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 Javier Fuster**

Clinical Leaders: **Valentín Fuster and Inés García Lunar**

The **Novel Mechanisms of Atherosclerosis Program** aims to advance our understanding of the pathophysiology of atherosclerosis, the main cause of cardiovascular and cerebrovascular diseases. Despite effective interventions targeting traditional risk factors, a significant residual risk of atherosclerotic cardiovascular disease persists, even in individuals with well-controlled cholesterol levels and low estimated cardiovascular risk. This highlights the need to explore non-conventional risk factors and underlying disease mechanisms to develop novel strategies for the prediction, prevention, and treatment.

The program brings together multidisciplinary teams that integrate experimental research in animal models and human studies, leveraging data from the Progression of Early Subclinical Atherosclerosis [PESA] cohort and other populations. Research efforts are focused on two main areas:

Inflammatory Mechanisms of Atherosclerosis: Atherosclerosis is firmly established as an inflammatory disease driven by maladaptive immune responses to chronic cardiovascular risk factor exposure. However, translating inflammation-targeted therapies into clinical practice remains challenging, underscoring the need for a deeper understanding of the complex inflammatory pathways involved.

Additional Key Mechanisms: Other research groups explore vascular cell biology, mechanosensing in the vascular wall, microbiota-derived metabolites, and the identification of circulating and imaging biomarkers to improve risk assessment and early atherosclerosis detection.

KEY ACHIEVEMENTS IN 2024

Clonal Hematopoiesis and Atherosclerosis

Program scientists uncovered critical links between atherosclerosis and acquired mutations driving clonal hematopoiesis—an emerging cardiovascular risk factor. This work also identified the anti-inflammatory drug colchicine as a promising personalized preventive strategy for individuals carrying specific mutations (Nature Medicine 2024; European Heart Journal 2024).

Endothelial Dysfunction in Hutchinson-Gilford Progeria Syndrome

Program researchers elucidated the molecular and cellular mechanisms linking endothelial dysfunction to accelerated atherosclerosis in Hutchinson-Gilford progeria syndrome, a rare genetic disorder characterized by premature aging and early death, primarily due to atherosclerosis-related complications (Circulation 2024; Journal of Clinical Investigation 2024).

Subclinical Atherosclerosis and Mortality Risk

Findings from the BioImage US cohort revealed that noninvasive imaging, particularly vascular ultrasound, significantly predicts all-cause mortality risk beyond traditional risk factors. Atherosclerosis progression over time also emerged as an independent predictor of mortality (Journal of the American College of Cardiology 2024).

Macrophage Imaging in Cardiovascular Disease

A study on macrophage imaging via positron emission tomography in mouse models of cardiovascular disease was recognized as Paper of the Year 2024 by npj Imaging.

Additional High-Impact Publications

Additional research was published in leading journals in 2024, including Nature Cardiovascular Research, Nature Communications, and Proceedings of the National Academy of Sciences.

OUTREACH AND ENGAGEMENT

The Program remains committed to sharing knowledge and engaging with the scientific community and the public. In 2024, program researchers presented their findings at prestigious international conferences, including:

The European Society of Cardiology annual congress

The European Hematology Association annual congress

The 2nd International Conference on Mesenchymal Cells in Health and Disease

The 29th Krakow Conference on Endothelium

In addition to contributing to these global forums, Program scientists played a key role in organizing the CNIC Conference: Understanding Immunity in Cardiovascular Disease, which convened leading national and international experts to discuss advances in cardiovascular immunology and immunotherapy.

The Program also emphasized public outreach, participating in events designed to make cardiovascular research accessible to a broader audience. Highlights included:

Participation in the ***Bringing Researchers Into the Light*** event, held at CaixaForum Madrid during European Researchers' Night 2024.

Leading the ***Mini-Cardioguía Infantil y Juvenil***, an interactive event hosted at the CNIC as part of the Madrid Science and Innovation Week, reaching over 100 children and adults.

RESEARCH GROUPS

Vicente Andrés
Molecular and Genetic Cardiovascular Pathophysiology

Jacob Fog Bentzon
Experimental Pathology of Atherosclerosis

Miguel Angel del Pozo
Mechanoadaptation and Caveolae Biology

José Javier 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

David Sancho
Immunobiology

Jesús Vázquez
Cardiovascular Proteomics

These efforts foster awareness and dialogue on cardiovascular health, inspiring the next generation of researchers and clinicians.

HIGHLIGHTED PUBLICATIONS

Circulation. 2024 150(20):1612-1630. doi:10.1161/CIRCULATIONAHA.

European Heart Journal. 2024 45(43):4601-4615. doi:10.1093/eurheartj/ehae546.

Journal of Clinical Investigation. 2024 134(22):e173448. doi:10.1172/JCI173448.

Journal of the American College of Cardiology. 2024 84(15):1391-1403. doi:10.1016/j.jacc.2024.06.045.

Nature Cardiovascular Research. 2024 3(2):203-220. doi:10.1038/s44161-023-00412-w.

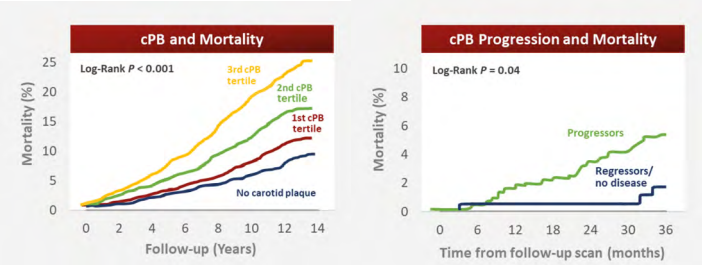
Nature Communications. 2024 15(1):10102. doi:10.1038/s41467-024-54224-y.

Nature Medicine. 2024 30(10):2857-2866. doi:10.1038/s41591-024-03213-1.

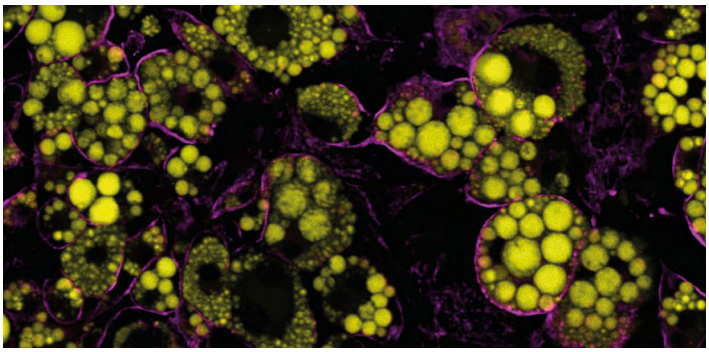
npj Imaging. 2024 2(1):12. doi:10.1038/s44303-024-00009-3.

Proceedings of the National Academy of Sciences. 2024 121(18):e2400752121. doi:10.1073/pnas.2400752121.

Nature Medicine cover (Vol. 30, No.10, 2024). Researchers in the Program found that clonal hematopoiesis confers an increased risk of atherosclerosis development, whereas atherosclerosis does not affect the risk of clonal hematopoiesis, indicating a unidirectional connection between these conditions (*Díez-Díez et al, Nature Medicine 2024, 30:2857-866*). *Image: Rosa Moro, CNIC*.



Influence of subclinical atherosclerosis burden and progression on mortality. The Bioimage study enrolled 5,716 asymptomatic individuals who underwent non-invasive imaging assessment to assess subclinical atherosclerosis. Carotid plaque burden (cPB) was quantified, and cardiovascular risk factors were evaluated. A subset of 732 participants underwent a second assessment after a median follow-up of 8.9 years. All participants were followed for all-cause mortality. Higher atherosclerosis burden at baseline, and more importantly, cPB progression over time, were significantly associated with mortality, independently of conventional cardiovascular risk factors and medication. *Modified by Rosa Moro (CNIC) from Fuster V et al JACC. 2024, 84:1391–1403*.



Confocal microscopy imaging of cultured adipocytes (fat cells) containing fat droplets (yellow) and the caveolin 1 protein in the cell membrane (magenta). Scale bar, 100 microns. Researchers in the Program found that genetically modified adipocytes expressing a non-phosphorylatable form of caveolin 1 accumulate caveolae, because they are unable to flatten in response to fat accumulation. Mutations in this protein are associated with human lipodystrophy. *Aboy-Pardal et al. Nature Communications 2024, 15:10102*.

2.1.2 MYOCARDIAL HOMEOSTASIS AND CARDIAC INJURY (MERCURY)

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

The **MERCURY research program** focuses on uncovering the pathomechanisms of heart diseases to develop new therapeutic strategies, with activities organized around two main focus areas:

- The mechanisms underlying the cardiotoxic effects of anti-tumor treatments
- The genetic and molecular causes of inherited cardiomyopathies

KEY ACHIEVEMENTS IN 2024

MERCURY program scientists made significant strides last year in all these areas, contributing to our overarching goal of developing targeted treatments to effectively prevent and manage heart disease.

Cardiotoxicity Induced by Anti-Tumor Treatments

Research on cardiac atrophy associated with anthracycline treatment has revealed that a high-protein diet before, during, and after doxorubicin administration mitigates cardiac atrophy. Preliminary proteomics data suggest this effect is linked to sarcomeric modifications. Current studies are analyzing sarcomeric protein phosphorylation, conformational changes in cardiac myosin (using the Mant-ATP assay), genetic mechanisms, and titin cleavage to better understand the structural and functional changes caused by anthracycline treatment.

Using engineered mouse models, we have found that individuals carrying gene variants associated with hypertrophic cardiomyopathy may also face a higher risk of fatal chemotherapy-induced cardiotoxicity. Clinical validation of these findings is ongoing. Additionally, we have uncovered a novel role of the giant protein titin in mediating pathogenic remodeling in both skeletal and cardiac muscle. This discovery opens new opportunities for developing drugs to prevent damaging remodeling processes in musculoskeletal and cardiac diseases.

In a large animal model of anthracycline-induced cardiotoxicity in pigs, chronic treatment with the sodium-glucose co-transporter 2 (SGLT2) inhibitor empagliflozin completely prevented cardiotoxicity. Our findings suggest that empagliflozin shifts cardiac metabolism toward increased ketone body utilization, offering a potential cardioprotective mechanism. These results highlight the therapeutic potential of SGLT2 inhibitors in preventing chemotherapy-induced heart damage.

Genetic and Molecular Causes of Inherited Cardiomyopathies

Dilated Cardiomyopathy (DCM)

Our research has advanced the understanding of the genetic basis of DCM by analyzing the rate at which genetic carriers develop the disease and identifying risk factors associated with its progression. These findings have laid the groundwork for the **EarlyGene trial**, coordinated by the CNIC. This trial integrates genetic screening with advanced imaging techniques to evaluate whether early treatment of genetic carriers can modify the natural progression of DCM, potentially enabling preventive interventions in at-risk populations.

Arrhythmogenic Cardiomyopathy (ACM)

We have developed innovative treatments for ACM, a condition often caused by genetic mutations. Our studies identified SGK1 as a critical regulator of QRS complex prolongation in a PKP2 CT-mutant ACM mouse model. SGK1 activation leads to Cx43 hyperphosphorylation and lateralization, disrupting gap junctions and worsening conduction defects. Uing AAV-driven SGK1 activation, we replicated these abnormalities, confirming SGK1's pathogenic role. Importantly, both genetic and pharmacological inhibition of SGK1 restored proper Cx43 localization and significantly reduced QRS prolongation, suggesting that SGK1 inhibition could be a promising therapeutic approach for ACM.

We have also developed a novel therapy for the aggressive ACM caused by the S358L mutation in TMEM43. Building on a mouse model that faithfully replicates the human disease, we generated gene therapy tools to overexpress the wild-type TMEM43 protein. This approach significantly improved cardiac function, highlighting the potential of gene therapy for TMEM43-related ACM.

