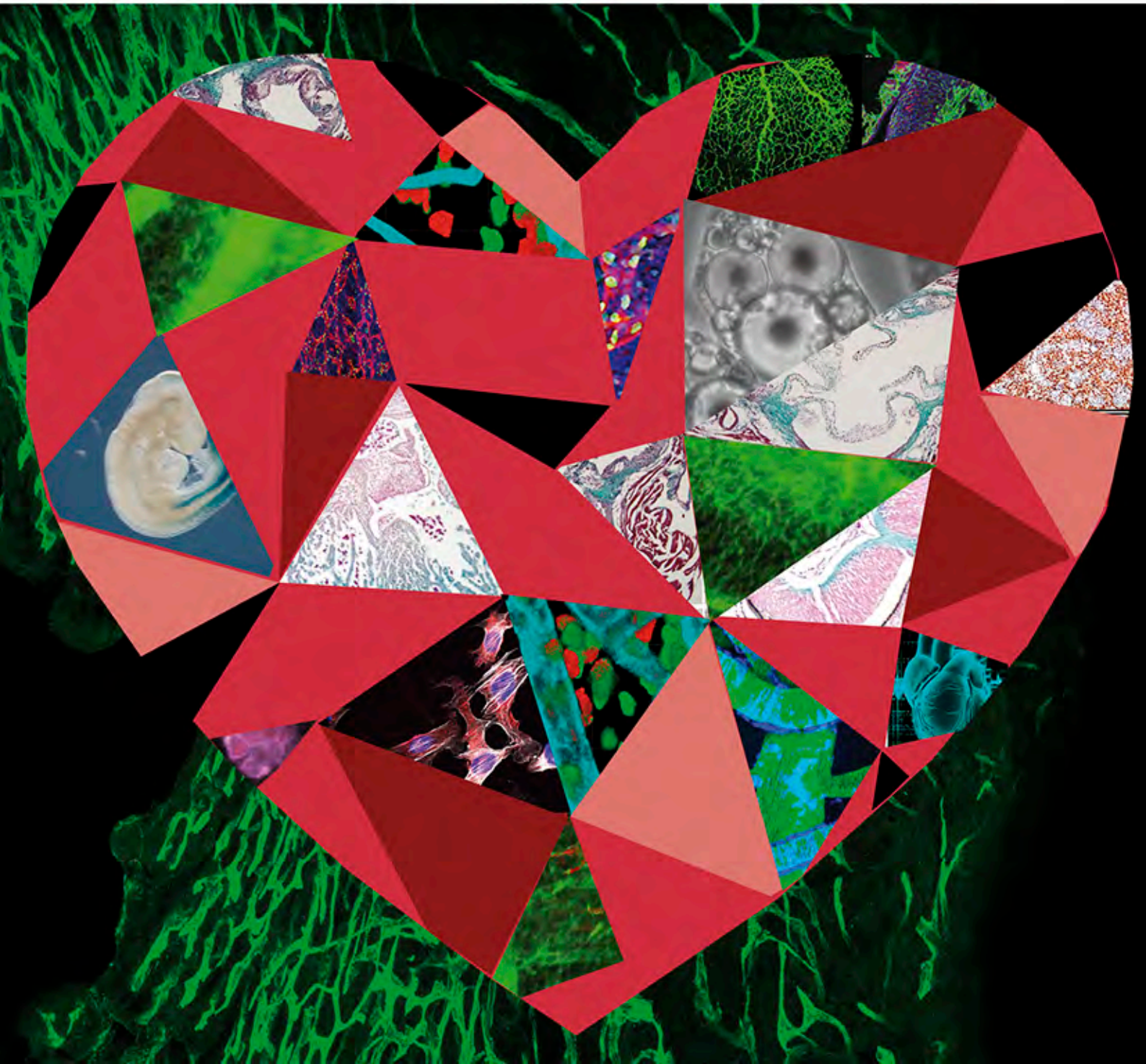
SCIENTIFIC REPORT 2015

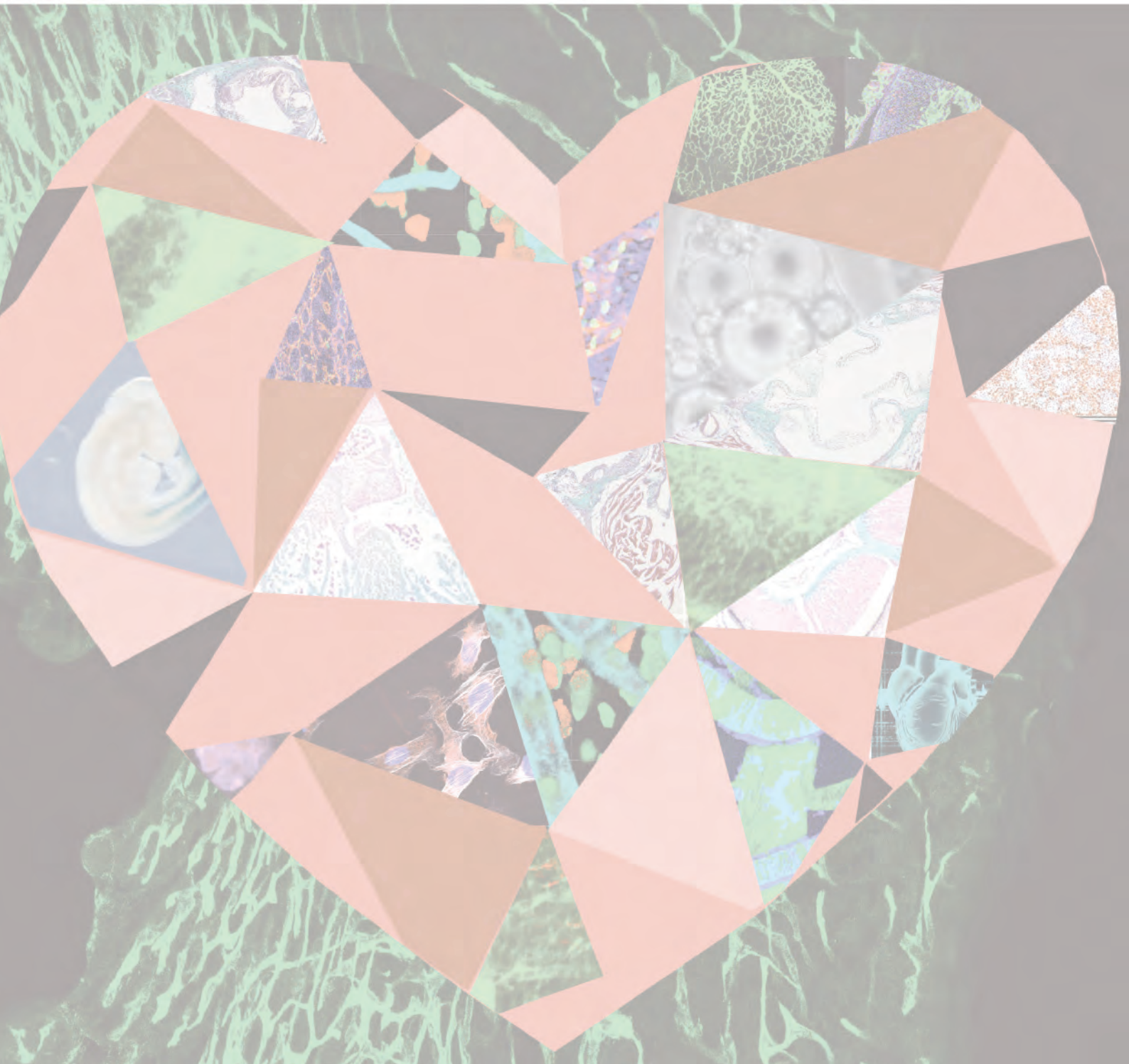
cnic

Fundación
Centro Nacional de
Investigaciones
Cardiovasculares
Carlos III

EXCELENCIA SEVERO OCHOA
2012 - 2019

HR EXCELLENCE IN RESEARCH





Valentín Fuster
General Director

A research center, like any other institution, needs to rejuvenate its internal organization as it matures. One of the CNIC's great strengths is its promotion of open and fluid interactions between clinical and basic researchers. To ensure that this flexibility continues and deepens, at the beginning of 2015 the Center dispensed with the old linear organization into research departments. In its place, CNIC research is now coordinated by two research directors, Borja Ibañez directing Clinical Research and Vicente Andrés Basic Research. Within this framework, research is broadly organized into three Research Areas (Vascular Pathophysiology, Myocardial Pathophysiology, and Cell & Developmental Biology), each bringing groups with a more clinical focus together with others oriented more toward basic research. The goal of this reorganization is to potentiate translational interactions between researchers with complementary skills and knowhow.

The CNIC's position as a leading center in Spain and internationally was reinforced last year with a slew of major awards. In October, the Center's status as a *Severo Ochoa* Center of Excellence was renewed for a further 4 years. Also in October, the CNIC was confirmed as the leading Spanish recipient of research funding from the European Union Horizon 2020 Programme (H2020) under the Societal Challenge "Health, Demographic Change and Wellbeing". The CNIC's success in securing funding for 10 H2020 projects so far builds on its prior record of 37 projects under the Seventh Framework Programme (FP7). The Center's EU funding includes 5 European Research Council projects under FP7 and 5 awarded under H2020. Other important international awards included 2 projects funded by the Progeria Research Foundation, supporting research into the premature aging and cardiovascular complications associated with this devastating condition.

As ever, it was a pleasure last year to welcome new investigators. Jacob Fog Bentzon and Juan Bernal bring expertise in the research and therapeutic applications of transgene and viral gene transfer to atherosclerosis and inherited cardiomyopathies. Héctor Bueno, an expert in acute coronary syndromes, is the first fellow on the new Valentín Fuster Program, and joins the CNIC through an agreement with the *Hospital 12 de Octubre*. We also welcomed visiting scientist Sandeep Pandit, who brings expertise in arrhythmias through an agreement with the University of Michigan and the *Fundación Jesús Serra*.

The Center's pioneering commitment to biomedical imaging was strengthened through a new partnership with leading imaging and biomaterials research center CIC biomaGUNE. And during a visit to the CNIC's installations, Valentín Fuster and British ambassador Simon Manley affirmed the importance of international collaboration in science, laying the ground for possible future partnerships with businesses and research institutes in the United Kingdom.

