAPI); red (plasma membrane–WGA-TexasRed); green (mitochondria–Dendra2-COXVIII). Arrows pointing at integrated mitochondria subject tof orthogonal view visualization.



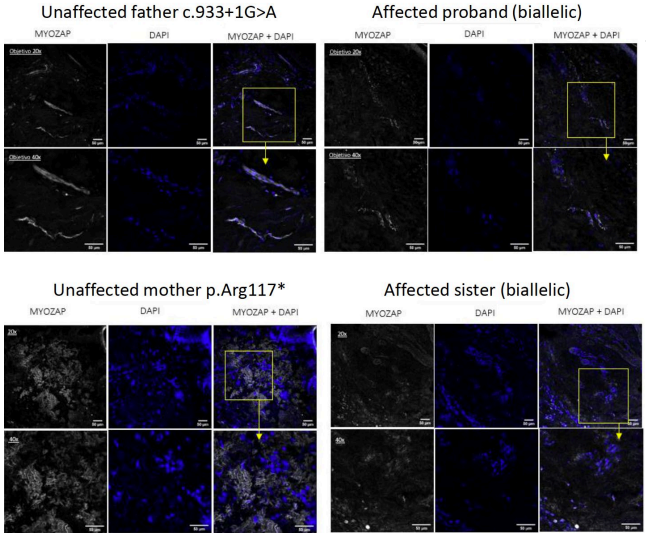
Positron emission tomography (PET) image of a mouse heart showing glucose (18F-FDG) uptake. Glucose uptake is severely reduced after treatment with anthracyclines, drugs commonly use for the treatment of cancer that have secondary effects on the heart.

MERCURY Program achievements in 2023

- Experiments in a large animal model of cardiotoxicity secondary to anthracyclines demonstrated that oral treatment with SGLT2 inhibitors prevents the onset of cardiac dysfunction by preserving myocardial metabolism.
- Gene sequencing in individual patients and their relatives identified new genetic mechanisms of inherited cardiomyopathy. The sequencing included thousands of genetic variants of titin that can cause dilated cardiomyopathy, and the information obtained will help in the diagnosis of a disease that is the number one cause of heart transplantation worldwide.
- MERCURY Program investigators established a national myocarditis registry that includes more than 25 centers.
- Research into links between actomyosin dysregulation and cardiac systolic dysfunction identified a potential treatment for arrhythmogenic cardiomyopathy.
- New gene therapy tools for inherited cardiomyopathy were successfully tested in mice.



Force-generating structures in isolated murine neonatal cardiomyocytes. Green: titin. Red: connexin 43. Blue: nucleus (DAPI).



Biallelic probands carrying pathogenic variants in the gene MYZAP, which encodes a protein of the intercalated discs, develop severe dilated cardiomyopathy. The figure shows MYZAP immunostaining in skin samples from affected siblings and unaffected parents to illustrate the loss or mislocalisation of the protein. The. Skin biopsies were immunostained with anti-MYZAP antibody (white). Nuclei were stained with DAPI (blue). Images were acquired with a 20X objective. Magnifications of the selected areas (yellow squares) were acquired with a 40X objective.
Note: this figure is form a published paper (by Pablo Garcia-Pavia and Enrique Lara-Pezzi).

2.1.3 CARDIOVASCULAR REGENERATION

Coordinator: **Miguel Torres**
Clinical leader: **Hesham Sadek**

The Cardiovascular Regeneration (CVR) Program aims to understand basic principles of heart and vasculature development and regeneration and to use this knowledge to develop new therapeutic strategies to cure cardiovascular diseases. Within this field, the CVR Program has a strong focus on the metabolic regulation of cardiac regenerative ability.

In the metabolic regulation area, a CVR collaborating group led by Dr Mercedes Ricote demonstrated that the gamma-linolenic acid–retinoid X receptor axis is a key transcriptional regulatory mechanism underlying the maternal control of perinatal cardiac metabolism. The study showed that RXRs in cardiomyocytes relay signals form maternal milk-derived fatty acids to enable mitochondrial maturation and metabolic adaptation in neonatal hearts. These results reinforce the idea that mother–infant interactions in early life are major drivers of organismal physiology and cardiac health and address a crucial period of heart development during which the natural regenerative ability of the fetal heart is lost. Along these lines, the CVR group led by Dr Jose Antonio Enríquez continued efforts to understand and control the regulation of mitochondrial oxidative phosphorylation (OxPhos). Their work demonstrated the role of cox7a1 in striated muscle maturation through regulation of the respiratory chain, uncovering a direct connection between mitochondrial flavin homeostasis and complex I stability and assembly. This finding paves the way for novel pharmacological strategies to regulate respiratory complex I. In a further collaborative study, Drs Enríquez and Muñoz-Cánoves showed that the loss of mitochondrial fission in skeletal muscle satellite cells—due to aging or genetic impairment—dysregulates the mitochondrial electron transport chain, leading to inefficient OxPhos metabolism, mitophagy, and increased oxidative stress. These findings have implications for regeneration therapies in sarcopenia. Also in this area, Drs Nadia Mercader and Enríquez used the zebrafish model to examine the effects of cox7a1 and cox7a2l loss-of-function on the physiology and injury response of skeletal muscle and myocardium, revealing a high Cox7a-isoform specificity in the control of supercomplex assembly and striated muscle metabolism.

Mechanistic studies of heart diseases with a developmental basis included a study led by Dr Rui Benedito which showed that single-cell transcriptional states, or the dysregulation of transcriptional factor networks, often do not correlate with vascular pathophysiological cell states, a finding that challenges the sole use of scRNAseq transcriptome analysis to predict vascular phenotypes, states, or diseases.

In another study, the group led by José Luis de la Pompa uncovered a new mechanistic basis for congenital heart disease, establishing the causal relationship between disrupted NOTCH-MIB1 signaling and human congenital heart defects (left ventricular noncompaction and bicuspid aortic valve). In a second study, these scientists identified a mechanistic link between NOTCH, NRG1, and HIPPO signaling in the regulation of cardiomyocyte proliferation and cellular dynamics during early cardiac chamber development.

2023 was a transitional year for the CVR Program, with several groups leaving and others joining. Dr Andrés Hidalgo has been transitioning to his new location at Yale University but has continued to participate in the CVR Program. His group has continued its studies of the roles of neutrophils and macrophages in vascular health and cardiac homeostasis, discovering that the contribution of macrophages to cardiomyocyte proteostasis extends to other organs and is adaptable to the metabolic status of the organ. The groups of Pura Muñoz-Cánoves and Mercedes Ricote also left the CNIC last year, but both maintain collaborative bonds with the CVR Program. Dr Florian Weinberger joined the Program as a new group leader in September 2023. He leads the Cardiac tissue engineering and regenerative therapies group, which aims to develop stem-cell based strategies to remuscularize the failing heart. Their research combines cardiac tissue engineering with chemo- and optogenetic tools to dissect mechanisms of cardiac regeneration. Dr Weinberger´s incorporation contributes essential expertise in IPSC-derived cardiomyocytes, supporting CVR Program plans to set up drug screening, in vitro disease modeling, and regenerative strategies. Recent recruit Dr Hesham Sadek consolidated his group at the CNIC during 2023 and is developing projects aimed at discovering new drugs for heart regeneration based on the transcriptional control of cardiomyocyte proliferation.

In the area of technology development, Dr Nadia Mercader established a screening pipeline that uses zebrafish larvae to assess the effect on cardiovascular development of compounds used to treat COVID-19. The pipeline can be expanded to perform further drug screens within the CVR Program. In addition, the Program is developing centralized technology platforms and has hired a technician for animal models and non-invasive imaging and a senior postdoc to set up an IPSC-derived cardiomyocyte high-throughput screening platform.

HIGHLIGHTED PAPERS:

Nature Cardiovasc. Res. (2023) 10.1038/s44161-023-00272-4

Circulation 2023 Jan 3;147(1):47-65. doi: 10.1161/CIRCULATIONAHA.121.058767

Nature 2023 Jan;613(7942):169-178. doi: 10.1038/s41586-022-05535-x

Nature 2023 Jun;618(7964):365-373. doi: 10.1038/s41586-023-06068-7

JAMA Cardiol. 2023 Aug. 1; 8(8): 721-731. DOI: 10.1001/jamacardio.2023.1469

Cell Stem Cell 2022 Sep 1;29(9):1298-1314.e10. doi: 10.1016/j.stem.2022.07.009

Circ Res 2023 Nov 10;133(11):927-943. doi: 10.1161/CIRCRESAHA.123.323321

Circ Res 2023 Dec 8;133(12):1022-1039. doi: 10.1161/CIRCRESAHA.123.323474

RESEARCH GROUPS

- **Rui Benedito**
  Molecular Genetics of Angiogenesis
- **Jose Luis de la Pompa**
  Intercellular Signaling in Cardiovascular Development and Disease
- **Jose Antonio Enríquez**
  Functional Genetics of the Oxidative Phosphorilation System GENOXPHOS
- **Andrés Hidalgo**
  Imaging the Cardiovascular Inflammation and the Immune Response
- **José Jalife**
  Cardiac Arrhythmia
- **Nadia Mercader**
  Development of the Epicardium and its Role during Regeneration
- **Pura Muñoz**
  Tissue Regeneration
- **Mercedes Ricote**
  Nuclear Receptor Signaling
- **Hesham Sadek**
  Myocardial Regeneration Vía Cardiomyocyte Cell Cycle Regulation
- **Miguel Torres**
  Genetic Control of Organ Development and Regeneration
- **Florian Weinberger**
  Cardiac Tissue Engineering and Regenerative Therapies