Right Ventricular Dysfunction

Research by MERCURY program scientists has identified metabolic changes critical to right-ventricular dysfunction under pressure overload conditions. These findings may lead to new opportunities for prevention and treatment.

HIGHLIGHTED PUBLICATIONS

JACC CardioOncol 6:217-232 (2024). doi: 10.1016/j.jaccao.2024.02.005

J Am Coll Cardiol 83:1640-1651 (2024). doi: 10.1016/j.jacc.2024.02.036.

Basic Res Cardiol Jun;119(3):419-433 (2024). doi: 10.1007/s00395-024-01041-5.

OUTREACH

"DNA-Adventure: explore DNA and edit!". European Researchers' Night, CNIC, 2024.

"DNA-Adventure: DNA extraction workshop". Science and Innovation Week, CNIC, 2024.

Interactive workshops for children. Science and Innovation Week, CEIP Rabindranath Tagore, Madrid, 2024.

RESEARCH GROUPS

Jorge Alegre-Cebollada
Molecular Mechanics of the Cardiovascular System

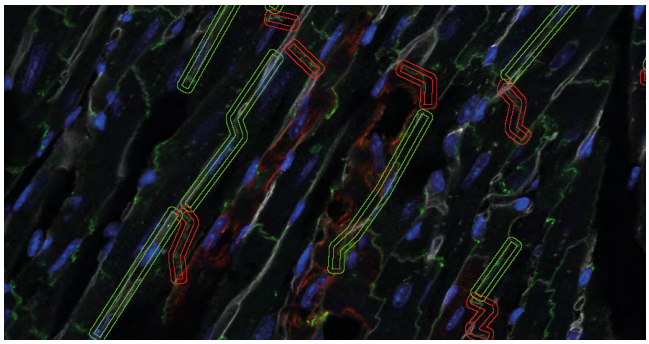
Ana Devesa
Cardiometabolic Disease and Advanced Imaging

Ana García Álvarez
Heart Failure and Pulmonary Hypertension Translational Research

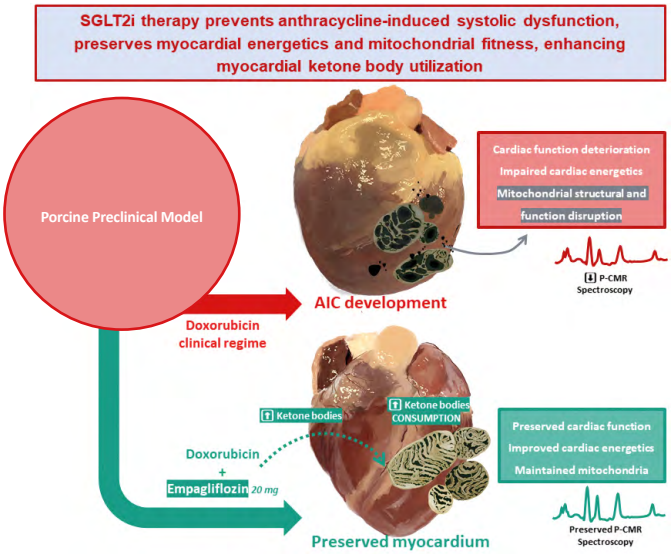
Pablo García-Pavía
Inherited Cardiomyopathies

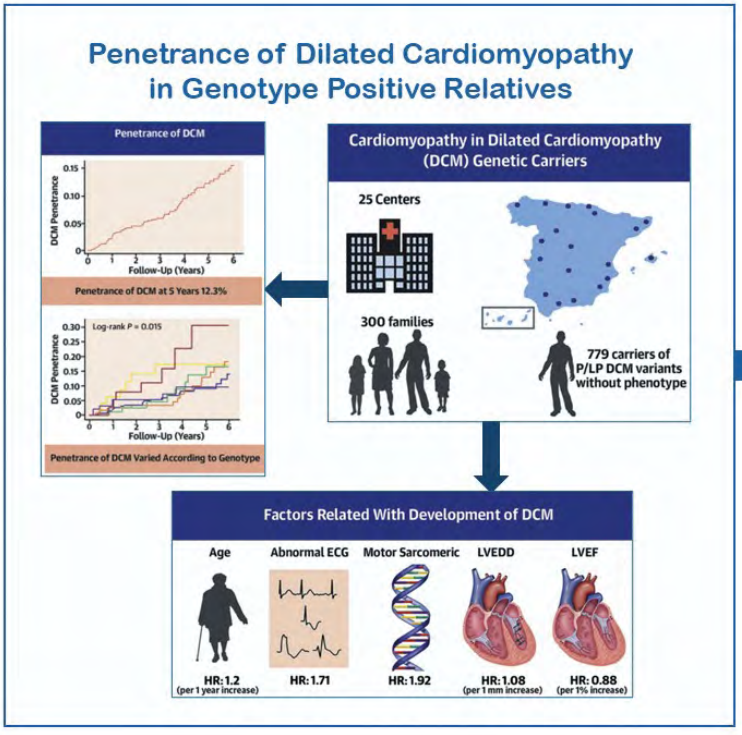
Borja Ibáñez
Translational Laboratory for Cardiovascular Imaging and Therapy

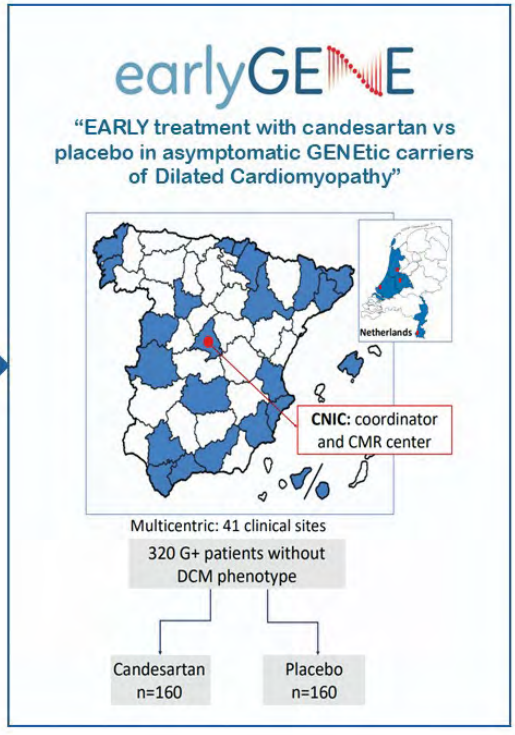
Enrique Lara-Pezzi
Molecular Regulation of Heart Failure



Histological section of murine myocardium. Red and green geometrical shapes are used to quantify subcellular location of proteins involved in the electromechanical coupling of cardiomyocytes, which is affected during myocardial disease







2.1.3 CARDIOVASCULAR REGENERATION

Coordinador: **Miguel Torres**
Clinical Leader: **Hesham Sadek**

The **Cardiovascular Regeneration Program (CVRP)** aims to understand the fundamental principles of heart and vasculature development and regeneration, using this knowledge to develop new therapeutic strategies for cardiovascular diseases. A key focus of the program is the metabolic regulation of cardiac regenerative ability. In 2024, the CVRP made significant progress, uncovering new targets and therapeutic approaches.

KEY ACHIEVEMENTS IN 2024

Metabolic Regulation of Cardiac Regeneration

Mitochondrial Complex I Discovery: Jose Antonio Enríquez's group identified an unsuspected role for mitochondrial complex I, revealing that the long-sought sodium channel in the mitochondrial inner membrane is embedded within complex I. This paradigm-shifting discovery changes our understanding of how the mitochondrial membrane protential is built and has important implications for its modulation (Cell, 2024).

Cox7a1-Deficient Zebrafish: Nadia Mercader and Jose Antonio Enríquez's groups joined forces to study Cox7a1-deficient zebrafish, demonstrating the critical role of ***Cox7a1*** in striated muscle homeostasis. Interestingly, the ***Cox7a1***-deficient fish exhibited improved cardiac regeneration capacity, highlighting the importance of the modulation of super-complex their formation for the control of cardiomyocyte proliferation, as well as identifying a potential target for pro-regenerative strategies (Developmental Cell, 2024).

Cardiomyocyte Proliferation and Regeneration

FDA-Approved Drugs for Regeneration: Hesham Sadek and colleagues developed a virtual drug screening approach to identify compounds that disrupt the transcriptional activity of the Meis1/Hoxb13 heterodimer, a known barrier to cardiac regeneration. The team identified two aminoglycoside antibiotics capable of inhibiting Meis1/Hoxb13 activity and promoting cardiomyocyte proliferation and heart regeneration in mice and pigs. These drugs are already approved for human use in other settings, and the findings are poised to move to clinical trials (Nature Cardiovascular Research, 2024a).

Mechanistic Studies in Heart Disease and Development

Single-Cell Resolution Tools: Rui Benedito's group developed advanced tools in mouse models, enabling precise genetic dissection of molecular pathways at singe-cell resolution in vivo (Nucleic Acids Res, 2024; Nature Methods, 2024).

Bicuspid Aortic Valve Genetics: Jose luis de la Pompa's group uncovered new genetic determinants of bicuspid aortic valve and began characterizing genes involved in ventricular wall maturation and cardiomyopathy (Disease Models and Mechanisms, 2024).

Cell Competition in Cardiomyocytes: Miguel Torres and colleagues identified regulatory elements in the *Myc* gene responsible for cell competition, a mechanism that enables the replacement of cardiomyocyte populations in the adult heart (Nature Communications, 2024).

Cardiomyocyte Electrical Activity and Disease

Models of Rare Genetic Cardiac Disorders: Dr José Jalife and colleagues developed new mouse models that provide mechanistic insights into Andersen-Tawil Syndrome (Circulation Res, 2024) and Type-III Short-QT Syndrome(Cardiovascular Research, 2024a).

Meis Transcription Factors: Miguel Torres' group described the first models of Meis transcription factor deficiency in the cardiac conduction system, demonstrating the essential role of Meis proteins in pacemaker activity and atrioventricular conduction (Cardiovascular Research, 2024b).

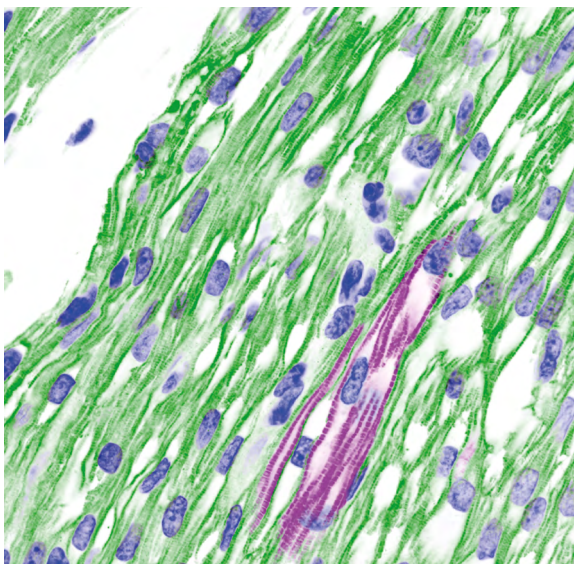
IPSC-Derived Cardiomyocytes: Florian Weinberger's team established IPSC-derived cardiomyocyte studies at the CNIC, showing that these cells can promote electrical impulse initiation and propagation when transplanted to infarcted rodent hearts (Stem Cell Reports, 2024). Weinberger also published a comprehensive review IPSC-derived cardiomyocyte transplantation strategies (Nature Cardiovascular Research, 2024b).

HIGHLIGHTED GRANTS IN 2024

Leducq Transatlantic network: Jose Luis de la Pompa secured $1.12 million for the **PlacHeart Network**, focusing on the placenta's role in maternal and fetal cardiovascular health and disease (January 2025-December 2030).