The CNIC continued its strong scientific production, including high-impact publications in *Science*, *Nature Cell Biology*, *Journal of the American College of Cardiology*, *Journal of Clinical Investigation*, and many others, as detailed in this report; the Center's wide-ranging publications bear testimony to the breadth of its research effort. Scientific production was accompanied by continuing strong performance in knowledge transfer. The CNIC now holds 20 patent families, and several extensions and new applications were filed in 2015. Four of the CNIC's patent families are licensed for commercial development, and last year 4 new agreements were signed with industrial partners for the development of joint projects.

Vicente Andrés
Basic Research Director

Borja Ibáñez
Clinical Research Director

The Fuster-CNIC-Ferrer Polypill, the first polypill approved in Europe for secondary prevention of cardiovascular events, was officially presented on March 3 by Carmen Vela, Spanish Secretary of State for Research, Development and Innovation. And the Polypill also won praise from the European Parliament for the innovative public-private partnership between the CNIC and pharmaceutical company Ferrer. Research into the Fuster-CNIC-Ferrer Polypill continues with the H2020-funded SECURE trial, testing the impact on hard cardiovascular outcomes over 2-4 years of follow-up. The kick-off meeting of the SECURE project was held in Madrid in May.

The CNIC continues to innovate through its other clinical studies. The EARLY BAMI trial builds on the success of the now completed METOCARD-CNIC trial, testing the effect of early intravenous metoprolol on infarct size in a less restricted patient population and preparing the ground for a larger multinational events-powered clinical trial in the future. Work also continues on the PESA study of subclinical atherosclerosis, and the PESA population is being incorporated into the international project TANSNIP (Transatlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis & Prevention), which pools American and European expertise and data to improve risk stratification and prevention.

The highlight of our public educational program last year was the international launch of the free mobile application *Circle of Health*. Through this application, developed by Dr. Fuster and the Pro-CNIC Foundation, users learn about the 6 variable risk factors, how to prevent or manage them, and how to live a healthier and longer life. The international version of the app was released on December 17.

Among the CNIC scientists who received prizes and honors last year, María Mittelbrunn was 1 of 5 winners of the 2015 *Bolsa de Investigación* award, part of the L'Oréal–UNESCO program for Women in Science. One of us (VF) was honored with the *Severo Ochoa* prize for Biomedical Research from *Fundación Ferrer* and also with *La Gran Cruz de la Orden Civil de Sanidad*. And everyone at the CNIC can feel very proud of the prize from the *Cátedra de Educación Médica Fundación Lilly-Universidad Complutense de Madrid*, awarded in recognition of our flagship postgraduate training program.