PRIZES:

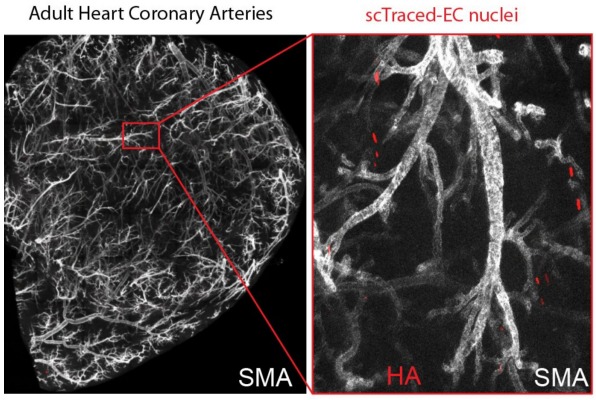
* Macarena Fernández Chacón obtained the WERNER RISAU PRIZE 2023 for outstanding study in vascular biology

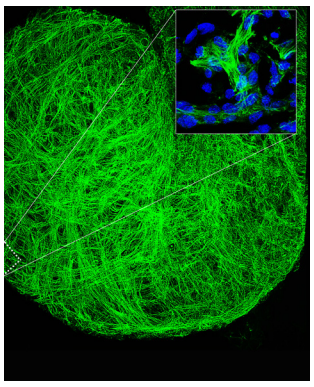
* Ana Paredes obtained the International Birnstiel Award for Doctoral Studies in Molecular Life Sciences 2023

HIGHLIGHTED GRANTS:

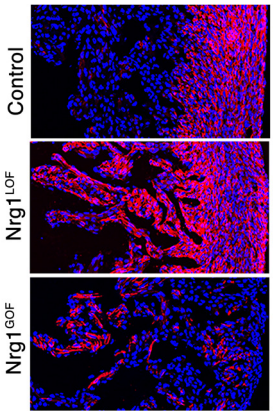
"La Caixa" Health Research Grant 2023. Applying DNA and optical barcoding to study endothelial progenitor cells in physiology and disease. 1 Million Euros; 3 groups- Rui Benedito (partner): Exec.: 12/2023-12/2026. (To Rui Benedito)

"La Caixa" Research Health Foundation. Novel genetic and mechanistic studies of hypertrophic cardiomyopathy. 1,000,000€, 3 members consortium. José Luis de la Pompa (coordinator). Exec.: 9/2023-9/2026

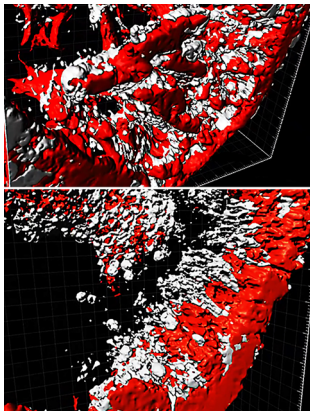




Neuregulin-1 induces actin filament changes in the myocardium during ventricular maturation. General view of a E9.0 wild type heart stained with anti-actinin (green) showing a striated pattern corresponding to the mature trabecular sarcomeres. Inset shows total actin filament (Phalloidin, green), differences in brightness highlights the more organized actin filament network in trabecular vs. compact myocardium. For details see paper by Grego-Bessa et al. in this issue.



Nrg1 is essential for ventricular patterning. Top to bottom: Sections of E16.5 control, Nrg1 loss-of-function (LOF) and Nrg1 gain-of-function (GOF) left ventricles stained for smooth muscle actin (SMA) and Dapi. Control heart shows SMA staining confined to the immature compact myocardium, Nrg1 LOF heart shows SMA expansion to trabeculae, while Nrg1 GoF shows very reduced SMA expression in compact myocardium and somewhat ectopic signal in a few trabeculae. The paper by Grego-Bessa et al. reveals the molecular and cellular mechanisms that Nrg[1] signaling uses to control ventricular patterning.



Nrg1 is essential for trabecular birth, growth and patterning. Top to bottom, 3D reconstructions (Amira) of smooth muscle actin (SMA, red) and isolectin B4 (IB4, white) staining of E10.5 control and Nrg1 deficient ventricles, showing the complex trabecular network of the normal heart and the primitive one in Nrg1 mutants. The paper by Grego-Bessa et al. reveals the molecular and cellular mechanisms that Nrg1 signaling uses to control ventricular patterning.

2.1.4 NOVEL ARRHYTHMOGENIC MECHANISMS

Coordinator: **Silvia Priori**
Clinical leader: **David Filgueiras**

PHENOTYPES ELICITED BY DISRUPTION OF INTRACELLULAR CA2+ REGULATION

Cardiac arrhythmias may be partially linked to underlying non-arrhythmogenic phenotypes. We have used mouse models of catecholaminergic polymorphic ventricular tachycardia (CPVT1) and the recessively inherited triadin knockout (KO) syndrome to detect the presence of uncharacterized non-arrhythmogenic phenotypes that might contribute to the arrhythmias observed in these diseases. In both models, we detected dysfunctional phenotypes in mitochondria. Characterization of the mitochondrial phenotype in cardiomyocytes from the CPVT1 model revealed that CPVT1 mitochondria are calcium-overloaded, have disrupted structure and ATP synthesis, and generate elevated amounts of mitochondrial reactive oxygen species (mitoROS). These mitoROS have the potential to diffuse out from mitochondria, increasing the probability of arrhythmias. A similar analysis is underway to characterize disturbed mitochondrial function in Triadin-KO hearts.

We have also observed a pronounced destabilization of the tubulin cytoskeleton in cells from triadin-KO hearts (Figure 1). This microtubular destabilization leads to mistargeting and degradation of calsequestrin 2, a protein that stabilizes the sarcoplasmic reticulum calcium release complex. Thus, in triadin-KO hearts, a cytoskeletal phenotype could be partially responsible for the observed changes in calcium handling that trigger arrhythmias.

These experiments are the subject of two Ph.D. theses. The project investigating CPVT1 has already been defended, while the one investigating triadin-KO syndrome is in progress.

NEW TARGETS & ADVANCED THERAPIES FOR INHERITED CHANNELOPATHIES

We have created the first knock-in swine model of Timothy Syndrome type 1, a devastating inherited arrhythmogenic disease associated with a median life expectancy <5 years. Extensive characterization of the arrhythmogenic substrate in single cells and whole hearts identified 3 previously uncharacterized mechanisms in the context of this disease that contribute to arrhythmia. The first is an unexpected calcium–overload-dependent and CaMKII-dependent slowing of conduction that promotes re-entrant arrhythmias. The other 2 mechanisms are an increased sodium late current and the fusion of late-systolic calcium sparks (leading to enhanced Na–Ca exchange). Together with the increased calcium current characteristic of Timothy Syndrome, these newly identified mechanisms contribute to dispersion of repolarization throughout the heart.

We have further tested 5 pharmacological therapies aimed at 3 separate points along the intricate pathway leading to arrhythmias in Timothy Syndrome, while simultaneously demonstrating the suitability of a whole-heart metric for improved diagnosis of arrhythmogenic risk (difficult to assess in patients) (Figure 2). These findings were recently published in Nature Cardiovascular Research alongside an accompanying editorial.

To model Timothy Syndrome in a human context, we have developed a human iPSC-derived cardiomyocyte cell line that replicates the phenotype.

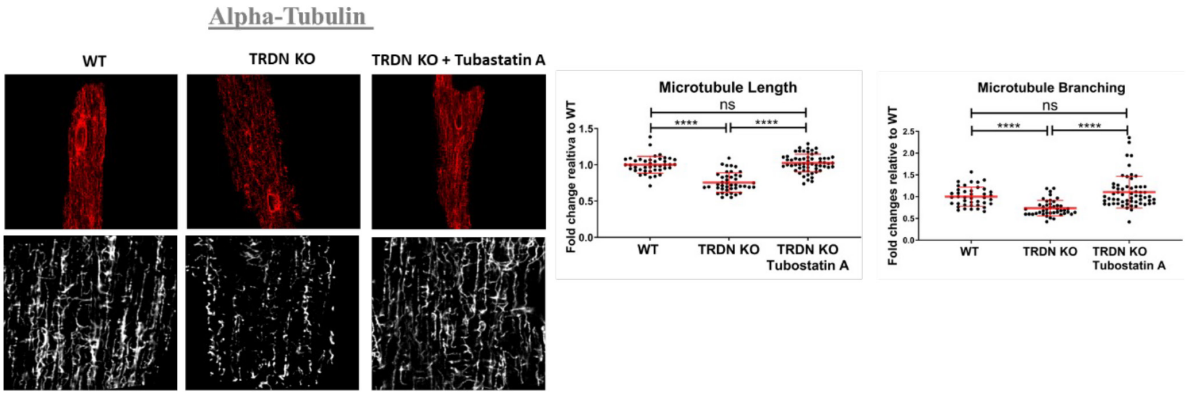


Figure 1: Immunostaining of fixed cardiomyocytes with a specific antibody against alfa-tubulin, a component of the microtubular cyto-skeleton, demonstrates a previously uncharacterized non-arrhythmogenic phenotype in mice with triadin-KO syndrome. Compared to wild-type (WT) cardiomyocytes, cells from triadin-KO hearts (TRDN KO) have shorter and less-branched microtubules. Treatment with the drug Tubastatin A rescues the microtubular defects in TRDN-KO cells. Unpublished data.