La Caixa Research Grant: Hesham Sadek received funding for research into **a new therapeutic strategy to combat heart disease**.

Advanced ERC Grant. Miguel Torres was awarded €2.5 million for the REACTIVA project, investigating the transcriptional regulation of cardiomyocyte polyploidization and its relevance in cardiac regeneration (October 2024–September 2029).



Engrafted human induced pluripotent stem cell-derived cardiomyocytes expressing the atrial (pink) or ventricular (green) myosin-light chain isoform respectively.

RESEARCH GROUPS

Rui Benedito
Molecular Genetics of Angiogenesis

José Luis de la Pompa
Intercellular Signaling in Cardiovascular Development and Disease

José Antonio Enríquez
Functional Genetics of the Oxidative Phosphorilation System (GENOXPHOS)

Andrés Hidalgo
Imaging the Cardiovascular Inflammation and the Immune Response

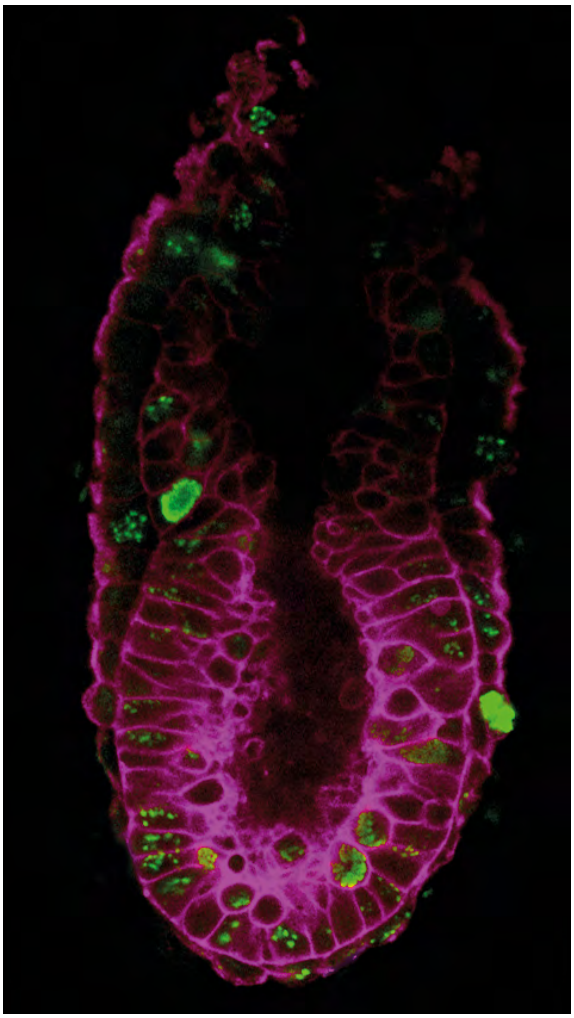
José Jalife
Cardiac Arrhythmia

Nadia Mercader
Development of the Epicardium and its Role during Regeneration

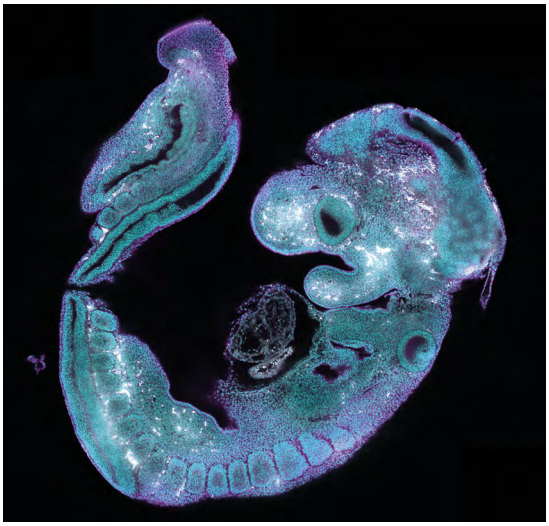
Hesham Sadek
Myocardial Regeneration Vía Cardiomyocyte Cell Cycle Regulation

Miguel Torres
Genetic Control of Organ Development and Regeneration

Florian Weinberger
Cardiac Tissue Engineering and Regenerative Therapies



Pluripotent Cells in Action. The image shows an optical section of the gastrulating mouse embryo showing very active proliferative activity (green) of the naturally occurring pluripotent cells. Cell membranes appear in pink.



Myc expression in the mammalian embryo. A saggital section of the 9-day mouse embryo reveals the expression of Myc, one of the pluripotency reprogramming factors (pink), in stem cells of different organs.

HIGHLIGHTED PUBLICATIONS

Cardiovascular Research. 2024a 120:490-505. doi: 10.1093/cvr/cvae019

Cardiovascular Research. 2024b cvae258, doi:10.1093/cvr/cvae258

Cell. 2024 187:6599-6613.e21. doi: 10.1016/j.cell.2024.08.045

Circulation Research. 2024 134:e52-e71. doi: 10.1161/CIRCRESAHA.123.323895

Developmental Cell. 2024 59:1824-1841.e10. doi: 10.1016/j.devcel.2024.04.012

Disease Models and Mechanisms. 2024 17:dmm050934. doi: 10.1242/dmm.050934

Nature Communications. 15:3931. doi: 10.1038/s41467-024-48258-5

Nature Cardiovascular Research. 2024a 3:372-388.doi: 10.1038/s44161-024-00450-y

Nature Cardiovascular Research. 2024b 3:515-524. doi: 10.1038/s44161-024-00472-6

Nature Methods. 2024. doi:10.1038/s41592-024-02534-w

Nucleic Acids Research. 2024. doi:10.1093/nar/gkae472

Stem Cell Reports. 2024 19:1053-1060. doi: 10.1016/j.stemcr.2024.06.012

2.1.4 NOVEL ARRHYTHMOGENIC MECHANISMS

Coordinator: **Silvia Priori**

Clinical leader: **David Filgueiras**

The **Novel Arrhythmogenic Mechanisms Program** aims to understand the relationship between non-arrhythmogenic phenotypes and cardiac arrhythmias in inherited animal models of cardiac disease. The program focuses on three key areas.

PHENOTYPES ELICITED BY DISRUPTION OF INTRACELLULAR CALCIUM REGULATION

Program scientists are leveraging a mouse model of Triadin-knockout syndrome, a recessive inherited disorder characterized by life-threatening diastolic arrhythmias due to the absence of triadin, a protein essential for heartbeat regulation that resides in the Ca2+ release complexes of cardiomyocytes.

Key findings in this area include:

- Electron microscopy studies revealed that mitochondria in Triadin-knockout myocytes are smaller and more abundant compared with wild-type cells, suggesting increased mitochondrial fission during disease progression (Figure 1).
- Confocal microscopy confirmed that the mitochondria of Triadin-knockout cells have a heightened tendency to accumulate Ca^{2+}, supporting the hypothesis that Ca2+ overload contributes to mitochondrial fission in Triadin-knockout syndrome.

Ongoing research is investigating the molecular factors driving mitochondrial Ca^{2+} overload in Triadin-knockout cells and how this mishandling contributes to diastolic arrhythmogenesis.

NEW TARGETS AND ADVANCED THERAPIES FOR INHERITED CHANNELOPATHIES

The program is dedicated to developing innovative concepts, tools, and therapies for inherited channelopathies, with a focus on Timothy Syndrome 1, a severe inherited arrhythmogenic disorder with a median life expectancy of less than 5 years in humans.

Using experimental data from Timothy Syndrome 1 knock-in swine myocytes and their wild-type littermates, we developed a mathematical model of ventricular cardiomyocyte electrophysiology and Ca^{2+} handling. This model successfully reproduced key Timothy Syndrome 1 phenotypes, including:

- Action potential elongation
- Cellular Ca^{2+} overload
- Increased sensitivity to early-after depolarizations (a trigger for life-threatening arrhythmias)
- Reduced depolarization reserve, a novel discovery contributing to conduction blocks

The model has also served as a predictive tool for evaluating the efficacy of current therapies (e.g., verapamil, mexiletine, ranolazine) and potential future strategies (e.g., gene therapy, IKr activators, repolarizing currents, CaMKII inhibition) (Scientific Reports, 2024).

FIBER DISORGANIZATION IN THE GENESIS OF COMMON ARRHYTHMIAS

The program has made significant contributions to integrating imaging into routine procedure planning for patients undergoing ventricular tachycardia (VT) ablation. A novel systematic strategy was developed and validated, based on scanning and processing multiple signal intensity cut-off ranges on 3D late-gadolinium enhancement cardiac magnetic resonance (LGE-CMR) images.

Key advantages of this approach include:

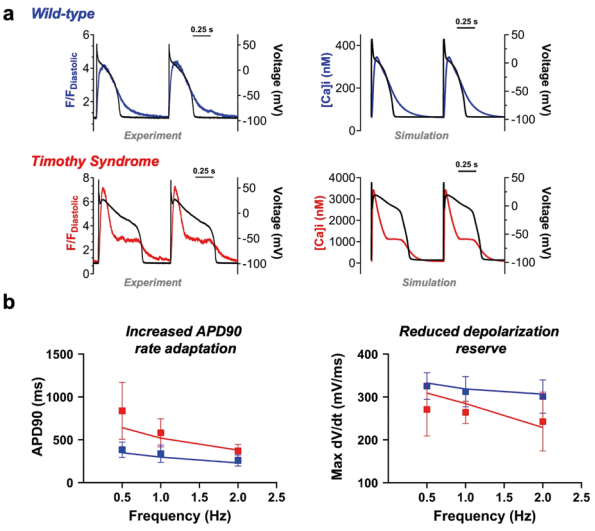
- Elimination of bias associated with manual selection of signal intensity cut-off ranges
- Simplified visualization of complex data from different myocardial layers and signal intensity cut-off ranges
- Clinically relevant assessment of infarct-related substrates and identification of myocardial regions associated with critical VT isthmus sites

This methodology is particularly valuable for planning substrate-based ablation procedures, especially in cases of unmappable VT episodes or multiple VT morphologies (Europace, 2024).