Across the spectrum of its activities, the CNIC works to maintain a transverse management culture that fosters fruitful translational interactions among clinicians and basic researchers, delivers excellence in training, and leads to real advances in the knowledge, prevention and treatment of cardiovascular diseases.

C O N T E N T S

RESEARCH AREAS

Translational Coordination

1. Myocardial Pathophysiology

2. Vascular Pathophysiology

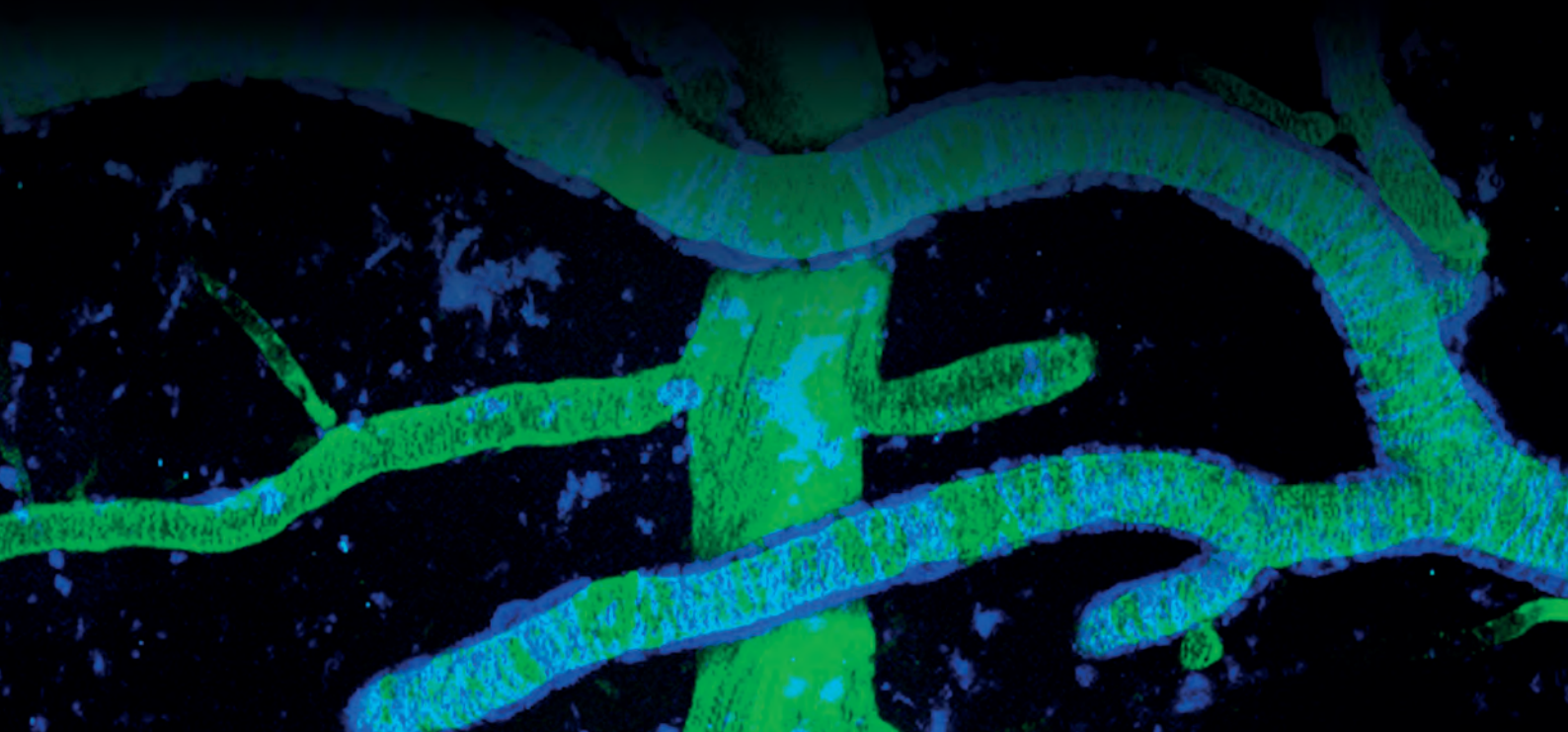
3. Cell and Developmental Biology

CLINICAL STUDIES

TECHNICAL UNITS

APPENDIX

Translational Coordination



Cardiovascular imaging and population studies

Head of Laboratory

Valentín Fuster (CNIC, Mt. Sinai Medical Center, New York)

Research Scientists:

Antonio Ignacio Fernández Ortiz
(CNIC, Hospital Clínico San Carlos Research Agreement)
José Mª Ordovás Muñoz
(CNIC, Tufts University, Boston Research Agreement / IMDEA-Food, Madrid)
José María Castellano Vázquez
(CNIC, Hospital Universitario HM Montepríncipe)
Héctor Bueno Zamora
(CNIC, Hospital 12 de Octubre Research Agreement)
Martín Laclaustra Gimeno
Luis Jesús Jiménez Borreguero
(CNIC, Hospital La Princesa Research Agreement)
Javier Sánchez González
(CNIC, Philips Healthcare)
Silvia Martín Puig
(Independent Research line)

Cardiologists:

Ana García Álvarez
(CNIC, Hospital Clinic de Barcelona)
Leticia A. Fernández Friera
(CNIC, Hospital Universitario HM Montepríncipe)
Javier Higueras Nafría
(CNIC, Hospital Clínico San Carlos)
Beatriz López Melgar
(CNIC, Hospital Universitario HM Montepríncipe)
Inés García Lunar
(CNIC, Hospital Quirón de Madrid)
Sandra Gómez Talavera
(CNIC, Hospital Universitario de Fuenlabrada)

Project Managers:

Ester Cunha Pavón
Laura García Leal
Evelyn Cárdenas Marín
Jacinto Marcelo Cabanillas Martín
Luz Álvarez Vilela

Administrative Assistant:

Marta García Mateos

Study Nurses:

Maite Dubraska Rodríguez Cabrera
Miriam Fernández Gallardo

Postdoctoral Researchers:

Marta Cortés Canteli
Juan Miguel Fernández Alvira

Predoctoral Researchers:

Irina Uzhova
Leda Yamilee Hurtado Roca
(CNIC, Boca Ratón Clinical Research Global, Perú)
Iván Menéndez Montes
(SMP Research Line)