FIBER DISORGANIZATION IN THE GENESIS OF COMMON ARRHYTHMIAS

Ventricular remodeling after myocardial infarction generates a potential proarrhythmic substrate. However, the interplay between the structural and functional substrates for ventricular tachycardia (VT) maintenance is not completely understood. During the last few years, we have studied the scar and fiber disorganization substrate associated with functional alterations during ventricular pacing and VT mapping. Fourteen pigs with an infarct-related substrate were investigated by state-of-the-art cardiac imaging and invasive mapping procedures to identify the functional and structural properties associated with abnormal wave-front propagation and VT maintenance. The results show that deceleration zones during ventricular pacing and critical isthmus sites during VT mapping share similar underlying scar properties and myocardial fiber disorganization. However, deceleration zones derived from one pacing location were not sufficient to identify critical VT isthmus sites in all animals. These data indicate that proper identification of the substrate associated with VT isthmus sites during pacing may require pacing maneuvers from more than one location. This work was presented in the Rosanna Degani Young Investigator Finals at the 2023 Computing in Cardiology Meeting in Atlanta (https://cinc.org/final_program_papers_2023/).

RESEARCH GROUPS

David Filgueiras
Advanced Development in Arrhythmia Mechanisms and Therapy

Silvia Priori
Molecular Cardiology

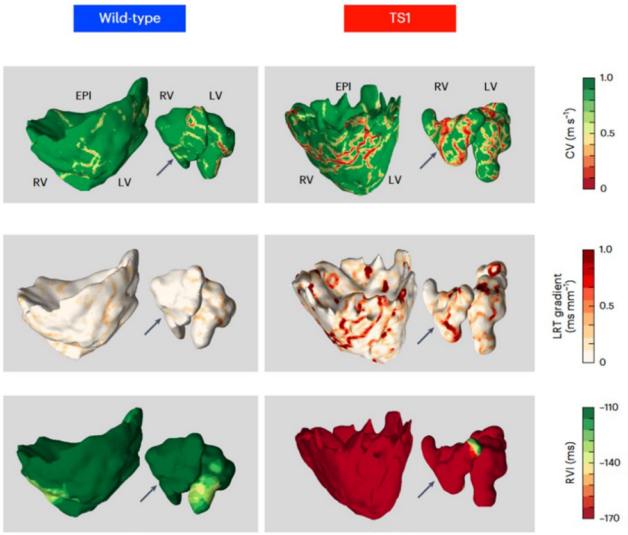


Figure 2: Advanced mapping metrics obtained from ultra-high-density electro-anatomical mapping studies confirmed the presence of a substrate vulnerable to reentry in a swine model of Timothy Syndrome. The application of 3 premature stimuli caused the appearance of areas of slow conduction (<0.2 m/s) in Timothy Syndrome hearts (top right), but had no effect on wild-type hearts (top left). The appearance of these areas coincided with a heterogeneous abbreviation of local repolarization time (LRT), as demonstrated by steep LRT gradients (center right). This had the effect of creating a substrate vulnerable to the development of functional reentry, as documented by extremely low reentry vulnerability index (RVI; bottom right). Black arrows indicate the pacing site in the right ventricular endocardium. Figure reproduced and legend modified from Porta-Sanchez et al. (2023), https://doi.org/10.1038/s44161-023-00393-w, published in open access under Creative Common License 4, http://creativecommons.org/licenses/by/4.0/).

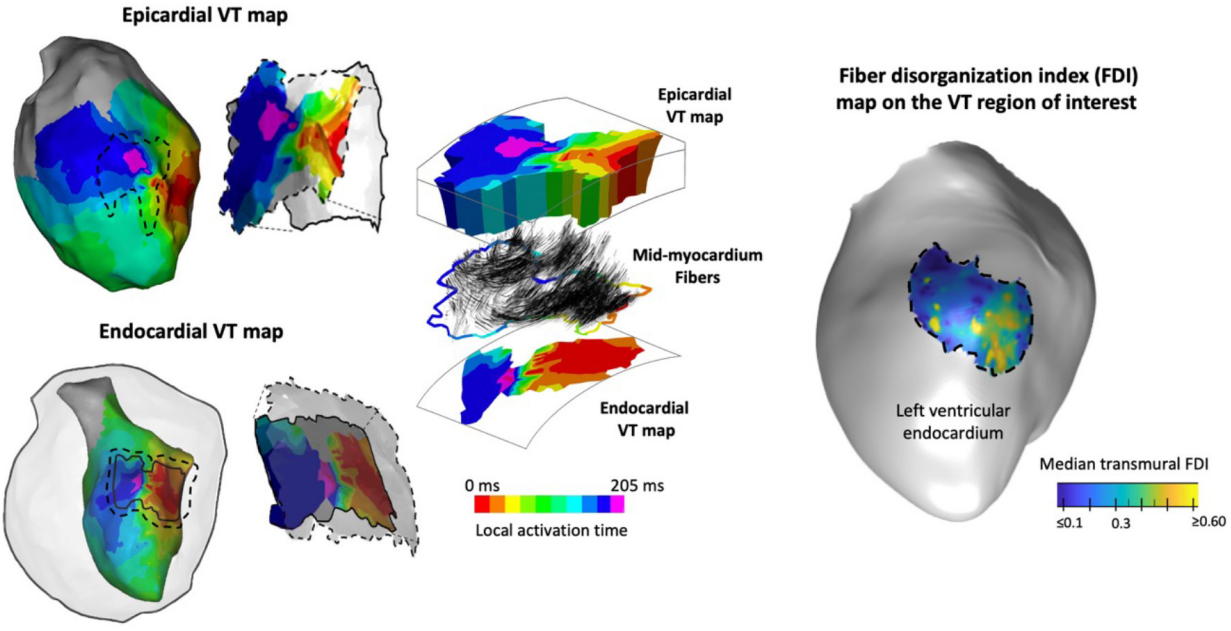


Figure 3. Left, Activation maps of the left ventricular epicardium and endocardium during a ventricular tachycardia (VT) episode in a pig with an infarct-related substrate. The central images show a 3-dimensional reconstruction of the myocardial fibers in the VT region of interest containing the protected isthmus site (critical for VT maintenance). Right, Sample fiber disorganization index (FDI) map of the VT region of interest.

2.1.5 CARDIOVASCULAR RISK FACTORS AND BRAIN HEALTH

Coordinator: **María Ángeles Moro**
Clinical leader: **Valentín Fuster**

This program studies the mechanisms involved in the cognitive impairment associated with cardiovascular disease and its risk factors, such as hypertension, high cholesterol, obesity, and metabolic syndrome. Our aim is to use the knowledge gained to promote cardiovascular health and to develop strategies to prevent the appearance of cognitive impairment with age.

Cardiovascular disease and dementia often coincide in advanced stages, and controlling cardiovascular risk factors in midlife may lower dementia risk later on. However, little attention has been paid in the past to how atherosclerosis and its risk factors affect brain health in midlife. In a first cross-sectional study of participants with subclinical atherosclerosis from the PESA study, researchers from the Cardiovascular Risk Factors and Brain Health Program (CRFBP) found that subclinical atherosclerosis and vascular risk were associated with cerebral hypometabolism in asymptomatic individuals aged 50 years (Cortés-Canteli et al. JACC 2021). In 2023, a longitudinal study of a subgroup of PESA participants with subclinical atherosclerosis (n=370) showed that those with persistently high cardiovascular risk experienced a faster decline in brain glucose consumption, partly as a consequence of neurodegeneration. Progression of subclinical carotid atherosclerosis was also linked to further declines in brain metabolism, particularly in areas vulnerable to Alzheimer's disease. These findings highlight the importance of maintaining cardiovascular health in midlife as a way to potentially reduce neurodegenerative diseases later on (Tristao-Pereira et al. Lancet Healthy Longev. 2023 Sep;4(9):e487-e498; Fig. 1).

Stroke is a model condition for studying the impact of cardiovascular risk factors on the cerebral vascular system and its neurobiological targets. Previous research by program members showed an association between post-stroke cognitive impairment and aberrant hippocampal neurogenesis (Cuartero et al. JCI 2019). Building on this finding, CRFBP scientists recently used noninvasive magnetic resonance spectroscopy to show that this cognitive impairment is mediated by an ipsilesional metabolic imbalance, thus providing novel diagnostic and therapeutic targets for the prevention of post-stroke cognitive impairment (Torres et al. Stroke. 2023 Oct;54(10):2652-2665; Figs 2 & 3).

Program members also contributed to the development of a new drug with demonstrated efficacy in reducing mortality and disability in stroke patients (Hernández-Jiménez et al. JAMA Neurol. 2023 Aug 1;80(8):779-788; Fig. 4).