HIGHLIGHTED PUBLICATIONS

Europace. 2024 26 (10): euae244. doi: 10.1093/europace/euae244

Scientific Reports. 2024 14: 29792. doi: 10.1038/s41598-024-80726-2

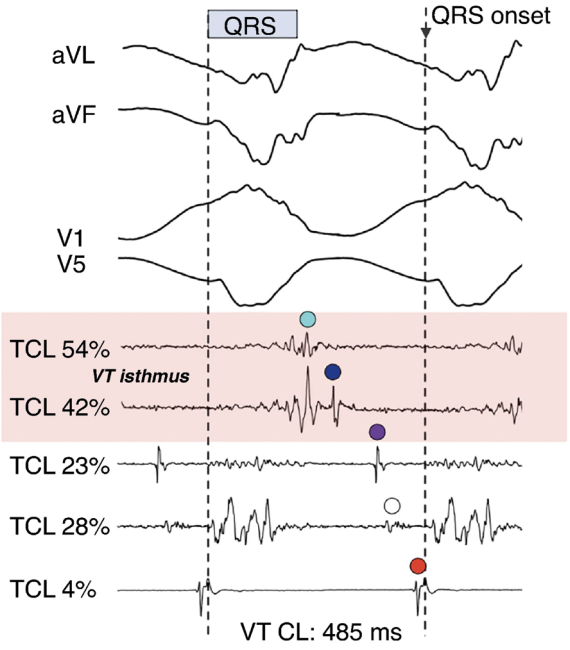


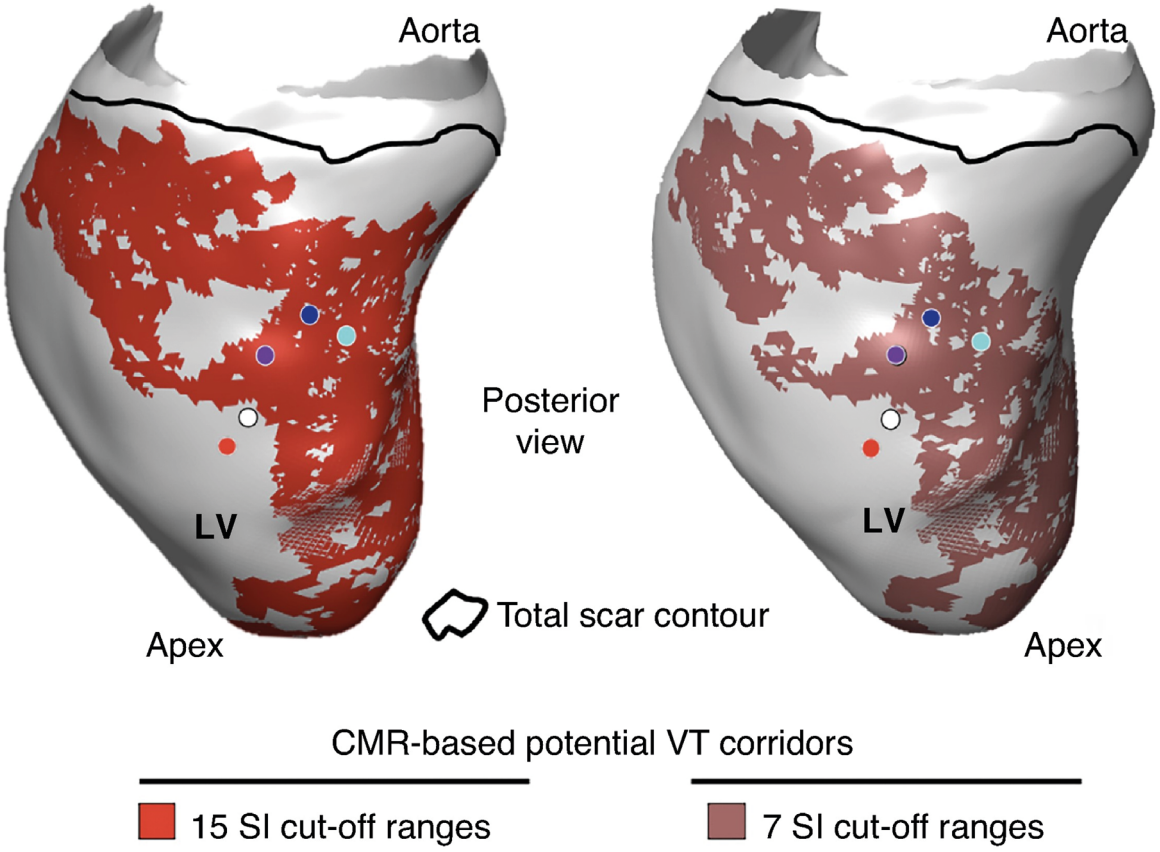
In silico modeling of the responses of ventricular myocytes from wild-type and Timothy Syndrome 1 swine. (a) Comparison between experimental (left) and simulated (right) action potentials and Ca^{2+} transients at 1 Hz pacing. Membrane voltage is plotted on the left axis, whereas cytosolic Ca^{2+} concentration (or fluorescence for experimental data) is plotted on the right axis. (b) Experimental data (squares, mean±SD) and simulated traces (lines) for action potential duration at 90% repolarization (APD90, left) and maximum upstroke velocity (Max. dV/dt), an indicator of depolarization reserve (right). Reproduced from Scientific Reports, 2024) in compliance with the terms of the article's Creative Commons Attribution 4.0 International License.

RESEARCH GROUPS

David Filgueiras
Advanced Development in Arrhythmia Mechanisms and Therapy

Silvia Priori
Molecular Cardiology





The left panels shows sample tracings during ventricular tachycardia (VT) with color-coded dots that indicate intracardiac electrograms (EGMs) at different activation times relative (%) to the tachycardia cycle length (TCL). QRS onset was used as the reference point for assigning activation times. Color-coded dots indicate different EGM–QRS times and their relative (%) activation time with respect to the TCL. The center and right panels show spatial positioning of the color-coded electrograms on the endocardial surface of the left ventricle (LV) together with corresponding imaging-derived potential VT corridors obtained at 15 (middle) and 7 (right) signal intensity (SI) cut-off ranges. Reproduced from Ramos-Prada et al. (2024), EP Europace, https://doi.org/10.1093/europace/euae244, in compliance with the terms of the article's Creative Commons Attribution 4.0 International License.

2.1.5 CARDIOVASCULAR RISK FACTORS AND BRAIN HEALTH

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

The **Cardiovascular Risk Factors and Brain Health Program** investigates how cardiovascular disease and its risk factors—such as hypertension, high cholesterol, obesity, and metabolic syndrome—affect brain function. Our aim is to use this knowledge to develop strategies for preventing cerebrovascular disease and cognitive impairment with age.

KEY ACHIEVEMENTS IN 2024

PESA-Brain Study

Program members Valentín Fuster and Marta Cortes-Canteli published the rationale and design of the PESA-Brain study, which targets 1,000 participants at the 10-year follow-up PESA visit. The study includes comprehensive neuropsychological testing, advanced multimodal neuroimaging, and analysis of blood-based biomarkers of neuropathology, including Alzheimer´s disease (AD). This research is uncovering novel relationships between cardiovascular and brain alterations during the health-to-disease transition, with important implications for interventional and therapeutic approaches (American Heart Journal, 2024).

In a related, these groups found that apolipoprotein E4 (APOE4), a major genetic risk factor for AD, is also associated with an increased risk of subclinical atherosclerosis in middle age. Conversely, the APOE2 variant, which protects against AD, was shown to reduce the risk of subclinical atherosclerosis (Circulation Research, 2024a).

Brain–Body Interactions

Several groups in the Program explore brain-body interactions in health and disease. The teams led by Guadalupe Sabio (now at the CNIO) and María Ángeles Moro described how exercise activates muscle p38γ, increasing locomotor activity through the secretion of interleukin-15, which acts on the motor cortex. This mechanism plays a crucial role in reducing the risk of diabetes and liver steatosis, revealing a vital muscle–brain communication pathway with significant clinical implications for obesity and metabolic diseases (Science Advances, 2024).

Themed Issue of The British Journal of Pharmacology

Drs. Moro and Cortes-Canteli edited a themed issue of the British Journal of Pharmacology entitled ***From Alzheimer's Disease to Vascular Dementia: Different Roads Leading to Cognitive Decline*** (British Journal of Pharmacology, 2024a). This special issue reviewed several topics of core interest to Program researchers:

- The clinical evidence on the effect of anticoagulants on the development of AD and other dementias (British Journal of Pharmacology, 2024b).
- The complex interplay between microbiota and brain health, including the role of gut dysbiosis in cerebrovascular disease and its possible implications for post-stroke cognitive impairment and dementia (British Journal of Pharmacology, 2024c).
- The contribution of the peripheral myeloid lineage to AD and vascular dementia, with a special focus on post-stroke cognitive impairment (British Journal of Pharmacology, 2024d).

Additionally, María Ángeles Moro's team provided an overview of the circadian control of immune–vascular interactions both in steady-state and in pathological cardiovascular conditions, such as atherosclerosis and infarction (Circulation Research 2024b).

INTERNATIONAL ACTIONS AND RECOGNITION

During 2024, Program members participated in several international actions, including:

- **MSCA-COFUND CURE HEARTandBRAIN** (Grant Agreement 101126521; María Ángeles Moro)
- The **Leducq-funded Stroke-Impact consortium** (TNE19-CVD01) and the **Leducq Circadian Network** (TNE21-CVD04) (María Ángeles Moro)
- The **EU Joint Action on Cardiovascular Diseases and Diabetes** (JACARDI, EU4H-2022-JA-03) (Hector Bueno).

Program members also received prestigious awards, including:

- The **2024 ESC President's Award** to Hector Bueno
- The nomination of María Ángeles Moro as a **Numerary Member of the Spanish National Royal Academy of Pharmacy**.
- Ignacio Ruiz-Fernandez, from Pilar Martín's group, received the **Best Poster Presentation Award** at the 2024 CNIC Cardioimmunology meeting and the Young Investigator Award at the Heart Failure Association (ESC) Winter Meeting in Nice (April 2024).

HIGHLIGHTED PUBLICATIONS

American Heart Journal. 2024 278:195-207. doi: 10.1016/j.ahj.2024.09.028

British Journal of Pharmacology, 2024a. 181:755–759. doi: 10.1111/bph.16292

British Journal of Pharmacology, 2024b 181:760–776. doi: 10.1111/bph.16032

British Journal of Pharmacology, 2024c 181:816–839. doi: 10.1111/bph.16167

British Journal of Pharmacology, 2024d 181:777-798. doi: 10.1111/bph.16159

Circulation Research. 2024a 134(4):411-424. doi: 10.1161/CIRCRESAHA.123.323921

Circulation Research 2024b 134(6):791-809. doi: 10.1161/CIRCRESAHA.123.323619

Science Advances, 2024 10(33) eadn5993. doi: 10.1126/sciadv.adn5993

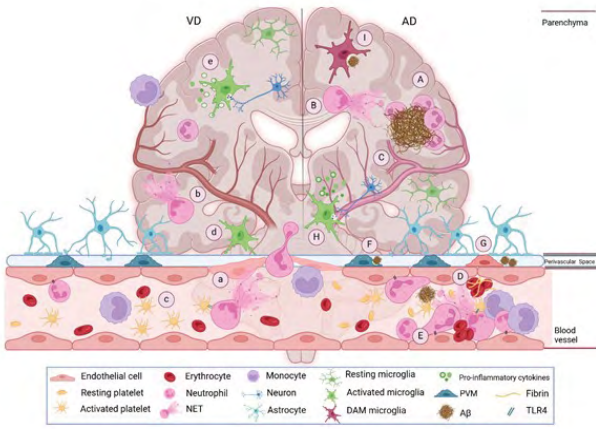
RESEARCH GROUPS

Héctor Bueno
Multidisciplinary Translational Cardiovascular Research (MTCR)

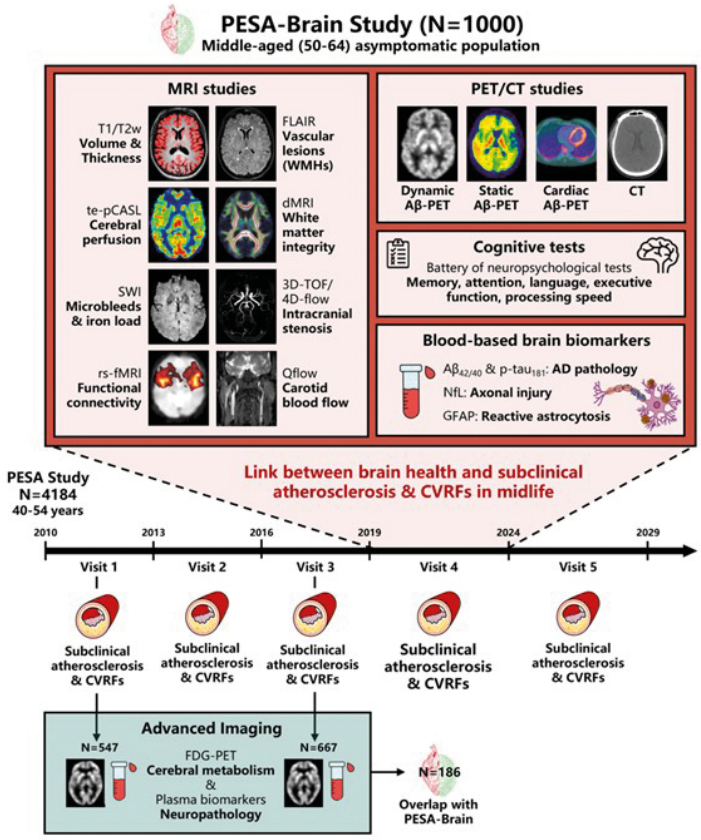
Valentín Fuster
Cardiovascular Imaging and Population Studies