Res@CNIC Fellows:

Cristina Asla Ormaza
Paula Mendoza Cuartero
Álvaro Marco del Castillo

Study Psicologists:

Silvia Santiago Sacristán
Carolina Rojas Murcia
María Isabel Martínez Castro

Biostatistician:

Belén Oliva Pellicer

Technicians:

Ángel Macías Hernán
Braulio Pérez Asenjo
Natalia Serrano Juzgado
Virginia Mass Ruiz
Clara Teresa Hernández Sánchez
Sergio Cárdenas Melero
Sara García Ortega
Ricardo Ponce Sánchez
Lorena Flores Ruiz
Ana Vanesa Alonso López
Aurora Del Barrio Mantecas
María José Diego Rubio
Tamara Guillén Casla
Rosa Villa Pobo
Rosario Pérez Rubiño
Alberto Ávila Morales
Laura Cepeda Denche
Beatriz Escobar Rodríguez
(SMP Research Line)
Beatriz Palacios Argandoña
(SMP Research Line)

Master Students:

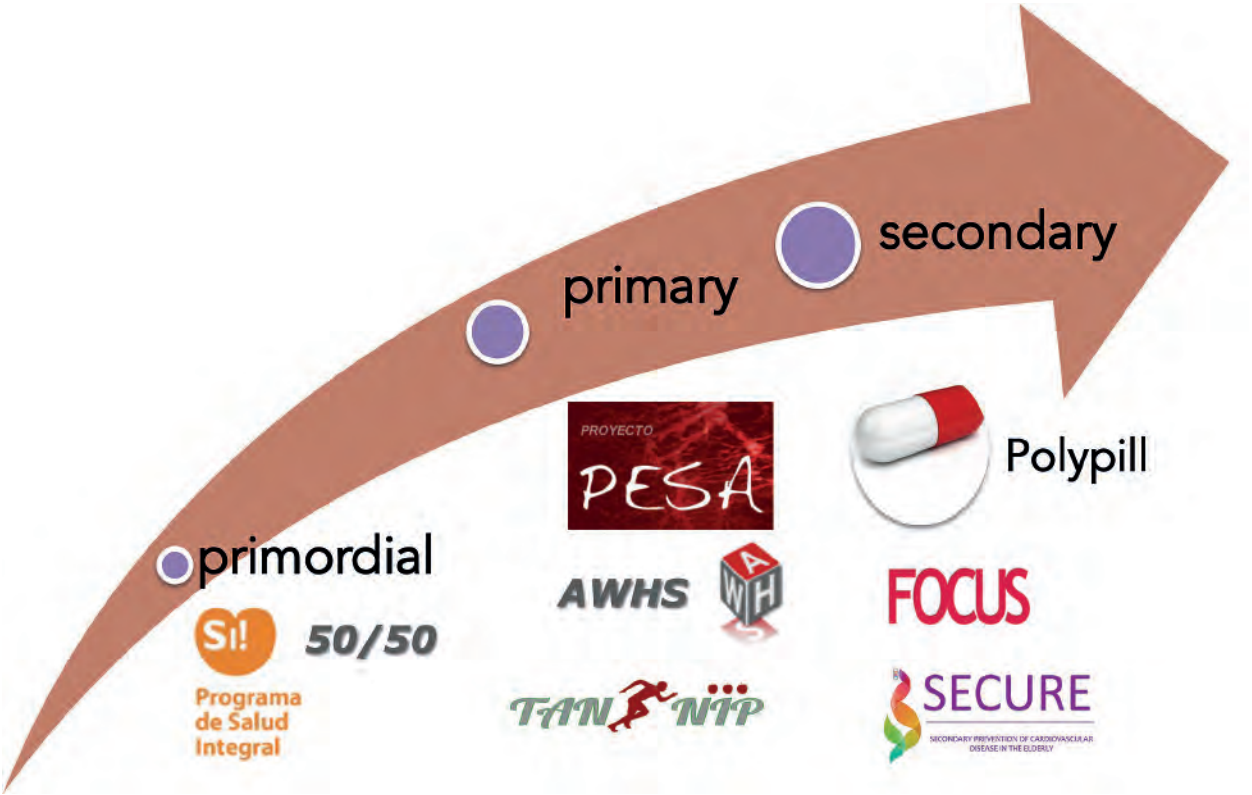
Nerissa Álvarez Chicote
Rocío Peiró Gómez
Verónica García López
(SMP Research Line)

Visiting Scientists:

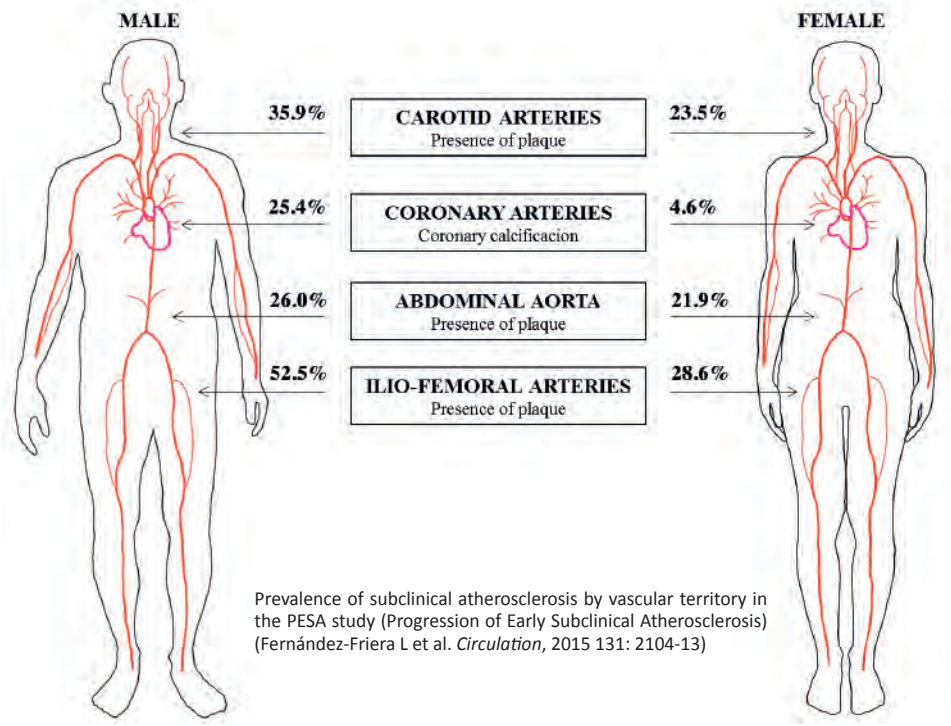
Stuart John Pocock
(CNIC, London School of Hygiene and Tropical Medicine, London)
Jennifer Kim Coffeng
(VUmc Amsterdam, Holland)
Gabriela Guzmán Martínez
(Hospital Universitario La Paz, Madrid)
Jose Luis Peñalvo García
(CNIC, Tufts University, Boston)
Manuel Franco Tejero
(University of Alcalá, Madrid)
Julia Díez Escudero
(University of Alcalá, Madrid)
Sameer Bansilal
(Mount Sinai School of Medicine, New York)
Nils Nothnagel
(CNIC, Philips Healthcare)
Paula Montesinos Suárez de la Vega
(CNIC, Philips Healthcare)
Daniel Tello Pernas
(Hospital Universitario Santa Cristina, Madrid) (SMP Research Line)
Oscar Yang Li
(Hospital Universitario Santa Cristina, Madrid) (SMP Research Line)