Another area of interest is how brain function is impacted by obesity and how this interaction is influenced by exercise. A study currently under editorial review characterizes the signaling pathway modulated by the myokine Il15, which plays a crucial role in this communication network, particularly affecting the motor cortex. (Herrera-Melle et al., Remodeling p38 Signaling in Muscle Controls Locomotor Activity via IL-15. Sci Adv under 2nd editorial review).

Several program members participated last year in the production of a series of articles for a themed issue of Br. J. Pharmacol. published in January 2024) called *From Alzheimer's disease to vascular dementia: Different roads leading to cognitive decline (Fig. 5).*

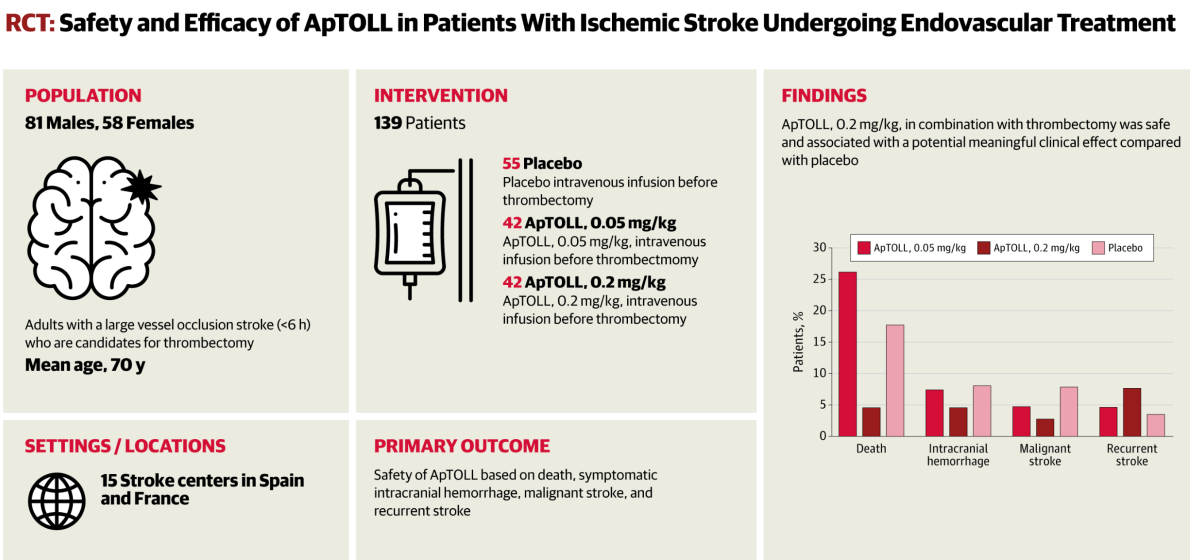


Fig. 4. The TLR4 antagonist ApTOLL (0.2 mg/kg), administered within 6 hours of stroke onset in combination with endovascular treatment, was safe and was associated with a potentially meaningful clinical effect, reducing mortality and disability at 90 days compared with placebo.

RESEARCH GROUPS

Héctor Bueno
Multidisciplinary Translational Cardiovascular Research (MTCR)

Valentín Fuster
Cardiovascular Imaging and Population Studies

Pilar Martín
Regulatory Molecules of Inflammatory Processes

María Ángeles Moro
Neurovascular Pathophysiology

Juan Miguel Redondo
Gene Regulation in Cardiovascular Remodelling and Inflammation

Guadalupe Sabio
Stress Kinases in Diabetes, Cancer and Cardiovascular Disease

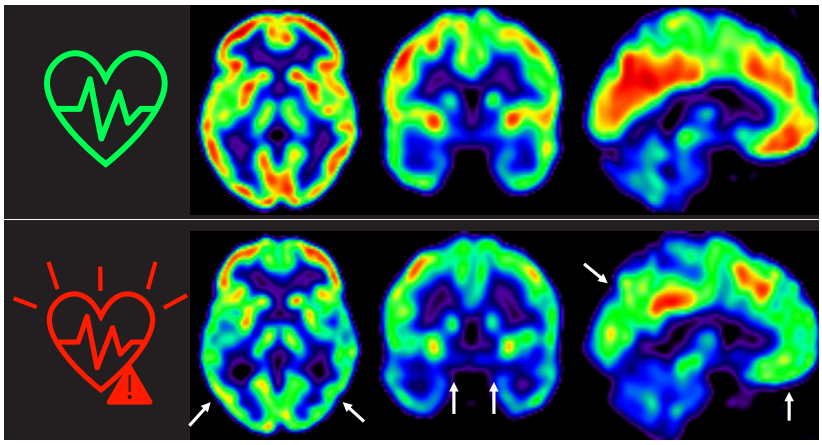


Fig. 1. Glucose uptake in the brain measured by positron emission tomography in middle-aged individuals with low (top) or high (bottom) sustained cardiovascular risk over 5 years. Individuals with persistent cardiovascular risk have lower cerebral glucose consumption (red areas).

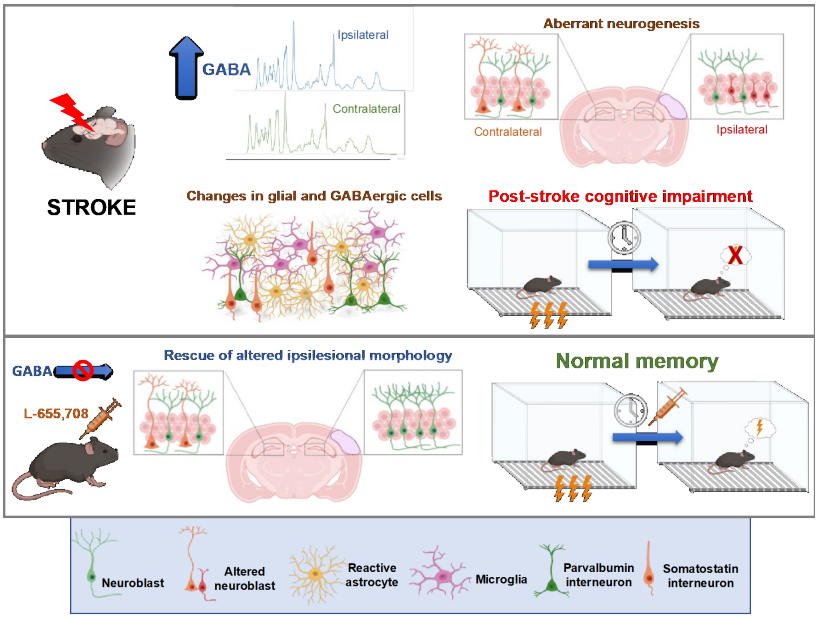


Fig. 2. Post-stroke worsening of cognitive function after middle cerebral artery occlusion (MCAO) in mice is associated with abnormalities in newborn hippocampal neurons. Magnetic resonance spectroscopy revealed significant ipsilesional changes in hippocampal metabolites, with higher ipsilesional hippocampal GABA levels associated with worse cognitive outcomes. Blocking GABAergic neurotransmission using the GABAA-R inverse agonist L-655,708 ameliorated memory deficits and abnormal neurogenesis, supporting GABA as a diagnostic and therapeutic target for post-stroke cognitive impairment.

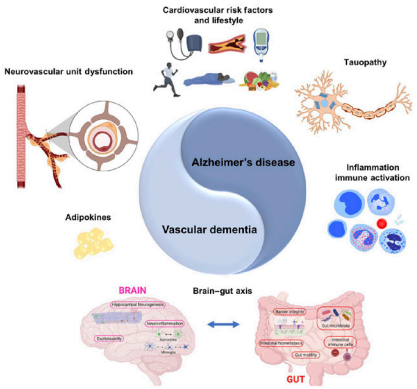


Fig. 5. Multiple shared risk factors and pathogenetic mechanisms contribute to degeneration and cognitive impairment in Alzheimer's disease and vascular dementia. From the Br. J. Pharmacol. themed issue: From Alzheimer's Disease to Vascular Dementia:Different Roads Leading to Cognitive Decline.

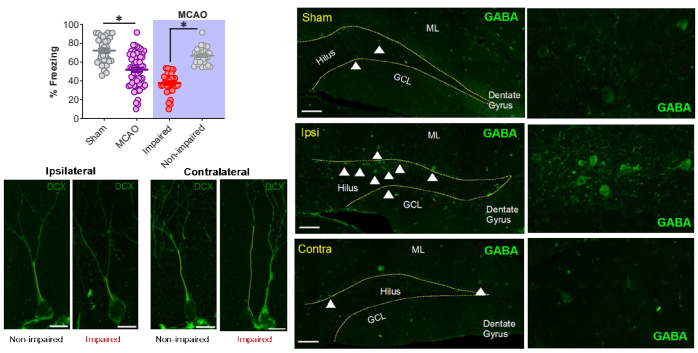


Fig. 3. Post-stroke cognitive decline after stroke is dependent on defective hippocampal neurogenesis and increased GABAergic cell numbers in the ipsilesional hemisphere. Top left: Percentage of memory (freezing), distinguishing two subsets in the MCAO group (*p<0.05; sham, n =37; MCAO, n =63). Bottom left: Representative images of immature newborn neurons (DCX+) of non-memory-impaired and memory-impaired MCAO mice. Right: Representative images of GABA+ cells at the ipsilateral and contralateral hippocampi of sham-operated and MCAO mice.