Pilar Martín
Regulatory Molecules of Inflammatory Processes

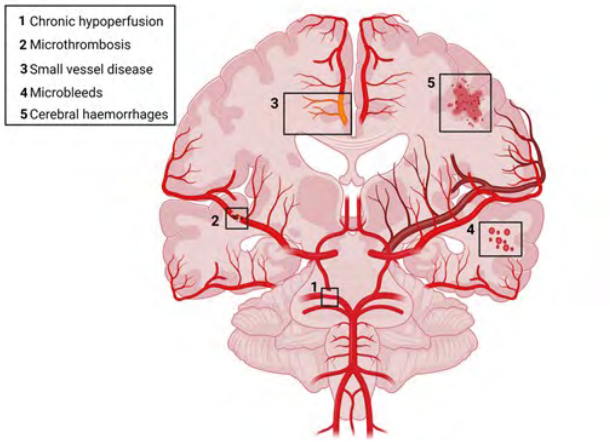
María Ángeles Moro
Neurovascular Pathophysiology



Effects of myeloid cells in Alzheimer's disease (AD) and vascular dementia (VD).
AD: (A) Neutrophils infiltrate the brain parenchyma located close to amyloid-β (Aβ) plaques. (B) These infiltrating neutrophils are activated by inflammatory mediators (possibly released by microglia) and can produce neutrophil extracellular traps (NETs). (C) Monocytes may participate directly in the removal of Aß-plaques. (D) NETs are also produced by neutrophils adhered to the vessel wall; production of these intravascular NETs may involve a contribution from activated platelets via TLR4. E) Platelets may also be responsible for the accumulation of Aβ in blood clots inside and around cerebral blood vessels. (F) Aβ phagocytosis in the perivascular space by perivascular macrophages (PVMs). (G) Intravascular Aβ crosses the vessel wall, enters the perivascular space, reaches PVMs, induces ROS production, and alters neurovascular function. (H) Excessive microglia activation causes neurotoxicity and synaptic loss. (I) Disease-associated microglia (DAM) involved in Aβ clearing.
VD: (a) Parenchymal and (b) intravascular NETs impair vascular remodeling during stroke recovery. (c) Platelet activation is still present in the chronic phase of ischemic stroke. (d) Vessel-attached microglia play a beneficial role in maintaining blood–brain barrier (BBB) integrity. (e) Activation of harmful microglia leads to pro-inflammatory cytokine release and the phagocytosis of astrocyte endfeet and axons, aggravating BBB damage.
(Figure modified from British Journal of Pharmacology, 2024d)



PESA-Brain Study Design.
Imaging Techniques: FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; mS-DTI, multishell diffusion tensor imaging; PET, positron emission tomography; rs-fMRI, resting-state functional MRI; SWI, susceptibility-weighted MRI; te-pCASL, time-encoded pseudo-continuous arterial spin labeling; TOF, time-of-flight.
Biomarkers: Aβ42/40, amyloid-β ratio 42/40; FDG, [18F] fluorodeoxyglucose; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau181, phosphorylated tau 181.
Conditions: AD, Alzheimer's disease; CVRF, cardiovascular risk factor.



Pathophysiological processes of vascular origin in vascular cognitive impairment.
1. Chronic hypoperfusion: Reduced blood flow and oxygen supply due to conditions like chronic hypertension or atherosclerosis.
2. Microthrombosis: Formation of small blood clots in brain vessels.
3. Small vessel disease: Characterized by damage to small blood vessels, reducing blood flow.
4. Microbleeds: Rupture of small brain vessels due to hypertension or cerebral amyloid angiopathy.
5. Cerebral hemorrhages: Bleeding in the brain from ruptured blood vessels.
(Figure modified from British Journal of Pharmacology, 2024d)

2.1.6 CARDIOVASCULAR HEALTH PROMOTION

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

Cardiovascular disease (CVD) remains one of the leading causes of death and disability worldwide, and its high prevalence and impact are largely driven by modifiable risk factors, such as smoking, unhealthy diets, and physical inactivity. Alarmingly, the problem is expected to worsen due to rising rates of unhealthy lifestyles and obesity, particularly among children.

The **Cardiovascular Health Promotion Program (CHPP)** addresses this challenge through multidisciplinary studies and clinical trials conducted in collaboration with schools and communities. These initiatives target both children and adults, aiming to implement early prevention strategies and develop noninvasive technologies to support translational research and population studies on preclinical atherosclerosis.

The ultimate goal of the CHPP is to reduce the personal and societal burden of CVD through health promotion and prevention strategies, potentially increasing life expectancy and reducing the risk of other diseases, such as dementia and cancer. The program focuses on three key objectives:

Refining primordial prevention strategies in children and adolescents.

Improving global primary prevention by addressing the subclinical development and progression of atherosclerosis in young adults.

Translating health promotion initiatives to the general population.

The CHPP comprises 2 research groups: Cardiovascular Imaging and Population Studies (PI, Valentin Fuster) and Cardiovascular Health and Imaging (PI, Rodrigo Fernández-Jiménez). In 2024, the program achieved significant scientific advances, highlighted below.

KEY ACHIEVEMENTS IN 2024

SI! Program for Elementary Schools Trial Results

CHPP scientists reported the primary results of the SI! Program for Elementary Schools trial, a 6-year cluster randomized intervention in 48 state-funded elementary schools in the Madrid region. The trial evaluated the impact of time-varying exposures to a multicomponent school-based health promotion program. Children exposed to the SI! Program throughout all 6 elementary school years or during the first 3 elementary school years showed significant improvements in abdominal adiposity markers (Figure 1) (Journal of the American College of Cardiology, 2024a).

Addressing Health Disparities in Adolescents

Analysis of data from the SI! Program for Secondary Schools trial revealed that adolescents from low socioeconomic backgrounds and migrant families were more likely to belong to groups with poorer cardiovascular health trajectories. These groups also had the highest prevalence of overweight/obesity and metabolic syndrome, underscoring the need for primordial prevention interventions targeting vulnerable populations (Figure 2) (Journal of Adolescent Health, 2024).

Sex-Specific Reference Values for Cardiac Strain in Adolescents

CHPP researchers established sex-specific reference values for biventricular strain using magnetic resonance imaging in adolescents aged 15 to 18 years. These values provide a valuable tool for detecting subtle changes at subclinical disease stages in pediatric populations (Journal of Magnetic Resonance Imaging, 2024).

Collaborative Program Highlights

Subclinical Atherosclerosis and Mortality

In collaboration with other CNIC research programs, CHPP researchers analyzed data from the BioImage study, demonstrating that subclinical atherosclerosis burden and progression in asymptomatic individuals were independently associated with all-cause mortality (Figure 3) (Journal of the American College of Cardiology, 2024b).

Apolipoprotein E and Cardiovascular Disease

Through analysis of data from the PESA study, CHPP researchers uncovered the role of apolipoprotein E in cardiovascular disease development, offering important insights for therapeutic and prevention strategies, particularly in early midlife (Circulation Research, 2024).

Outreach and Recognition

The CHPP recognizes the importance of effective communication to raise public awareness of cardiovascular health and healthy lifestyles. In 2024, program scientists participated in outreach activities, including the XXIV Science and Innovation Week in Madrid.

Additionally, CHPP researchers received prestigious awards, including:

The Gabriella Morreale National Youth Research Award in Medicine and Health Sciences, awarded to Dr. Rodrigo Fernandez-Jimenez by the Ministry of Science, Innovation, and Universities.

The Lifetime Achievement Award by the World Heart Federation, the AstraZeneca Foundation Honorific Award for Excellence in Scientific Research, and the Antonio M. Gotto Jr. Prize in Atherosclerosis Research by the International Atherosclerosis Society, all awarded to Dr. Valentin Fuster.

HIGHLIGHTED PUBLICATIONS

Circulation Research. 2024 134(4):411-424. doi: 10.1161/CIRCRESAHA.123.323921

Journal of Adolescent Health. 2024;74(5):1039-1048. doi: 10.1016/j.jadohealth.2023.12.016

Journal of Magnetic Resonance Imaging. 2024 60(6):2409-2420. doi: 10.1002/jmri.29334

Journal of the American College of Cardiology. 2024a 84(6):499-508. doi: 10.1016/j.jacc.2024.04.065

Journal of the American College of Cardiology. 2024b 84(15):1391-1403. doi: 10.1016/j.jacc.2024.06.045

RESEARCH GROUPS

Rodrigo Fernández-Jiménez
Cardiovascular Health and Imaging

Valentín Fuster
Cardiovascular Imaging and Population Studies

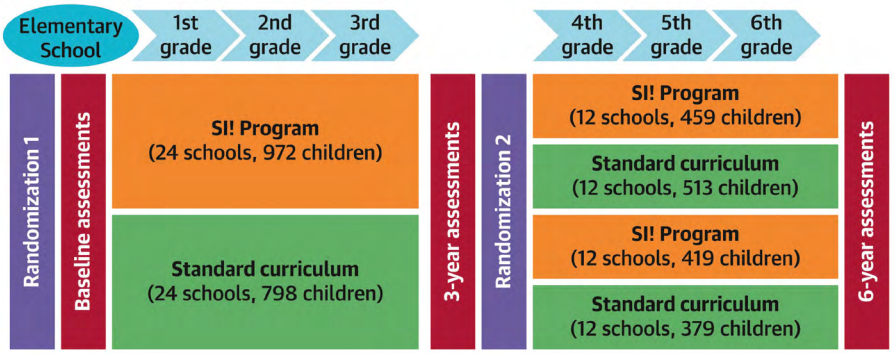


Figure 1. Study design of the SI! Program for Elementary Schools trial. The SI! Program curriculum covers diet, physical activity, body and heart health, and emotional management. The curriculum was implemented for the first 3 years (grades 1-3), the final 3 years (grades 4-6), or throughout all 6 years of elementary school. Control schools followed the standard curriculum. Primary outcomes included changes in adiposity markers. Reproduced from J Am Coll Cardiol, 2024a.

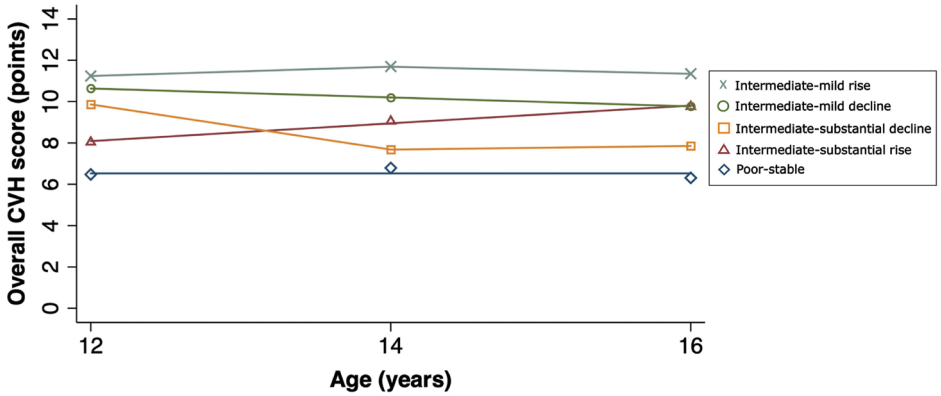


Figure 2. Cardiovascular health trajectories in adolescence. Based on American Heart Association thresholds, cardiovascular health (CVH) metrics (smoking, BMI, physical activity, diet, blood pressure, cholesterol, and blood glucose) were scored as 0 (poor), 1 (intermediate), or 2 (ideal). The overall CVH score thus ranged from 0 to 14 points, with higher scores indicating better CVH. Trajectories are ordered by baseline CVH scores. Reproduced from J Adolesc Health, 2024.