RESEARCH INTEREST

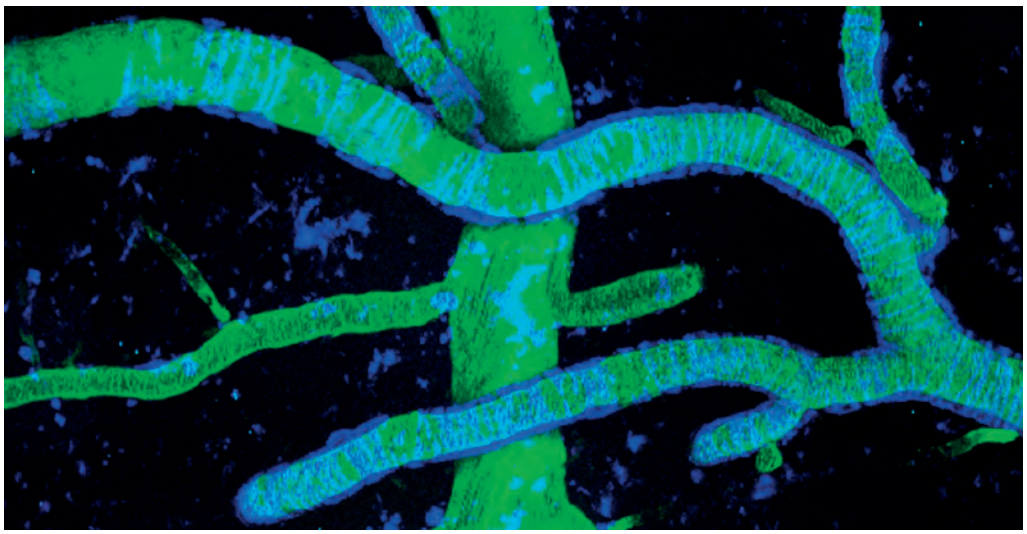
We are a highly multidisciplinary research group dedicated to conducting clinical studies for cardiovascular prevention using the latest advances in advanced imaging. Our research covers major CVD risk factors including diet, exercise, genetics and epigenetics, metabolic factors, the environment, and psychosocial factors. This work goes hand in hand with the development and research application of noninvasive, advanced imaging technologies for the early diagnostic and prognostic assessment of atherosclerosis. The group participates in the CNIC´s major population studies PESA (Progression of Early Subclinical Atherosclerosis), AWHS (Aragon Workers Health Study), TANSNIP (Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis and Prevention), SECURE (Secondary Prevention of Cardiovascular Disease in the Elderly Population, an EU Horizon2020-funded continuation of research into the successful Fuster-CNIC-Ferrer polypill concept), and SPHERE (testing the efficacy of a novel therapy discovered at the CNIC for the treatment of pulmonary hypertension). We are also involved in educational programs to promote healthy habits for cardiovascular prevention in children (Program SI!), and adults (50/50 Project, in collaboration with the *Observatorio de la Nutrición y de Estudio de la Obesidad*). Our most recent research line focuses on analyzing the vascular component present in Alzheimer disease (AD). The evidence indicates the presence of increased thrombosis, decreased fibrinolysis and a higher number of obstructed blood vessels in the AD brain. We use in vivo noninvasive imaging (PET, MRI) on small and large animal models to identify these phenomena in the AD brain. Chronic heart failure is recognized as a cause of cerebral hypoperfusion and dementia, establishing the fundamental importance of heart-brain integration in health and disease.



Research and educational programs for cardiovascular prevention from infancy to adulthood



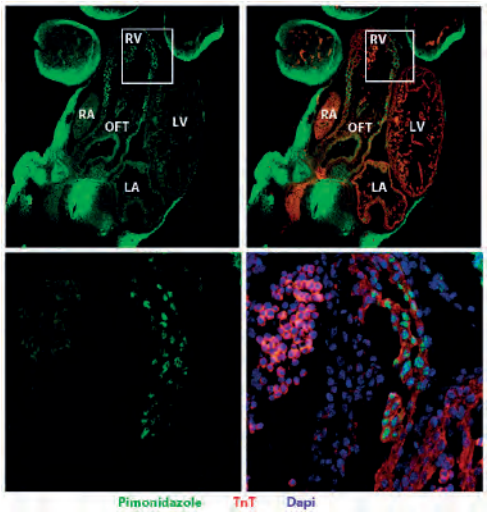
Prevalence of subclinical atherosclerosis by vascular territory in the PESA study (Progression of Early Subclinical Atherosclerosis) (Fernández-Friera L et al. *Circulation*, 2015 131: 2104-13)



Brain vasculature in Alzheimer disease (AD). Cranial windows were opened over the cortex of AD mice. Blood flow (green) and amyloid (blue) were visualized in vivo with a two-photon microscope. Blood vessels in the brain of AD mice are surrounded by cerebral amyloid angiopathy and by amyloid plaques in the brain parenchyma (Modified from Cortes-Canteli M et al. *Neuron* 2010 66: 695-709)

Another research line within the group is led by Dr Silvia Martín Puig. Hypoxia and HIF factors are proposed to play an important role in heart development and cardiovascular diseases, but the underlying molecular mechanisms and the cell populations involved in these processes remain largely unkown. Our main goal is to understand the role of the VHL-HIF pathway in cardiovascular homeostasis, with particular interest in heart development and homeostasis. We have generated several gain and loss of function mouse models of the hypoxia pathway, paying special attention to early cardiovascular populations that contribute to the various cell types of the mature heart. Using these genetic tools, we are interrogating whether changes in oxygen tension influence mammalian cardiogenesis. Our data indicate that hypoxia, through the VHL-HIF axis, is essential for the correct development of the ventricular chambers, myocardium maturation and the homeostasis of the coronary vasculature. We are currently unraveling the molecular mechanisms and functional adaptations underlying the phenotypes found in the different models generated, and we are analyzing the transcriptional responses mediated by HIF factors. We believe that our research has potential therapeutic applications and will help to elucidate the role of low oxygen tension and VHL-HIF signaling in congenital heart disease, opening up new perspectives in the arena of translational medicine.