2.1.6 CARDIOVASCULAR HEALTH PROMOTION

Coordinator: **Rodrigo Fernández-Jiménez**
Clinical leader: **Valentín Fuster**

Cardiovascular disease (CVD) is one of the leading causes of death and disability worldwide, and its high prevalence and impact are largely the result of risk factors that can be modified by changes in behavior (smoking, an unhealthy diet, physical inactivity, etc.). The problem is expected to get worse in the coming years due to the disturbing increase in the prevalence of unhealthy lifestyles and obesity, particularly among children.

The research teams in the Cardiovascular Health Promotion Program (CHPP) work on multidisciplinary studies and clinical trials in close collaboration with schools and communities. These studies target both children and adults and are aimed at implementing early prevention strategies and developing research applications for noninvasive technologies to support translational research and population studies on preclinical atherosclerosis.

The ultimate goal of the CHPP is to implement health promotion and prevention strategies as an effective means of reducing the personal and societal burden of CVD and potentially increasing life expectancy free of other diseases such as dementia and cancer. The key objectives of the Program are as follows:

1) To refine primordial prevention strategies in children and adolescents.

2) To improve global primary prevention by stemming the subclinical development and progression of atherosclerosis in young adults.

3) To translate health promotion initiatives to the general population.

The Program comprises 2 research groups: the *Cardiovascular Imaging and Population Studies* group (PI, Valentin Fuster) and the *Cardiovascular Health and Imaging Lab* (PI, Rodrigo Fernández-Jiménez). Projects and activities developed during 2023 generated relevant scientific advances, some of which are highlighted below.

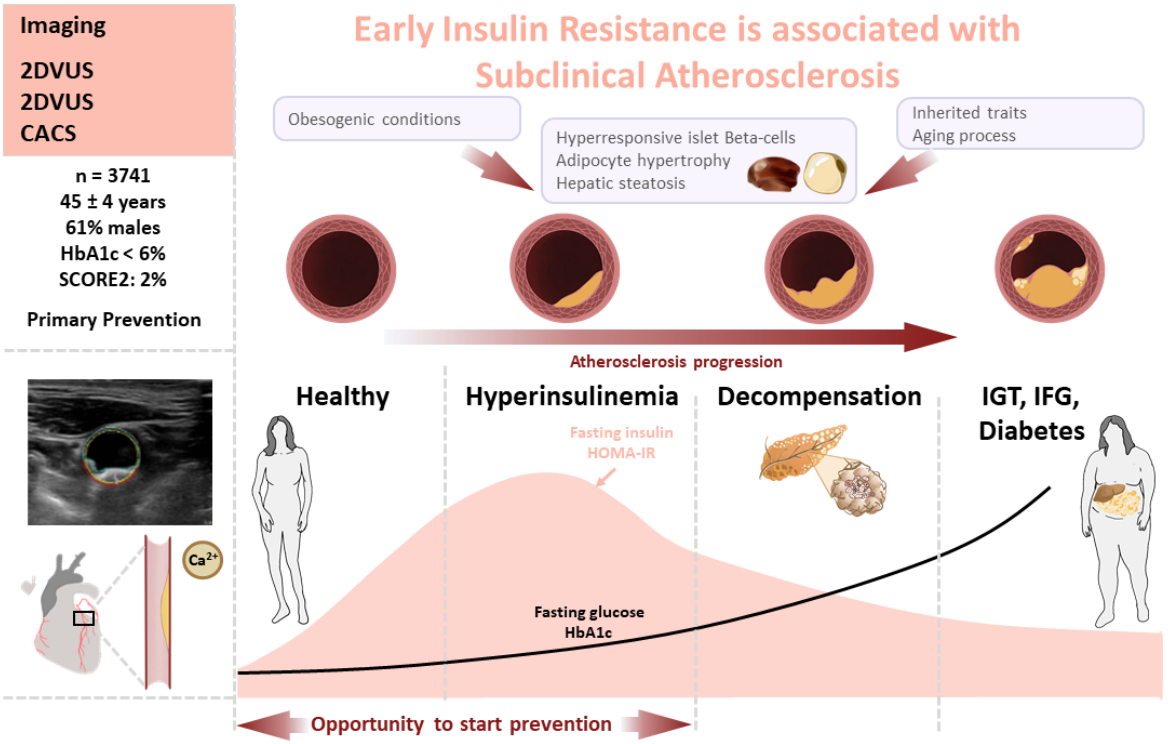


Figure 1. Early insulin resistance and subclinical atherosclerosis. This study examined 3,741 individuals with normal HbA1c < 6.0% <42 mmol/mol) and no known cardiovascular disease. Higher homeostatic model assessment of insulin resistance (HOMA-IR) values were associated with a higher subclinical atherosclerosis (SA) burden measured by extensive noninvasive imaging, and this association was maintained after adjusting for traditional cardiovascular risk factors and HbA1c. 2DVUS, 2-dimensional vascular ultrasound; 3DVUS, 3-dimensional vascular ultrasound; CACS, coronary artery calcium score; IGT, impaired glucose tolerance; IFG, impaired fasting glucose.

Through an analysis of data from the PESA study, CNIC researchers from the CHPP demonstrated that insulin resistance (measured as the homeostatic model assessment of insulin resistance index; HOMA-IR) identifies individuals with a low CVD risk who have an elevated burden of subclinical atherosclerosis, independently of the effects of key traditional cardiovascular risk factors and glycated hemoglobin. These findings suggest that HOMA-IR can be used as a simple measure to facilitate earlier implementation of primary cardiovascular prevention strategies in clinical practice [Cardiovasc Diabetol. 2023;22(1):350.doi: 10.1186/s12933-023-02090-1. Figure 1]

CHPP scientists also reported the primary results of the SI! Program for Secondary Schools trial, a 4-year cluster randomized intervention trial conducted in 24 secondary schools in Barcelona and Madrid. This trial assesses the effect of 2 multicomponent educational health promotion strategies of differing duration and intensity on cardiovascular health in adolescents. Although there was evidence of some beneficial effects at a point midway through the long version of the intervention, further implementation was affected by the COVID-19 pandemic and no benefit was noted at the end of the trial [JAMA Cardiol. 2023;8(9):816-824.doi: 10.1001/jamacardio.2023.2231]

In another study of data from the SI! Program for Secondary Schools trial, accelerometry-measured sleep duration was analyzed for cross-sectional and longitudinal associations with adiposity markers in adolescence. Strikingly, most adolescents did not meet sleep recommendations, and shorter sleep duration was independently and cumulatively associated with unfavorable adiposity markers [Eur J Prev Cardiol. 2023;30(12):1236-1244.doi: 10.1093/eurjpc/zwad137. Figure 2]. These results emphasize the importance of including good sleep habits as a key element of health promotion programs.

The CHPP recognizes the crucial importance of effective communication to increase public visibility and awareness of cardiovascular health and healthy lifestyle habits. Program scientists participated in several outreach activities during 2023, most notably *"Cardioguía: más allá del colesterol alto"* event, held as part of the XXIII Science and Innovation Week in Madrid.