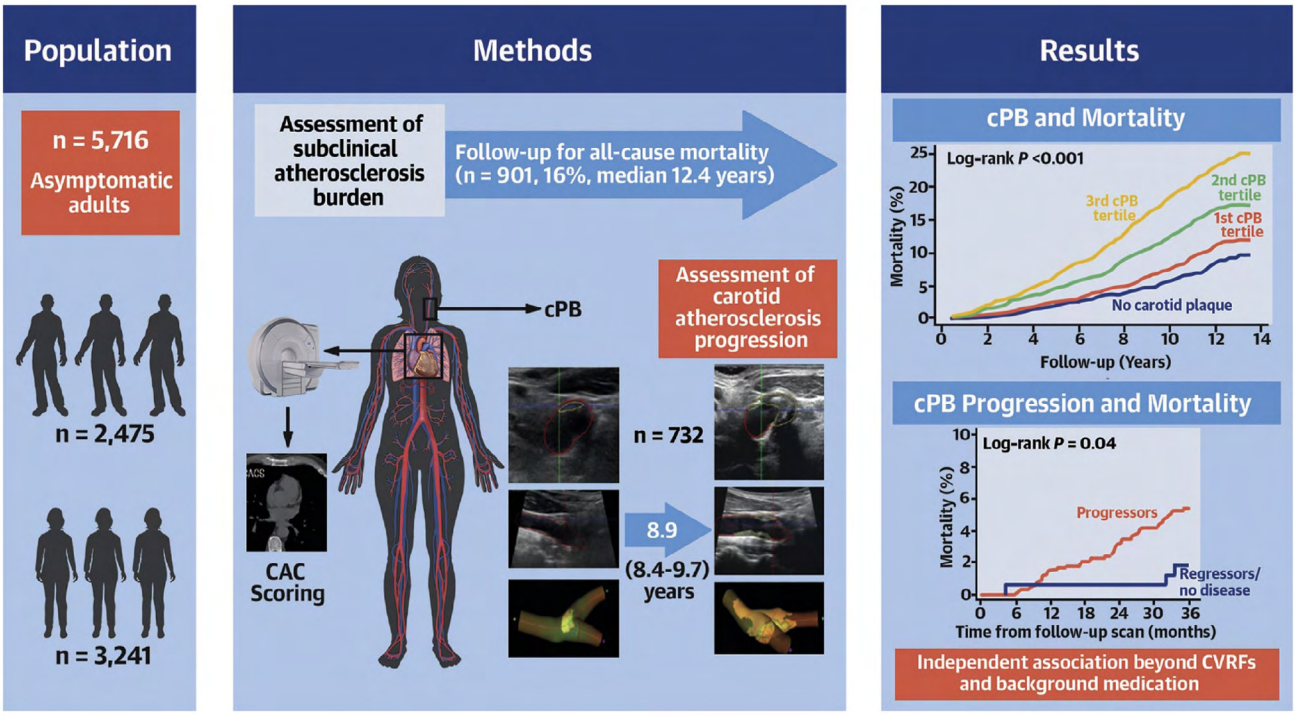


Figure 3. Study design and main results of the BioImage study. The study enrolled 5,716 asymptomatic individuals assessed for subclinical atherosclerosis (coronary artery calcium [CAC] score and carotid plaque burden [cPB]) and cardiovascular risk factors (CVRFs). A subset of 732 participants were reassessed after 8.9 years. Atherosclerosis burden and the progression of carotid atherosclerosis were independently associated with all-cause mortality. Reproduced from J Am Coll Cardiol, 2024b.

2.1.7 TECHNOLOGY DEVELOPMENT

Coordinator: **Beatriz Álvarez**

The **Technology Development Program** (TDP) comprises 11 Technical Units (TUs) that keep the center at the forefront of cardiovascular research by developing and implementing cutting-edge biomedical technologies. The TUs provide internal and external services, engage in training, and participate in scientific collaborations through funded projects. More information about the TUs can be found at https://www.cnic.es/en/investigacion/unidades-tecnicas.

Advanced imaging capabilities support detailed molecular and anatomical visualization, enabling comprehensive studies from whole-organ to cellular resolution. These efforts are complemented by units focused on cellular engineering and gene editing, which develop innovative genetically modified models—including large animal systems—to unravel disease mechanisms with high precision. Omics platforms, including cytometry, proteomics, and genomics, offer in-depth cellular and molecular characterization, while the bioinformatics infrastructure enables integration and analysis of complex datasets, driving data-driven discoveries. Collaborative developments in gene therapy vectors exemplify the translational potential emerging from this research framework. Together, these interconnected units form a dynamic and innovative environment that accelerates the transition from basic research to clinical applications, reinforcing the CNIC's leadership in cardiovascular science.

KEY ACHIEVEMENTS IN 2024

ISO Certification: All TUs obtained UNE-EN-ISO 9001:2015 certification, guaranteeing the quality of services and continuous process improvement.

High-Tech Equipment Acquisition: The TDP successfully acquired all five items requested in the competitive EQC2024 call, resulting in a total economic saving of €2.7 million for the center. The new equipment includes:

A high-tech axial computerized tomography system with a spectral detector.

A high-sensitivity mass spectrometer coupled to ultra-high-pressure liquid chromatography for metabolomics and targeted lipidomics.

A 3-4D multimodal-adaptive fluorescence microscope.

An expansion of the center's computing capacity with a new platform for the storage and analysis of biomedical images using artificial intelligence (AI).

Two newly acquired cutting-edge ultrasound imaging systems

TRAINING AND KNOWLEDGE DISSEMINATION

Training is a core activity at the CNIC, and the TDP is committed to supporting it through several actions:

Participation of the the TUs in the UAM Master's in Molecular Biomedicine (BMM7).

Involvement in scientific training and communication programs, such as Acércate and Res@cnic, as well as the seminar program.

Organization of Technodays, a 3-day event for sharing scientific and technical expertise. The 2024 edition focused on three key areas:

1. In Vivo and In Vitro Models
2. Omics
3. Cardiac and Brain Imaging

Coordination (by the Microscopy Unit) of the scientific committee of the SPAOM2024 congress (Spanish & Portuguese Advanced Optical Microscopy Meeting, Toledo, Nov 2024).

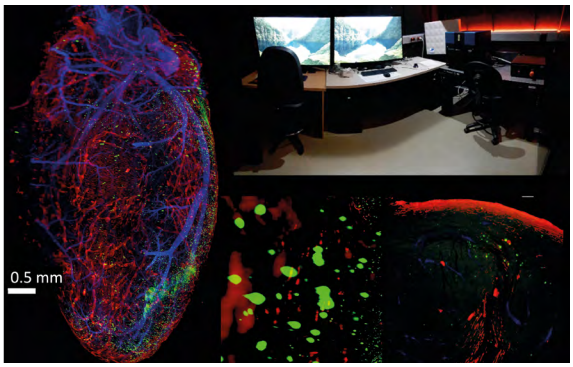
SCIENTIFIC OUTPUT IN 2024

The CNIC TUs contributed to the center's scientific production, with 102 articles published—accounting for 26% of the center's total output.

82% of publications appeared in first-quartile journals

42% were featured in first-decile journals.

47% were the result of international collaborations.



Microscopy analysis suite and images of a mouse heart labeled for endothelial cell nuclei (green), microvasculature (red), and arteries (blue). The heart images were generated in collaboration with Luis Diago and Rui Benedito of the Molecular Genetics of Angiogenesis Laboratory.

TECHNICAL UNITS

Fátima Sánchez Cabo
Bioinformatics

Antonio J. Quesada
Clinical Trial Coordination

Comparative Medicine

Beatriz Álvarez
Flow Citometry

Ana Dopazo
Genomics

Manuel Desco
Imaging

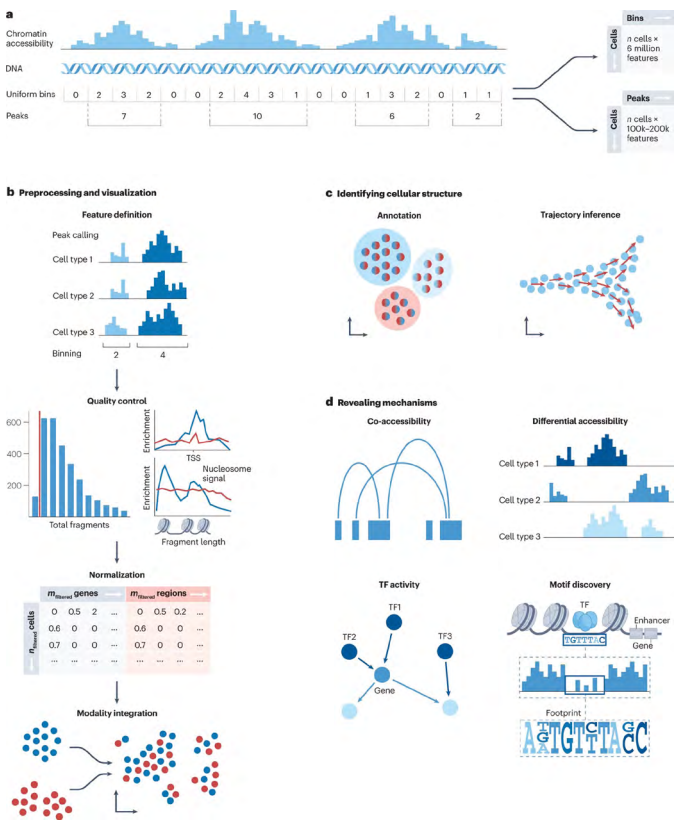
Valeria Caiolfa
Microscopy

Giovanna Giovinazzo
Pluripotent Cell Technology

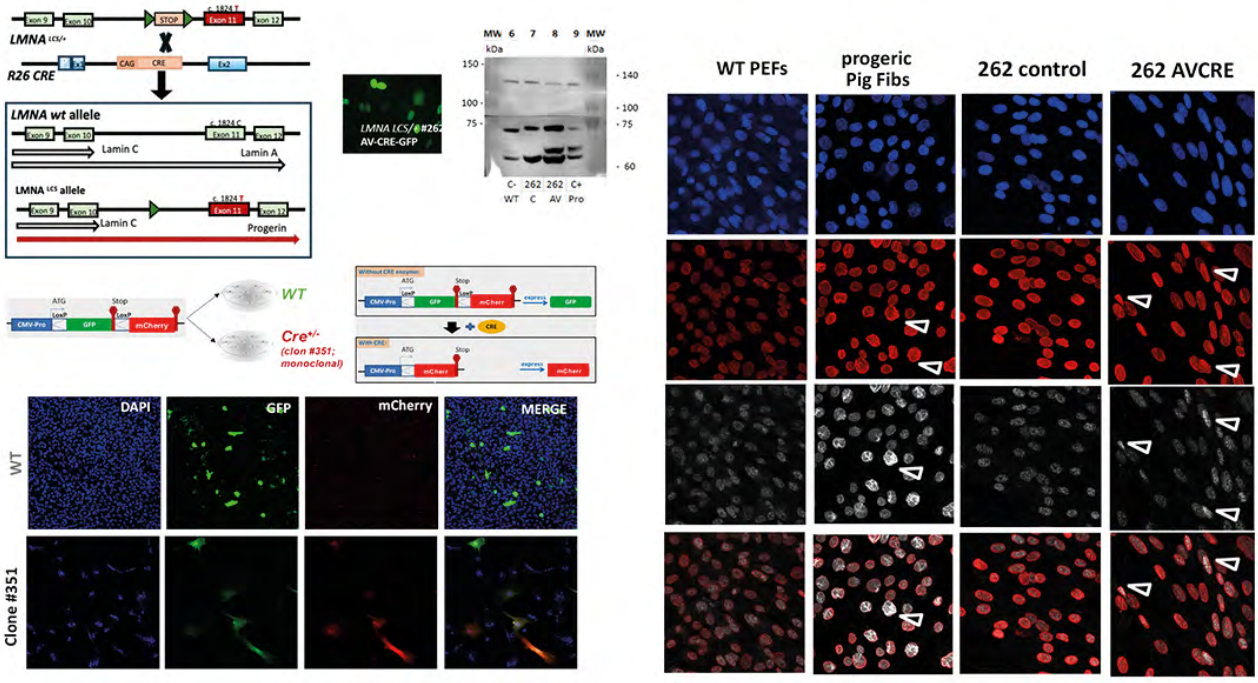
Juan Antonio López
Proteomics

Juan De Dios Hourcade
Transgenesis

Juan A. Bernal
Viral Vectors



Single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) is a method for measuring chromatin accessibility in individual cells.