Translational Coordination



Hypoxic regions at mid cardiogenesis. Representative E10.5 mouse heart sections showing low oxygen areas stained with the hypoxia probe pimonidazole (green) within the developing myocardium stained by immunofluorescence for troponin T (TnT, red). The pimonidazole signal is strong in the right ventricle (RV) and at the base of the outflow tract (OFT), with intermediate signal strength in the interventricular septum close to the left ventricle (LV). Both atria are negative for pimonidazole (LA, RA). Bottom panels show magnifications of the insets in the top panels.

MAJOR GRANTS

- *H2020-PHC-2014-two-stage (GA633765). PI: V Fuster*
- *NHLBI-BAA-10-08 Co-Project Director of Project #3: V Fuster*
- *NHLBI - 5U01HL114200-02 PI: V Fuster*
- *PESA CNIC-Santander. PI: V Fuster*
- *Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS). PI: V Fuster*
- *FP7-PEOPLE-2013-IIF (GA 624811). PI: M Cortés*
- *EU FP7. Marie Curie European Reintegration Grant (276891). PI: S Martín Puig*
- *Ministerio de Ciencia e Innovación. FIS (CP09/00100). PI: S Martín Puig*
- *Comunidad de Madrid: (S2010/BMD-2542). PI: S Martín Puig*
- *COST – European Cooperation in Science and Technology. PI: S Martín Puig*
- *Instituto de Salud Carlos III (PI13/02339). PI: A García*
- *Instituto de Salud Carlos III (PI15/02019). PI: L Fernández-Friera*
- *Instituto de Salud Carlos III (CP08/112). PI: M Laclaustra*
- *Instituto de Salud Carlos III (FIS PI14/00009). PI: M Laclaustra*
- *Instituto de Salud Carlos III (PI11/00403). PI: JL Peñalvo*

SELECTED PUBLICATIONS

Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A, Ibanez B, Lopez-Melgar B, Laclaustra M, Oliva B, Mocoroa A, Mendiguren J, Martinez de Vega V, Garcia L, Molina J, Sanchez-Gonzalez J, Guzman G, Alonso-Farto JC, Guallar E, Civeira F, Sillesen H, Pocock S, Ordovas JM, Sanz G, Jimenez-Borreguero LJ, Fuster V. Prevalence, vascular distribution and multi-territorial extent of subclinical atherosclerosis in a middle-aged cohort: The PESA (Progression of Early Subclinical Atherosclerosis) study. *Circulation* (2015) 131: 2104-13

Penalvo JL, Santos-Beneit G, Sotos-Prieto M, Bodega P, Oliva B, Orrit X, Rodriguez C, Fernandez-Alvira JM, Redondo J, Vedanthan R, Bansilal S, Gomez E, Fuster V. **The SI! Program for Cardiovascular Health Promotion in Early Childhood: A Cluster-Randomized Trial.** *J Am Coll Cardiol* (2015) 66: 1525-34

Vedanthan R, Seligman B, Fuster V. **Global perspective on acute coronary syndrome: a burden on the young and poor.** *Circ Res* (2014) 114: 1959-75

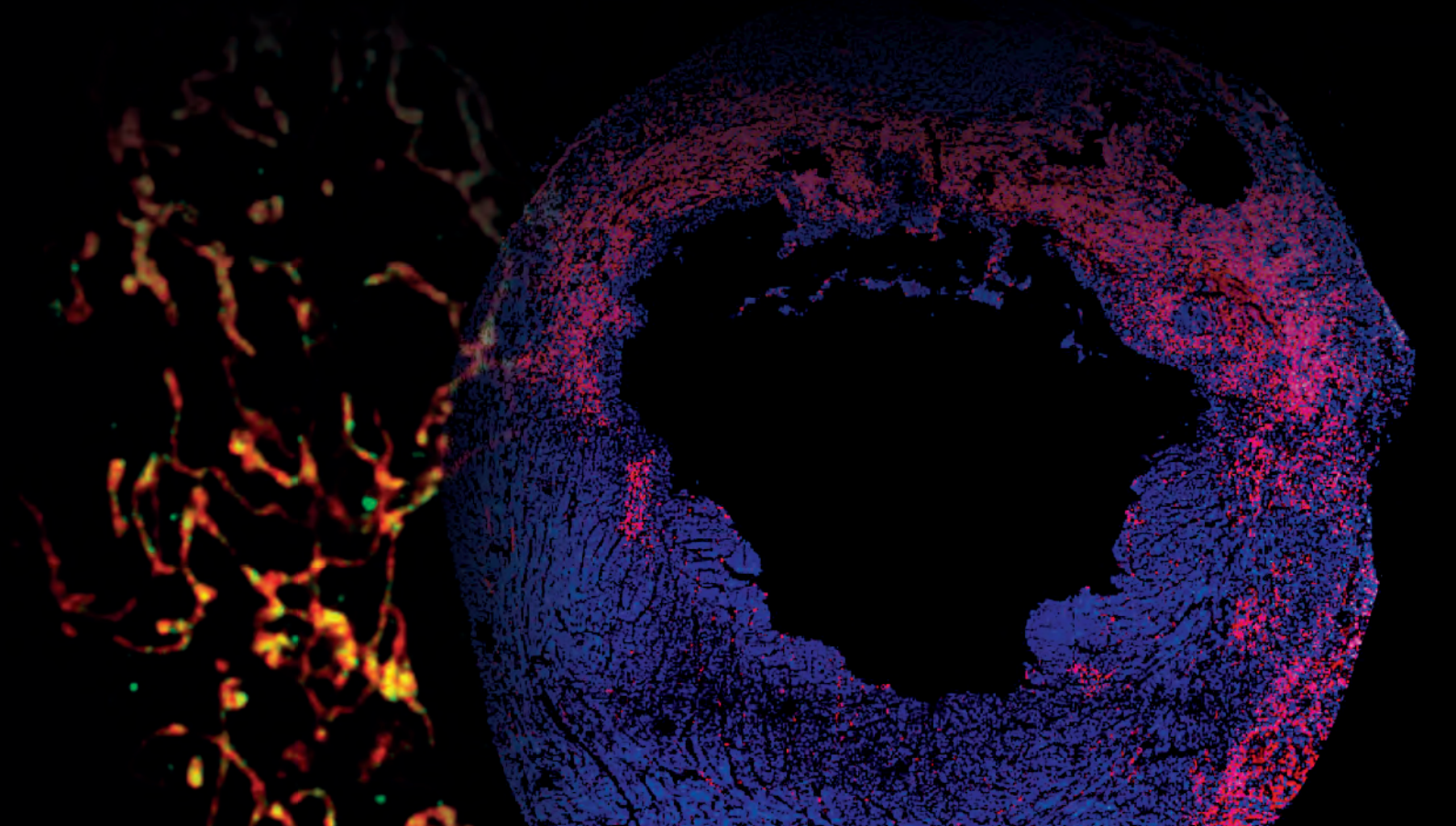
Castellano JM, Sanz G, Peñalvo JL, Fernández-Ortiz A, Alvarez L, Guzmán L, Linares JC, García F, D'Aniello F, Arnáiz JA, Varea S, Martínez F, Lorenzatti A, Imaz I, Sánchez-Gómez LM, Roncaglioni MC, Baviera M, Smith S, Taubert K, Pocock S, Brotons C, Fuster V. **A polypill strategy to improve adherence: results from FOCUS (Fixed-dose Combination Drug for Secondary Cardiovascular Prevention) Project**. *J Am Coll Cardiology* (2014) 64: 2071–82