RESEARCH GROUPS

Rodrigo Fernández-Jiménez
Cardiovascular Health and Imaging

Valentín Fuster
Cardiovascular Imaging and Population Studies

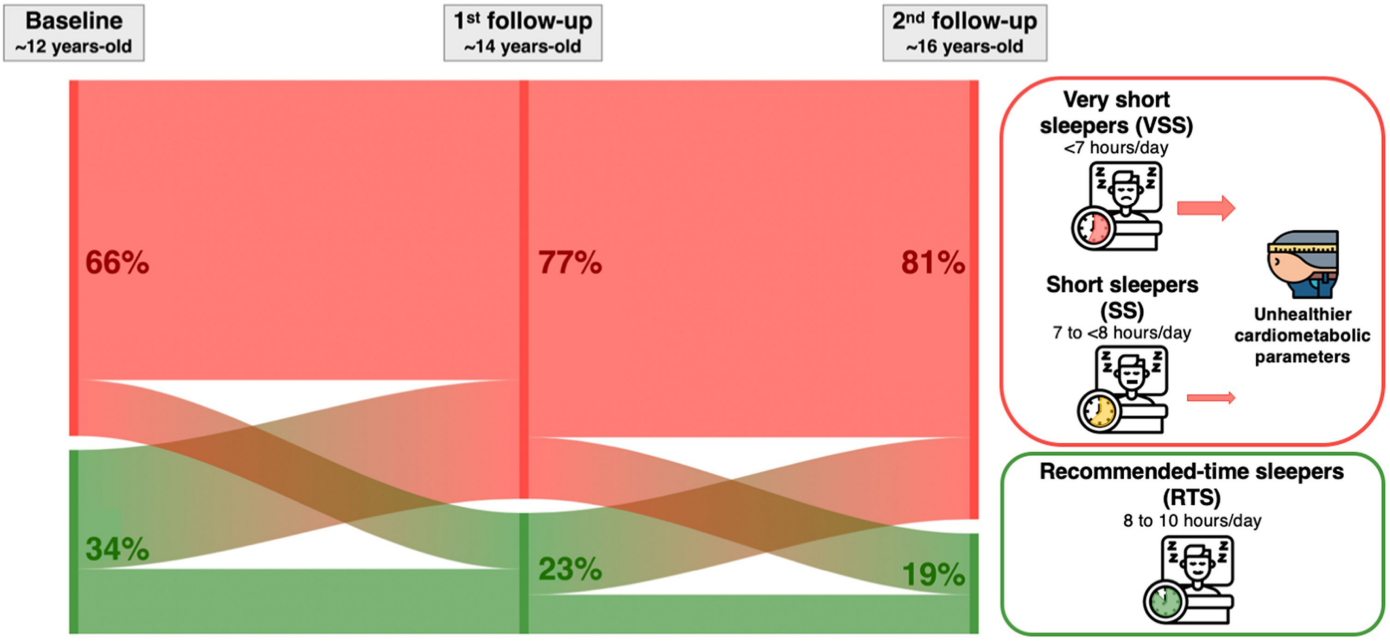


Figure 2. Changes in sleep duration between the ages of 12 and 16 years. The recommended sleep duration is between 8 and 10 h per day. Those sleeping 7 to <8 hours a day were considered short sleepers, and those sleeping <7 hours per day very short sleepers. At 12 years, only 34% of adolescents met sleep recommendations, and this percentage decreased with advancing age (23% at 14 and 19% at 16 years). Adolescents sleeping <8h a day were more likely to present overweight, obesity, or other adverse adiposity markers than their peers with sufficient sleep. This figure has been designed using images from Flaticon.com.

2.1.7 TECHNOLOGY DEVELOPMENT

Coordinator: **Beatriz Álvarez Flores**

The 11 Technical Units (TUs) that make up the Technology Development Program (TDP) work to keep the CNIC at the forefront of cardiovascular research by developing and implementing cutting-edge biomedical technologies, providing internal and external services, and engaging in training and scientific collaborations in funded projects (https://www.cnic.es/en/investigacion/unidades-tecnicas).

Our work falls into 4 key areas:

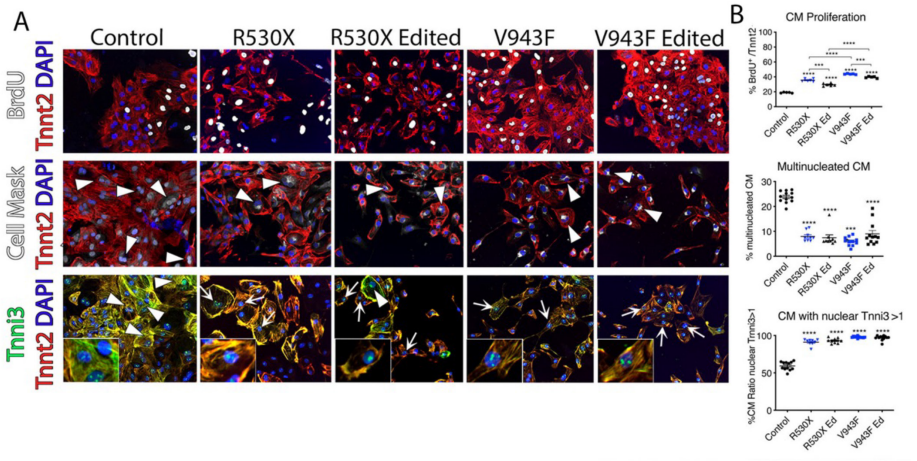
1. Contributing to the CNIC strategic plans by aligning our activities and vision to those the Center's Scientific Programs
2. Examining the latest technological advances relevant to the CNIC Scientific Programs for the purposes of upgrading and innovation
3. Improving communication and protocol flows in coordination with the CNIC management, administration, governance committees (Infrastructure, Computing, Innovation, etc.), and research groups
4. Guaranteeing ISO quality and reliability of infrastructure support and services

Training is one of the CNIC's core activities and enjoys the TDP's commitment and support through the participation of the TUs in the UAM Master's in Molecular Biomedicine (BMM7) and other scientific training and communication programs, such as Acércate, Res@cnic, and the seminar program. The TUs also participate in high-impact external activities such as the organization of the XVIII Congress of the Iberian Society of Cytometry; Cost Action (CA) 20140 (CorEuStem, The European Network for Stem Cell Core Facilities); CA21113 (GeneHumDi, Genome Editing for the Treatment of Human Disease Network); and CA21151 (HaploiPSCs, Generation of human induced pluripotent stem cells from haplo-selected cord blood samples).

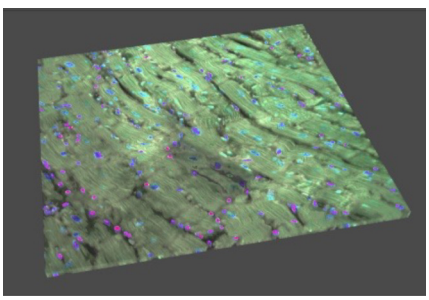
In 2023, the CNIC TUs published 82 articles, accounting for 28% of the total CNIC scientific production. Also last year, all the TUs for the first time applied jointly as the Technology Development Program to the ISCIII call for platforms.

Through its efforts to unify the organization of the TUs, the TDP has been able to make significant progress towards ISO certification, which is expected to be completed for all the TUs in 2024.

2023 HIGHLIGHTS



The Pluripotent Cell Technology Unit collaborated with CNIC researchers in the generation of *in vitro* and *in vivo* models by combining stem-cell and genome-engineering technology. Using CRISPR/Cas9 gene editing, the Unit was able to generate several mutant mESC lines and isogenic hiPSC lines, essential for in vitro modeling of cardiovascular disease.

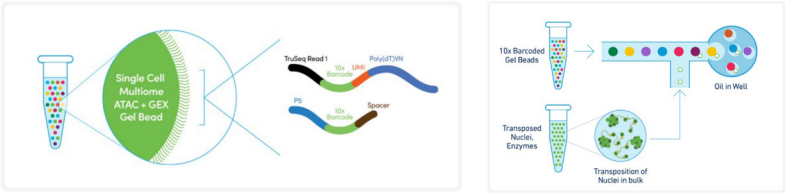


The Microscopy Unit launched a multiparametric platform that combines non-linear and linear visible light-imaging and is equipped for single-photon counting fluorescence lifetime analysis (FLIM) (funded by Grant EQC2021-007527-P [MCIN/AEI/10.13039/501100011033] and the European Union NextGenerationEU/PRTR). The image shows *in-situ* localization of doxorubicin and its metabolites in mouse heart sections after intraperitoneal treatment, detected by the intrinsic fluorescence lifetime changes of the drug under 2-photon excitation and phasor-FLIM analysis.

TECHNICAL UNITS

- **Fátima Sánchez Cabo**
  Bioinformatics
- **Antonio J. Quesada**
  Clinical Trial Coordination
- Comparative Medicine
- **Beatriz Álvarez**
  Flow Citometry
- **Ana Dopazo**
  Genomics
- **Manuel Desco**
  Imaging
- **Valeria Caiolfa**
  Microscopy
- **Giovanna Giovinazzo**
  Pluripotent Cell Technology
- **Juan Antonio López**
  Proteomics
- **Juan De Dios Hourcade**
  Transgenesis
- **Juan A. Bernal**
  Viral Vectors

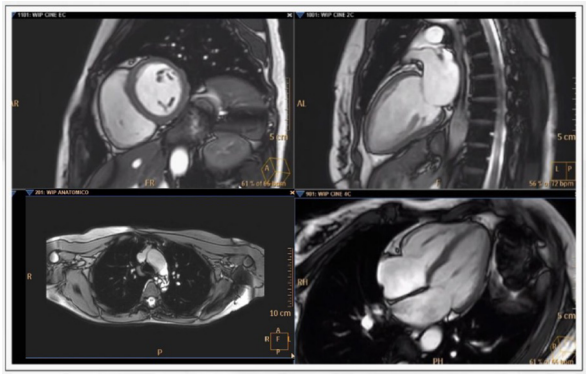
The CNIC Bioinformatics Unit has installed new computational infrastructure to keep the CNIC at the state-of-the art in the use of artificial intelligence and big data. This equipment was acquired with grants EQC2021-007294-P and TED2021-131611B-I00 (MCIN/AEI/10.13039/501100011033) and the European Union NextGenerationEU/PRTR



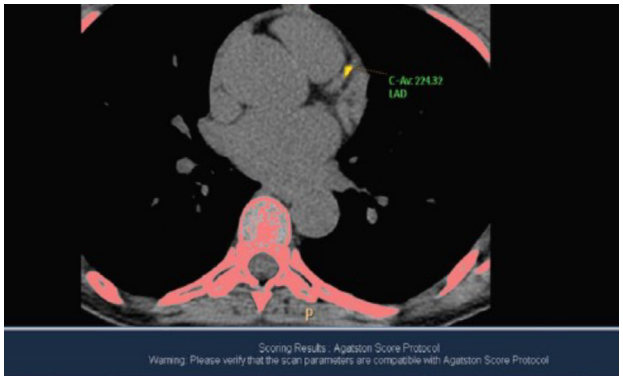
10xGenomics Single Cell Technology

In addition to its established scRNA-seq protocols, the CNIC Genomics Unit has continued to advance in the field of single-cell genomics by performing single-cell ATAC-seq (scATACseq) and single-cell multiomics (scRNAseq and scATACseq in the same cell). The Unit has also analyzed single adult cardiomyocytes (length and width approximately 100 and 25 µm) by Split-seq, a methodology that labels molecules with cell-specific combinations of barcodes after cell fixing and permeabilization, thus eliminating the need for the difficult task of capturing these large individual cells in droplets or microwells.