Design of gene editing strategies based on the CRISPR/Cas9 system and critical experiments for generating models of cardiovascular disease, in vivo models and in vitro models in hiPSCs.

2.2 CLINICAL STUDIES AT THE CNIC

Clinical-translational research plays a vital role in turning laboratory discoveries into real-world medical solutions. By bridging the gap between science and practice, this research helps develop new treatments, diagnostic tools, and prevention strategies that directly benefit patients. This approach combines insights from basic science with clinical expertise to accelerate medical progress and improve healthcare outcomes.

Clinical-translational research is a cornerstone of our work at the CNIC. We are committed to advancing these studies and ensuring their impact on patient care. Below, we highlight some of the clinical projects that were active in 2024.

PESA-HEALTH-CNIC-SANTANDER: EARLY DETECTION OF SUBCLINICAL ATHEROSCLEROSIS, DISEASE PROGRESSION, AND CARDIOVASCULAR HEALTH

Principal Investigator: *Valentín Fuster*

PESA Health Initiative

cnic Santander

The PESA-Health-CNIC-Santander study builds on the foundation laid by its predecessor, the PESA Study, which began in 2010 under the direction of the CNIC in collaboration with Santander Bank. The original study enrolled 4,184 asymptomatic individuals aged 40 to 55 and aimed to detect subclinical atherosclerosis (SA) long before symptoms appear, while also exploring the factors driving its development and progression.

The continuation of the PESA study, PESA-Health, extends this work by tracking the same participants over an additional 10 years. The new study broadens the scope to include new research areas, such as the links between SA and Alzheimer's disease or cognitive decline, the role of somatic mutations in aging, and how these mutations may influence cardiovascular events and SA progression.

As in the original PESA study, PESA-Health leverages advanced imaging technologies, including 3D vascular ultrasound of the carotid arteries and aorta, coronary artery calcium quantification via computed tomography, cardiac magnetic resonance, AngioTC, PET, and PET-amyloid analysis. In addition, biosampling is used for for extensive omics analysis, and new substudies are exploring the connection between SA and sleep apnea.

As the CNIC's flagship study, PESA-Health involves many of the center's clinical and basic research groups. Findings from the PESA study have already made significant contributions to understanding the origins and progression of atherosclerosis.

PESA-Health launched in February 2020, with 3,496 PESA participants continuing their involvement. By the end of 2024, the first round of visits was completed for 3,300 individuals, and the second round of visits had begun.

RESILIENCE: REMOTE ISCHEMIC CONDITIONING IN LYMPHOMA PATIENTS RECEIVING ANTHRACYCLINES

Principal Investigator: *Borja Ibáñez*
H2020 Grant# 945118

RESILIENCE

Anthracyclines are a widely used class of anticancer drugs, administered to over 3 million of the 4 million new cancer patients diagnosed annually in Europe. While effective, these drugs carry a significant risk: recent data show that >35% of patients treated with anthracyclines develop some form of cardiomyopathy. This trade-off between cancer treatment and chronic heart failure (HF) not only places a heavy psychological burden on cancer survivors but also poses a growing challenge for healthcare systems.

Remote ischemic pre-conditioning (RIPC) offers a potential solution. RIPC is a noninvasive, safe, and cost-effective technique that involves brief, reversible episodes of ischemia (e.g., in an arm) followed by reperfusion. This process can protect remote tissues and organs against injury in future ischemic events. Experimental studies in large animals have shown that 3 to 5 cycles of 5-minute limb ischemia followed by 5-minute reperfusion reduce the size of induced myocardial infarctions. Recent evidence suggests that, to be effective, RIPC must be initiated before the damaging event. Since chemotherapy is a planned procedure, anthracycline-induced cardiomyopathy provides an ideal setting to test this approach.

RESILIENCE is a multinational, phase II, double-blind, sham-controlled, randomized controlled trial evaluating the efficacy and safety of RIPC in patients with lymphoma or breast cancer and who are receiving anthracyclines. Eligible patients scheduled to receive ≥240 mg/m² of anthracyclines undergo baseline cardiac magnetic resonance (CMR) imaging and blood tests for high-sensitivity troponin (hsTn) and NT-proBNP. Patients with a left ventricular ejection fraction >40% confirmed by CMR are randomized 1:1 to receive either RIPC or a sham procedure.

Nine weeks after completing chemotherapy, patients undergo a final CMR and hsTn/NT-proBNP test. Clinical follow-ups are scheduled for 12, 18, 30, and 42 months until the last patient completes the final CMR.

The RESILIENCE Trial aims to enroll 608 patients across 22 sites in six European countries (Spain, Portugal, France, Germany, the Netherlands, and Denmark). Funded by the European Commission (Grant Agreement-945118-RESILIENCE), the trial began its grant period in June 2021, with patient recruitment starting in 2022. By the end of 2024, three additional

hospitals were added, all 22 sites were operational, and 284 participants had been enrolled.

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

Principal Investigator: *Borja Ibáñez*

The use of beta-blockers for patients after a myocardial infarction (MI) is largely based on evidence from trials conducted before the reperfusion era. While these drugs have proven benefits for post-MI patients with reduced ejection fraction, their effectiveness for patients with preserved ejection fraction remains unclear. Despite this lack of evidence, over 80% of post-MI patients in this category are prescribed beta-blockers for life.

The REBOOT trial addresses this gap by evaluating the need for beta-blocker therapy in post-MI patients with a left ventricular ejection fraction greater than 40%. This multinational study has enrolled 8,506 patients, who have been randomized to either receive beta-blockers (with the type and dose determined by their physician) or no beta-blocker treatment. The primary endpoint is a composite of all-cause death, reinfarction, or heart failure admission over a 3-year follow-up period.

Coordinated by the CNIC Clinical Trials Coordination Unit and conducted in collaboration with the Mario Negri Institute of Pharmacological Research in Milan, the trial involves 77 hospitals in Spain and 29 in Italy. This large-scale project has the potential to significantly influence clinical practice.

Patient enrollment began in October 2018 and has now been completed. The trial is currently in the event-adjudication process.

MRVALVE: MULTIMODALITY MYOCARDIAL TISSUE CHARACTERIZATION IN PATIENTS WITH SIGNIFICANT VALVULAR DISEASE

Principal Investigator: *Borja Ibáñez*

The MRVALVE study uses a multimodality imaging approach (cardiac magnetic resonance [CMR] and strain echocardiography) to better characterize left ventricular (LV) status in patients with significant valvular heart disease (VHD), focusing on aortic valve stenosis (AS) as a model of LV pressure overload and mitral regurgitation (MR) as a model of LV volume overload.

VHD significantly impacts LV dimensions, function, and tissue composition, all of which play a critical role in clinical decision-making. Current guidelines recommend surgical treatment for patients with significant VHD when symptoms develop or when there is evidence of LV remodeling or dysfunction. The most common forms of VHD are AS and MR. The progression from asymptomatic to symptomatic disease, or from normal LV function to LV dilatation, hypertrophy, and dysfunction, is driven by changes in tissue composition—primarily cardiomyocyte death, extracellular volume expansion, and fibrosis.

While surgery or percutaneous valve repair or replacement are effective treatments for severe VHD, interventions are typically based on the presence of symptoms or significant LV dysfunction. By the time these features appear, it is often too late to fully restore heart function. This highlights the need for tools for the early detection of myocardial involvement in asymptomatic VHD patients, allowing timely intervention before irreversible damage occurs.

CMR is the gold standard for anatomical and functional cardiac assessment. This methodology can detect focal fibrosis through late gadolinium enhancement and offers advanced tissue characterization using techniques like parametric T1/T2 mapping, absolute myocardial perfusion quantification, and extracellular volume calculation (a marker of diffuse fibrosis). These assessments require the use gadolinium-based contrast agents, which have a strong safety profile and are widely used in clinical practice. A blood sample is also needed to determine hematocrit for assessing diffuse fibrosis.

Strain echocardiography, the best imaging modality for evaluating active LV myocardial deformation, can detect impaired multidirectional strain even when overall LV function appears normal. In the MRVALVE study, we correlate imaging data with functional assessments from the 6-minute walking test, which provides an objective measure of exercise capacity. Additionally, cardiac computed tomography is used to assess calcium deposition in the coronary arteries and heart valves, providing a calcium score that serves as both a diagnostic and prognostic tool in AS patients.

To date, 71 patients have been recruited, and all have completed their 1-year follow-up visit.

MATRIX: NOVEL MITOCHONDRIA-TARGETED THERAPIES FOR CANCER TREATMENT–INDUCED CARDIOTOXICITY

Principal Investigator: *Borja Ibáñez*
ERC Consolidator Grant#819775

The MATRIX Project aims to develop innovative treatments for cardiotoxicity caused by certain cancer therapies. This initiative is a joint effort between the CNIC and ***Fundación Jiménez Díaz*** University Hospital, building on a collaborative framework established in 2015 to study myocardial diseases.

While significant advances in cancer treatment have improved outcomes for the 4 million new cancer patients diagnosed annually in Europe, these therapies often come with serious side effects. One of the most common is cancer treatment–induced cardiotoxicity (CTiCT), which affects up to 25% of patients treated with anthracyclines or trastuzumab. CTiCT can lead to severe complications, including chronic heart failure or even death, creating a devastating trade-off for cancer survivors.

CTiCT poses a significant challenge, as current therapies are suboptimal. Early detection methods are inadequate, and heart failure treatments are nonspecific. Recent findings from our group suggest that CTiCT is linked to altered mitochondrial dynamics, which triggers metabolic reprogramming in cardiomyocytes.

The MATRIX Project takes a holistic approach to addressing mitochondrial dysfunction in CTiCT. We propose that early-stage CTiCT could be reversed by metabolic reprogramming to alter mitochondrial substrate utilization. Using a novel imaging-based algorithm developed by our team, we aim to detect myocardial damage in patients receiving common anticancer drugs long before traditional clinical parameters show abnormalities. This early detection, currently unavailable, is critical for timely intervention.

For end-stage CTiCT, where mitochondrial dysfunction may be irreversible, we propose that replenishing the myocardium with healthy mitochondria through in-vivo mitochondrial transplantation could offer a radical new therapeutic option.

The MATRIX Project has broad translational potential, including the development of new therapeutic approaches, early diagnostic technologies, and insights into the basic mechanisms of CTiCT.

Patient recruitment began in 2020, and as of February 2025, 56 participants have been enrolled.

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

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

Acute myocarditis is challenging to diagnose due to its varied clinical presentation and the lack of rapid, accessible, and accurate diagnostic tools. Symptoms can range from atypical chest pain (resembling pericarditis or angina) to dyspnea, fatigue, palpitations, syncope, and, in severe cases, sudden death or shock. Early diagnosis is further complicated by the nonspecific results obtained with standard tests, such as ECG, echocardiography, and laboratory tests.