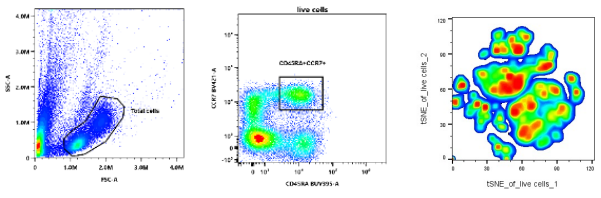
Clinical Trials Coordination Unit

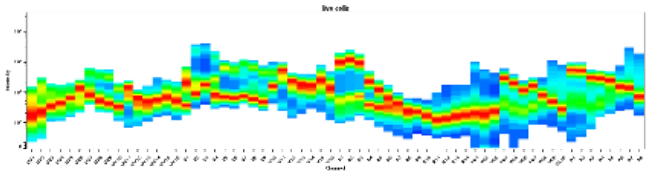


Different planes of a cardiac magnetic resonance scan



Calcium score quantification by computed tomography





The Flow Cytometry Unit has acquired a new full spectrum flow cytometry cell sorting platform. The spectral cell sorter flow cytometer was acquired with financing from Grant EQC2021-007031-P (MCIN/AEI/10.13039/501100011033) and the European Union NextGenerationEU/PRTR.
The CNIC Proteomics Unit has installed a new Thermo Orbitrap Eclipse mass spectrometer with FAIMS and an Evosep chromatographic system. The Thermo Scientific Orbitrap Eclipse mass spectrometer is a cutting-edge instrument designed to tackle complex analytical challenges. This equipment were acquired with grant EQC2021-007053-P (MCIN/AEI/10.13039/501100011033) and the European Union NextGenerationEU/PRTR.

2.2 CLINICAL STUDIES

EARLY DETECTION OF SUBCLINICAL ATHEROSCLEROSIS, DISEASE 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 begun in 2010 with the PESA Study, directed by the CNIC in collaboration with Santander Bank. In PESA-Health, the PESA participants enrolled in 2010 (4184 asymptomatic individuals between the ages of 40 and 55 years at enrollment) are being followed up over an additional 10 years.

The PESA study sought 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 expands these goals to new areas, including the correlation of SA with Alzheimer's and cognitive diseases, the acquisition of somatic mutations with aging, and the correlation of these mutations with increasing cardiovascular event rates and SA progression. PESA-Health continues to make good use of the opportunities offered by 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, PET, and PET-amyloid analysis. And, just as in the PESA study, PESA-Health includes biosampling for extensive omics analysis. New state-of-the-art substudies in PESA-Health include investigation of the relationship between sleep apnea and SA.

PESA-Health is the CNIC's flagship study, and several CNIC clinical and basic research groups participate in it. The PESA study findings are 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. By the end of 2023, 3484 participants had agreed to continue their participation, with around 3000 of them having completed their first PESA-Health visit.

REMOTE ISCHEMIC CONDITIONING IN LYMPHOMA PATIENTS RECEIVING ANTHRACYCLINES (RESILIENCE)

Principal Investigator: ***Borja Ibáñez***
H2020 Grant# 945118

Anthracyclines are a class of anticancer drugs that are widely used to treat many cancers. Of the 4 million new cancer cases diagnosed in Europe every year, >3 million receive anthracyclines (alone or in combination with other treatments). Very recent data show that >35% of patients receiving anthracyclines develop some form of cardiomyopathy. The trade-off between cancer and chronic heart failure (HF) places an immense psychological burden on cancer survivors, and for healthcare systems the growing incidence of chronic HF is a devastating consequence of cancer treatment.

Remote ischemic pre-conditioning (RIPC) is a process in which brief, reversible episodes of ischemia followed by reperfusion in one region (e.g. an arm) render remote tissues and organs resistant to injury. RIPC is safe and effective, noninvasive, feasible, and inexpensive. There is abundant experimental evidence that large animals undergoing 3 to 5 cycles of brief (5 min) limb ischemia followed by 5 min reperfusion have a degree of protection against subsequent induced myocardial infarction, having smaller infarcts than animals undergoing myocardial infarction without preceding cycles of RIPC. Recent evidence suggests that, to be protective, RIPC needs to be initiated before the index insult. Anthracycline-induced cardiomyopathy provides an ideal setting for testing this hypothesis because chemotherapy is a planned procedure.

RESILIENCE is a multinational prospective proof-of-concept phase II, double-blind, sham-controlled, randomized controlled trial evaluating the efficacy and safety of RIPC in patients with non-Hodgkin lymphoma (NHL) and receiving anthracyclines. Patients scheduled to undergo ≥3 chemotherapy cycles and fulfilling all inclusion and no exclusion criteria will be enrolled and undergo baseline cardiac magnetic resonance (CMR) imaging and a high sensitivity troponin (hsTn) and NT-proBNP blood test. Patients with confirmed LVEF >40% by CMR will be randomized 1:1 to RIPC or simulated RIPC (Sham).

Nine weeks after finishing chemotherapy, patients will undergo a final CMR+ hsTn/NT- proBNP test. All patients will be followed up for clinical events at 12, 18, 30, and 42 months until the last patient undergoes the final CMR.

The RESILIENCE Trial aims to recruit 608 patients in 6 European countries (Spain, Portugal, France, Germany, the Netherlands, and Denmark) and is funded by the European Commission (Grant Agreement-945118-RESILIENCE). The grant period began in June 2021, and in 2022 patient recruitment began at 11 sites. By the end of 2023 all 18 sites were opened, with 157 participants enrolled.

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

Principal Investigator: ***Borja Ibáñez***

The prescription of beta-blockers to patients after a myocardial infarction (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 a 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 8600 post-MI patients with a left ventricular ejection fraction >40%. Patients are 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 Pharmacological Research in Milan. In total, 77 hospitals in Spain and 29 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 8400 had been recruited by the end of January 2024. Enrollment will be completed during the first quarter of 2024.

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 VHD when symptoms develop or when there is LV remodeling or dysfunction. 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, to enable 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 the contrast agent gadolinium, which has a superior 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, which is 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, either AS (a paradigm of LV pressure overload) or MR (a paradigm of LV volume overload).

So far 71 patients have been recruited, and 34 have completed the 1-year follow-up visit.

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 the cardiotoxicity associated with some cancer treatments. MATRIX will be jointly run by 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 anticancer drugs, long before widely used clinical 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 transplantation. 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 February 2024 we had already hosted 50 participants.

PROSPECTIVE REGISTRY TO VALIDATE A NEW DIAGNOSTIC MARKER IN PATIENTS WITH CLINICAL SUSPECT OF MYOCARDITIS
(MYOCARDITIS-CNIC)

Principal Investigator: ***Mª Pilar Martín Fernández***
Co-Principal Investigator: ***Domingo Pascual Figar***

Acute myocarditis is difficult to diagnose because of its varied clinical presentation and the lack of rapid, accessible, and accurate diagnostic methods. The nonspecific manifestations of acute myocarditis include atypical chest pain (suggesting pericarditis or angina), dyspnea, asthenia, palpitations, syncope, and even sudden death or shock. The difficulty in achieving early diagnosis of myocarditis is caused by its varied presentation and the heterogenous and nonspecific findings in the usual tests (ECG, echocardiography, and laboratory tests).

Diagnosis of acute myocarditis typically requires either endomyocardial biopsy, which is invasive, or cardiovascular magnetic resonance imaging, which is not universally available, so there is a clear need for new approaches. Dr. Martín Fernández's group has identified a novel microRNA in mice and humans with myocarditis; the team's research shows that the human homolog (hsa-miR-Chr8:96) can be used to distinguish patients with myocarditis from those with myocardial infarction (N Engl J Med. 2021 May 27;384(21):2014-2027).

In the MYOCARDITIS-CNIC Registry, run by the CNIC in collaboration with Hospital Virgen de la Arrixaca, several Spanish hospitals (including Hospital de la Princesa and Clínica Universitaria de Navarra) will collect clinical data and biological samples from patients attending the emergency department with clinical signs of myocarditis. These data will provide valuable information on the early onset of myocarditis and will help in the validation of early clinical biomarkers. So far, 64 participants have been enrolled.

EFFECT OF REMOTE ISCHEMIC PRECONDITIONING ON COGNITIVE FUNCTION AND CEREBRAL VASCULATURE (PRECOGNITIVE)

Principal Investigator: ***Gonzalo Pizarro Sánchez***

Arterial hypertension can damage the cerebral vascular system, even when blood pressure values are normalized. Current treatments are aimed at controlling blood pressure and avoiding damage to target organs, such as the brain. Thirty years ago, remote ischemic preconditioning (RIPC) was shown to protect organs such as the heart and brain in animal models, and the procedure has subsequently been used in human patients. RIPC consists of inflating and deflating a tension cuff on the arm through 4 cycles of 5 minutes each. The ischemia generated in the arm can protect distant organs, such as the brain, against future ischemic episodes. Our group has made important contributions in this area, and our recent studies show that RIPC can improve cognitive performance and cerebral vascular function in patients with dementia of vascular origin.