Currently, diagnosing acute myocarditis typically requires invasive procedures like endomyocardial biopsy or advanced imaging techniques like cardiovascular magnetic resonance, which are not universally available. This highlights the urgent need for new, noninvasive diagnostic approaches.

Dr. Martín Fernández's research group has made significant progress in this area by identifying a novel microRNA in mice and humans with myocarditis. Their work, published in the New England Journal of Medicine (2021: 27;384(21):2014-2027), demonstrates that the human homolog (hsa-miR-Chr8:96) can effectively distinguish myocarditis patients from those with myocardial infarction.

The MYOCARDITIS-CNIC Registry, a collaborative initiative between the CNIC and ***Hospital Virgen de la Arrixaca***, aims to build on these findings. Several Spanish hospitals, including ***Hospital de la Princesa*** and ***Clínica Universitaria de Navarra***, are participating in the registry by collecting clinical data and biological samples from patients presenting to emergency departments with signs of myocarditis. This registry will provide critical insights into the early stages of myocarditis and help validate potential clinical biomarkers for early diagnosis.

To date, 75 participants have been enrolled in the registry.

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

Principal Investigator: *Gonzalo Pizarro Sánchez*

Arterial hypertension can cause damage to the cerebral vascular system, even when blood pressure effectively controlled. Current treatments for hypertension focus on managing blood pressure and preventing damage to target organs, such as the brain. Remote ischemic preconditioning (RIPC), a technique first shown to protect organs like the heart and brain in animal models 30 years ago, has since been adapted for use in human patients. RIPC involves inflating and deflating a blood pressure cuff on the arm through four cycles of 5 minutes each. The brief ischemia induced in the arm can protect distant organs, including the brain, from future ischemic damage.

Our research group has contributed significantly to this field, with recent studies demonstrating that RIPC can improve cognitive performance and cerebral vascular function in patients with vascular dementia.

The PRECOGNITIVE study is a proof-of-concept randomized trial involving 45 women with hypertension and evidence of target organ damage, such as left ventricular hypertrophy. Participants are divided into 3 groups:

1. The RIPC group undergo the RIPC procedure, with the cuff inflated to 20 mmHg above their systolic blood pressure.
2. The RIPC-Sham group follow the same procedure, but the cuff will only be inflated to 50 mmHg, insufficient to induce ischemia.
3. The control group do not receive any cuff therapy.

The study aims to determine whether RIPC has a significant protective effect on the cerebral vasculature in hypertensive patients without significant cognitive impairment. Outcomes will be assessed using comprehensive neurocognitive tests and noninvasive imaging techniques, including echocardiography, noncontrast brain magnetic resonance imaging, and transcranial Doppler ultrasound.

As of the end of 2024 15 patients have been recruited.

MACADAMIA: CHARACTERIZATION OF CARDIAC METABOLISM USING MULTIMODAL IMAGING IN IDIOPATHIC CARDIOMYOPATHY

Principal Investigator: *Borja Ibáñez*

Heart failure (HF) is one of the most significant health challenges in modern societies, placing a heavy burden on healthcare systems. To develop new therapies, it is essential to understand the mechanisms underlying the development and progression of HF.

The heart is the body's most energy-demanding organ relative to its size. Under normal conditions, it primarily relies on fatty acid beta-oxidation, which generates about 60% of the ATP needed for cardiac function. Carbohydrate metabolism via the Krebs cycle provides the second-largest energy source, while other nutrients, such as amino acids, contribute less than 1%.

In HF, however, the heart undergoes a metabolic shift, switching from fatty acids to glucose as its main energy source. This phenomenon, known as the metabolic switch, was initially thought to be a protective adaptation. However, recent evidence from animal and human studies shows that glucose metabolism produces 4-5 times less ATP than fatty acid metabolism, suggesting that this switch is harmful and contributes to the decline in cardiac contractile function.

Our group has demonstrated that the metabolic switch plays a key role in the progression of HF secondary to idiopathic dilated cardiomyopathy (IDCM). In mouse models, a diet rich in fatty acids reversed the metabolic switch and improved the IDCM phenotype. Similar results were observed in pigs, which have a metabolism comparable to humans.

Before conducting a clinical trial in patients with IDCM, it is critical to determine the prevalence of the metabolic switch in this population. This is the primary goal of the MACADAMIA study, an observational project involving a small cohort of IDCM patients. Using advanced imaging techniques—including transthoracic echocardiography with myocardial strain analysis, cardiac magnetic resonance (CMR), and positron emission tomography/computed tomography (PET/CT) with the radiotracer 18FDG—the study aims to characterize the metabolic profile of these patients without any intervention.

As of February 2025, 28 patients have been recruited. In the medium to long term, we plan to conduct a clinical trial in IDCM patients who exhibit the metabolic switch. Patients will be randomized to receive either a diet rich in fatty acids or a normal diet, and will be assessed for changes in cardiac function (by CMR) and metabolism (by PET/CT).

ATTRACKING CNIC: THE ATTRACKING REGISTRY

Principal Investigator: *Pablo García Pavía*

Tafamidis is a medication used to treat transthyretin cardiac amyloidosis (ATTR-CM), a rare and life-threatening disease caused by the buildup of of transthyretin amyloid fibrils in the heart. Clinical trials have demonstrated that Tafamidis reduces mortality and cardiovascular hospitalizations in patients with ATTR-CM, prompting its recent approval for use in Spain. However, clinical trials can have limitations, and their results may not always reflect real-world clinical outcomes.

Advances in diagnostic techniques have enabled earlier detection of ATTR-CM, resulting in a shift in patient profiles and improved prognoses compared with the population studied in the original clinical trial. This raises questions about the effectiveness and safety of Tafamidis in contemporary patients. The ATTRACKING CNIC study aims to address this uncertainty by evaluating Tafamidis in a real-world, unselected cohort of ATTR-CM patients.

Objectives

1. To characterize the real-world population of ATTR-CM patients in Spain prescribed 61mg Tafamidis.
2. To assess the safety of Tafamidis in a diverse, real-world Spanish cohort with ATTR-CM.
3. To compare the real-world clinical impact of Tafamidis therapy with the results of the ATTR-ACT clinical trial.

Study Design

ATTRACKING CNIC is a prospective, multicenter, non-randomized observational trial involving patients diagnosed with ATTR-CM who are starting daily treatment with 61 mg Tafamidis.

As of February 2025, contracts have been signed with 22 hospitals, and 195 participants have been recruited.

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

Principal Investigator: *David Filgueiras*
Co-Promoter: *Hospital Universitario Clínico San Carlos*

Pulmonary vein isolation (PVI) is the cornerstone of catheter-based ablation for patients with persistent atrial fibrillation (AF). However, over the past 20 years, the success rate of PVI in achieving long-term rhythm control for persistent AF has been suboptimal. Even with treatment, only about 40% of patients remain free of AF (without antiarrhythmic drugs) at 12 months post-ablation, highlighting the challenges of managing this condition.

To address this issue, our group has developed a computational tool that helps interventional electrophysiologists identify specific atrial regions, called driver regions, which are associated with the long-term persistence of AF. Using a conventional electroanatomic mapping system and multielectrode catheters, these regions can be pinpointed with novel signal processing algorithms. These algorithms, which are openly available to the scientific community (Quintanilla JG et al. Circ Res. 2019;125:609-27), analyze individual atrial signals to detect frequency and amplitude modulations (iFM and iAM) during fibrillatory activity. This allows for the precise localization of rotational activity and the identification of the leading drivers of persistent AF.

The primary goal of the TAILOR-AF study is to use iAM/iFM mapping to identify and ablate the leading drivers of AF in patients who continue to experience symptomatic recurrences of persistent AF despite undergoing 2 or more PVI procedures. Secondary objectives include analyzing blood biomarkers, atrial imaging parameters, and phenotypic features in the surface electrocardiograms associated with advanced atrial remodeling. In patients undergoing minimally invasive thoracoscopy-guided ablation of leading drivers, tissue samples from the left atrial appendage will also be collected to study the molecular mechanisms underlying AF maintenance.

As of the end of 2024, 22 patients had been enrolled in the study.

SIR-CVT: SPANISH IMMUNOTHERAPY REGISTRY – CARDIOVASCULAR TOXICITY

Co-Promoters: *Sociedad Española de Cardiología / Sociedad Española de Oncología Médica*

SIR-CVT
Spanish Immunotherapy Registry- Cardiovascular Toxicity

SIR-CVT is a non-interventional project with two primary objectives:

1. To evaluate risk factors and current practice management practices for cardiovascular (CV) toxicity in patients with solid organ cancer receiving immune checkpoint inhibitors (ICI) for approved indications.
2. To validate the human homolog of miR-721 as a biomarker for the early diagnosis of immunotherapy-induced myocarditis in these patients.

The study also addresses several secondary objectives:

- Identifying clinical, electrocardiographic, imaging, laboratory, and genetic markers for the early diagnosis of ICI-related myocarditis.

- Identifying markers for the early detection of other ICI-related adverse CV effects unrelated to myocarditis.

- Evaluating the relationship between previous chemotherapy regimens and ICI-related CV risk.

- Investigating the pathogenic mechanisms underlying myocarditis induced by immune checkpoint blockade (antiPD-L1/PD1 and anti-CTLA4).

- Assessing the impact of CV monitoring on patient quality of life and perceived quality of care.

The study involves of a baseline visit, after which patients are managed according to standard clinical protocols, including the frequency of follow-up visits. Participants will be followed until death, loss to follow-up, or the final visit 12 months after enrollment. All data collected will be used exclusively for research purposes.

As of February 2025, two patients have been enrolled and have undergone cardiac magnetic resonance and ultrasound imaging studies at the CNIC.

REACT: REVERSAL OF EARLY ATHEROSCLEROSIS THROUGH PERSONALIZED CURATIVE TREATMENT

Principal Investigator: *Borja Ibáñez*

REACT
Early Cure of Atherosclerosis

Atherosclerosis, the buildup of plaque in arteries, is the leading cause of cardiovascular diseases. This condition develops gradually as cholesterol, fat, blood cells, and other substances accumulate in the arterial walls, causing them to narrow and reducing blood flow to vital organs. Atherosclerosis contributes significantly to global morbidity and mortality, often beginning early in life and affecting multiple vascular systems.

To address this major health challenge, the CNIC and the Rigshospitalet in Copenhagen have launched Phase 1 of the REACT project (Reversal of Early Atherosclerosis through Personalized Curative Treatment), funded by the Novo Nordisk Foundation. The primary goal of this phase is to determine the prevalence of asymptomatic (silent) atherosclerosis in a diverse, European-ancestry population across a wide age range (18–69 years). This will be achieved using advanced biomedical imaging techniques and circulating biomarkers, alongside traditional risk factors.

The REACT project focuses on early detection of atherosclerosis through a combination of imaging techniques, including vascular ultrasound (3DVUS), calcium scoring, and angiography, as well as biomarker analysis. The first phase is a prevalence study involving 16,000 participants (8,000 in Spain and 8,000 in Denmark). This study will provide a detailed understanding of how common asymptomatic atherosclerosis is at different ages and help develop a more precise risk evaluation algorithm than those currently in use.

The findings from Phase 1 will inform the design of Phase 2, a randomized clinical trial that will assess whether early

intervention—guided by imaging and including lifestyle changes, non-pharmacological approaches, and lipid-lowering treatments—can reduce atherosclerotic plaque burden or even lead to regression or cure.

The first participant was recruited on December 30, 2024, and the goal is to enroll all 8,000 participants within 18 months. This initiative will create a unique resource for future basic and clinical research.