The PRECOGNITIVE study is a proof-of-concept randomized trial in which 45 women with hypertension and evidence of target organ involvement (such as left ventricular hypertrophy) will be randomized into 3 groups. Patients in the RIPC group will receive RIPC (blood pressure increased by 20 mmHg above their systolic blood pressure). The RIPC-Sham group will undergo the same procedure, but the cuff will not be inflated enough to induce ischemia (50 mmHg). The control group participants will not undergo cuff therapy.

The goal is to determine if RIPC has a significant effect on the cerebral vasculature in patients with hypertension without significant cognitive impairment. The effect of the treatment will be assessed through comprehensive neurocognitive tests and noninvasive imaging tests such as echocardiography, noncontrast brain magnetic resonance imaging, and trans-cranial Doppler ultrasound.

By the end of 2023 we had recruited 12 patients.

CHARACTERIZATION OF CARDIAC METABOLISM USING MULTIMODAL IMAGING IN IDIOPATHIC CARDIOMYOPATHY (MACADAMIA)

Principal Investigator: ***Borja Ibáñez***

Heart failure (HF) is one of the main health problems in contemporary societies and a major burden on healthcare resources. In order to develop new therapies, it is crucial to identify new mechanisms involved in the development and maintenance of HF.

The heart is the most energy-consuming organ in the body by weight. The primary energy source for the heart under physiological conditions is beta-oxidation of fatty acids, which generates approximately 60% of the total ATP consumed by the heart. The second energy source is carbohydrate metabolism via the Krebs cycle, while other nutrients such as amino acids contribute less than 1% of cardiac energy production.

In the altered physiological conditions of HF, there is a shift in nutrient consumption by the cardiac muscle, which begins to consume glucose instead of fatty acids, making carbohydrates the main energy substrate. This change is known as the "metabolic switch." Initially, this metabolic switch was considered a protective defense mechanism rather than a deleterious effect. However, recent data from animal and human models indicate that glucose metabolism produces 4-5 times less ATP than that of fatty acids, indicating that this metabolic switch, far from being beneficial, is harmful and contributes to the drop in cardiac contractile capacity.

Our group has demonstrated that the metabolic switch in HF secondary to idiopathic dilated cardiomyopathy (IDCM) is involved in the deterioration of ventricular function. We initially demonstrated this in a mouse model, where a diet rich in fatty acids was able to reverse the metabolic switch and the IDCM phenotype. This ability of a fatty diet to reverse deteriorated ventricular function in IDCM is also seen in pigs, which have a similar metabolism to humans.

The first step before performing a clinical trial in patients with IDCM is to study the incidence of the metabolic switch in this patient group. This is the goal of the MACADAMIA study. MACADAMIA is an observational study of a small number of patients diagnosed with IDCM. The study aims to characterize this population, without any intervention, using cardiac imaging techniques, including transthoracic ultrasound (TTE, including myocardial strain), cardiac magnetic resonance (CMR), and a metabolic study by positron emission tomography/computed tomography (PET/CT) using the radiotracer 18FDG.

By February 2024 we had recruited 28 patients. Our medium–long-term goal is to conduct a clinical trial in IDCM patients in whom these imaging techniques demonstrate a metabolic switch. These patients will be randomized to receive a diet rich in fatty acids or a normal diet, and we will assess changes in cardiac function (by CMR) and cardiac metabolism (by PET/CT) as a function of this dietary intervention.

THE ATTRACKING REGISTRY (ATTRACKING CNIC)

Principal Investigator: ***Pablo García-Pavía***

Tafamidis is a drug used to treat transthyretin cardiac amyloidosis (ATTR-CM), a rare, life-threatening disease resulting from the deposition of transthyretin amyloid fibrils in the heart. The effectiveness of Tafamidis has been demonstrated in clinical trials showing a reduction in mortality and cardiovascular hospitalizations in patients with ATTR-CM, and Tafamidis was recently marketed in Spain. However, clinical trials can have biases, and trial results are not always comparable to those obtained in real-world clinical practice. Crucially, improvements in diagnosis mean that ATTR-CM is now diagnosed earlier than in the past, and this has had the effect of altering the patient profile and improving prognosis compared with the study population in the clinical trial that ushered in the authorization of this medication. There is thus uncertainty about the effect of Tafamidis on patients with currently diagnosed ATTR-CM, and the aim of ATTRACKING CNIC is to evaluate the effectiveness and safety of Tafamidis in an unselected contemporary cohort of patients with this disease.

OBJECTIVES:

1) To evaluate the characteristics of a real-world population of ATTR-CM patients in Spain prescribed stabilizing treatment with 61mg Tafamidis.

2) To assess the safety of tafamidis in an unselected real-world Spanish cohort with ATTR-CM.

3) To analyze the real-world clinical impact of Tafamidis therapy in patients with ATTR-CM compared with the results of the ATTR-ACT clinical trial.

PROTOCOL

Prospective multicenter non-randomized study of an unselected cohort of patients diagnosed with ATTR-CM and initiating daily treatment with 61 mg Tafamidis.

At the end of 2023 we were finalizing contracts with 10 hospitals in preparation for the beginning of the registry early in 2024.

PATIENT-SPECIFIC ABLATION OF PERSISTENT ATRIAL FIBRILLATION DRIVERS GUIDED BY FREQUENCY AND AMPLITUDE MODULATION CRITERIA (TAILOR-AF)

Principal Investigator: ***David Filgueiras***

Co-Promoters: ***Centro Nacional de Investigaciones Cardiovasculares / Hospital Universitario Clínico San Carlos***

Pulmonary vein isolation (PVI) is considered the cornerstone of catheter ablation in patients with persistent atrial fibrillation (AF). However, the results in patients with persistent AF are suboptimal, with high recurrence rates after a single PVI procedure. In current guidelines for AF ablation in patients with persistent AF, an AF-free rate after 12 months without antiarrhythmic drugs of just 40% is considered acceptable, illustrating the difficulty of rhythm control in this type of arrhythmia.

Our group recently developed a computational tool that enables interventional electrophysiologists to identify specific atrial 'driver regions' during AF that are associated with long-term perpetuation of the arrhythmia. Using a conventional electroanatomic mapping system and multielectrode catheters, these regions can be identified with novel signal processing algorithms that have been made available to the scientific community (Quintanilla JG et al. Circ Res. 2019;125:609-27). The algorithms use individual atrial signals at each of the atrial mapping locations and provide an automatic and accurate analysis of frequency and amplitude modulations (iFM and iAM) that are present during the fibrillatory activity of the atria. This analysis enables localization of the footprint of rotational activity and determination of which activations drive persistent AF (the leading drivers).

The main objective of this study is to identify (through iAM/iFM maps) and ablate the leading drivers of AF in patients with symptomatic recurrences of persistent AF despite having undergone 2 or more PVI procedures. As secondary objectives, the study analyzes blood biomarkers, atrial imaging parameters, and phenotypic parameters in the surface electrocardiogram associated with advanced stages of atrial remodeling. In patients undergoing minimally invasive thoracoscopy-guided ablation of leading drivers, we also take tissue samples of left atrial appendage tissue to further analyze the underlying molecular mechanisms associated with AF maintenance. By the end of 2023, 15 patients had been included.

SPANISH IMMUNOTHERAPY REGISTRY – CARDIOVASCULAR TOXICITY (SIR-CVT)

Principal Investigators: ***Borja Ibáñez and Pilar Martín***
Co-Promoters: ***Centro Nacional de Investigaciones Cardiovasculares / Sociedad Española de Cardiología / Sociedad Española de Oncología Médica***

SIR-CVT is a non-interventional study with 2 primary objectives: to evaluate risk factors and daily practice management of cardiovascular toxicity in patients with solid organ cancer receiving immune checkpoint inhibitors (ICI) for approved indications, and to validate the human homolog of miR-721 as a biomarker for the early diagnosis of immunotherapy-induced myocarditis in these patients.

The study will also address the following secondary objectives:

- To define clinical, electrocardiographic, imaging, laboratory, and genetic markers for the early diagnosis of ICI related myocarditis
- To define clinical, electrocardiographic, imaging, laboratory and genetic markers for early diagnosis of ICI-related adverse CV effects unrelated to myocarditis
- To evaluate the relationship between previous chemotherapy protocols and ICI-related cardiovascular risk.
- To identify the pathogenic mechanism of myocarditis induction by immune checkpoint blockade (antiPD-L1/PD1 and anti-CTLA4).
- To define the impact of CV monitoring on patient quality of life and perceived quality of care.

The study consists of a baseline visit, and patients will be treated according to routine clinical protocols, including the frequency of follow-up visits. Patients will be followed up until death, loss-to-follow-up, of the final visit 12 months after enrollment. The information collected will be used exclusively for the purposes of the study.

By February 2024, 3 patients had been included and had undergone cardiac magnetic resonance and ultrasound imaging studies at the CNIC.