Castellano JM, Sanz G, Fernandez Ortiz A, Garrido E, Bansilal S, Fuster V. **A Polypill Strategy to Improve Global Secondary Cardiovascular Prevention: From Concept to Reality.** *J Am Coll Cardiol* (2014) 64: 613-621

Martin-Puig S, Tello D and Aragones J. **Novel perspectives on the PHD-HIF oxygen sensing pathway in cardioprotection mediated by IPC and RIPC.** *Front Physiol* (2015) 6: 137. doi: 10.3389/fphys.2015.00137

RESEARCH AREAS

1. Myocardial Pathophysiology
2. Vascular Pathophysiology
3. Cell and Developmental Biology



1 RESEARCH AREAS

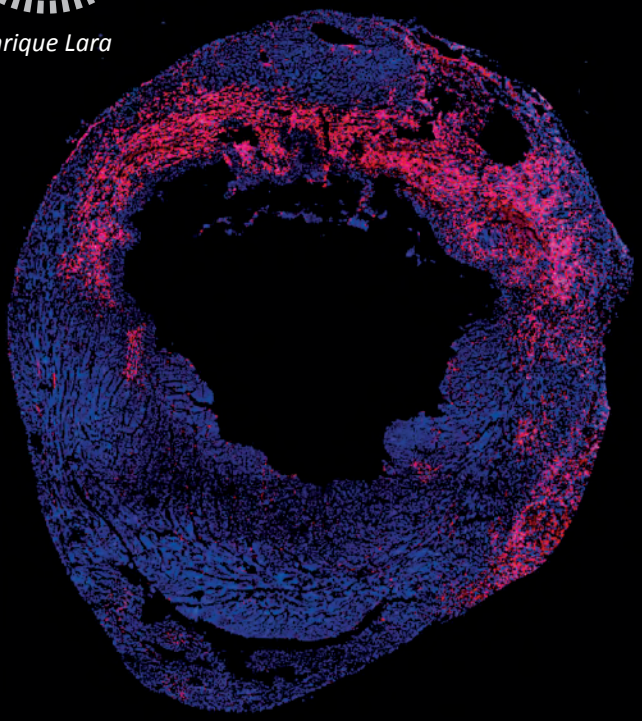
Myocardial Pathophysiology

Area coordinators:

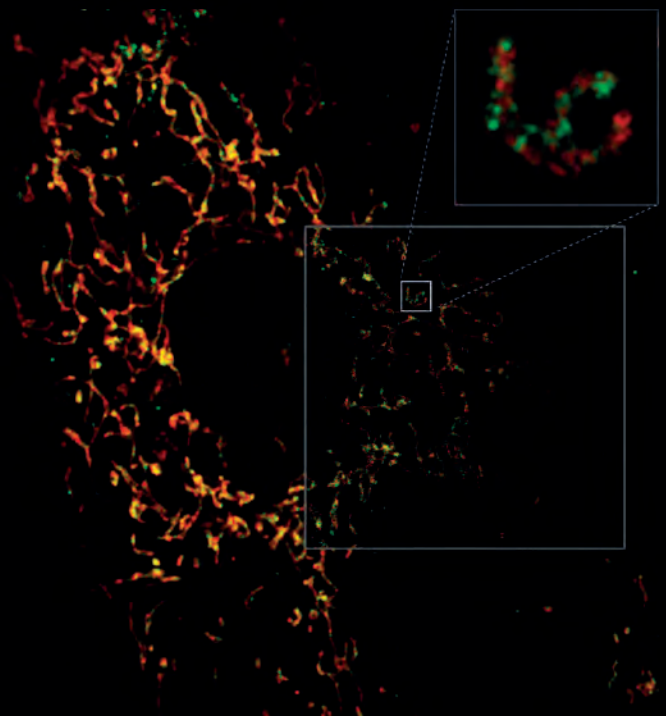
José Antonio Enríquez *Enrique Lara*

RESEARCH INTEREST

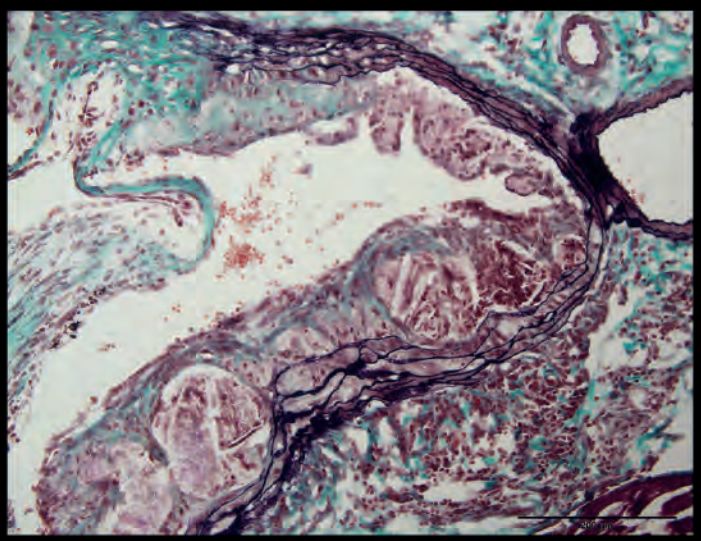
The Myocardial Pathophysiology Area brings together scientists from a broad range of disciplines. Basic scientists, cardiologists and engineers work together to generate invaluable knowledge about the molecular mechanisms regulating the cardiovascular system in homeostasis and disease. This strategy enables the development of noninvasive technologies through the identification of imaging, genetic, molecular and metabolic markers for understanding health and disease, and has the potential to lead to improvements in both diagnosis and treatment. Our experimental strategy comprises in vitro, animal and human studies, and the range of topics includes the oxidative phosphorylation system, the role of nuclear receptors in lipid metabolism and inflammatory responses, metabolic syndrome and stress kinases, immunobiology of inflammation, inherited cardiomyopathies, cardiac arrhythmias, epigenetic regulation, alternative splicing in cardiac development and heart disease, and cardioprotection during myocardial infarction.



Cross-section of a mouse heart 7 days after infarction, stained for nuclei (blue) and CD68 macrophages (red).



Colocalization of endogenous respiratory complex II subunit (anti-Fp70; red) and complex III subunit (anti-Rieske; green) in mouse fibroblasts. The large image shows a single confocal Z-stack, and the gray boxed area shows a single STED microscopy Z-stack. The white-boxed area in the STED image is shown at high magnification in the inset.



Masson's trichrome staining of an aortic root section from C57BL/6J mouse transduced with an adeno-associated virus (AAV) encoding D374Y-mutated proprotein convertase subtilin/kexin type 9 (AAV-C57-PCSK9^{DY}) and fed a high-fat diet.

Inherited cardiomyopathies

Head of Laboratory
Juan A. Bernal

Predoctoral Researchers:
Francisco M. Cruz
Marta Roche-Molina
Cristina del Carmen Roselló

Masters Student:
Silvia Sacristán

Technician:
Andrés González Guerra

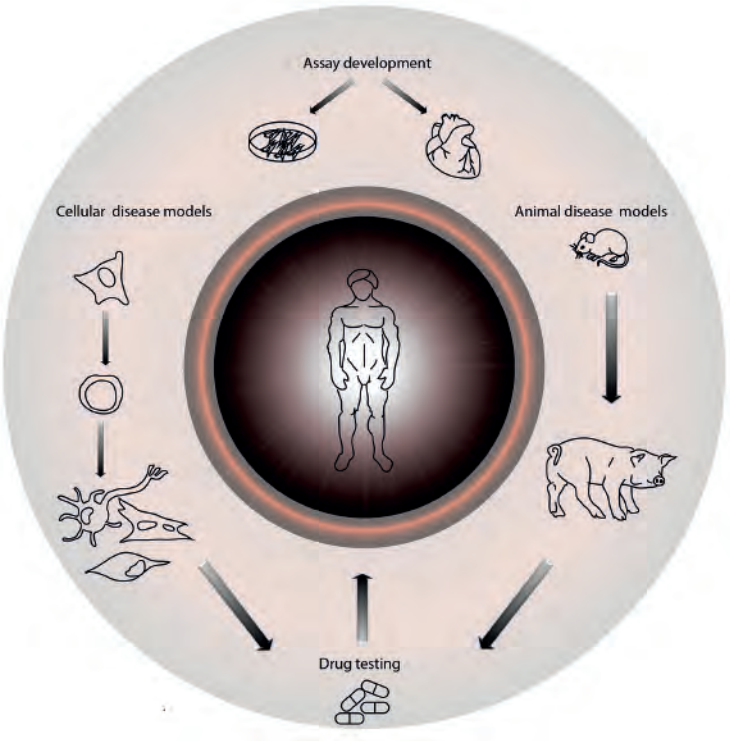
RESEARCH INTEREST

Our research into cardiovascular disease is based on a simple principle: create to understand, create to treat.

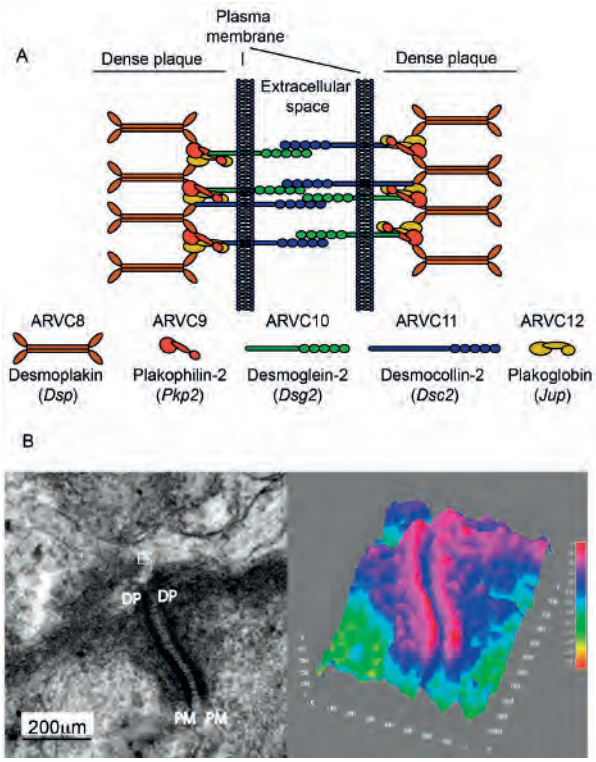
Animal models are essential investigative tools for expanding our understanding of disease; however, the generation and maintenance of genetically modified mouse colonies for research is costly. We have developed an alternative method that uses adeno-associated virus (AAV) vectors, widely used for gene-therapy approaches, to express disease-causing dominant-negative mutants to generate disease models in wild-type mice. Single systemic injection of AAV virus is more versatile, cost-effective, simpler, and time-efficient than transgenic approaches for generating mutant animals.

Our major area of interest is arrhythmogenic right ventricular cardiomyopathy (ARVC). This heart muscle disease is characterized by right ventricular anatomical abnormalities and ventricular arrhythmias that can lead to sudden cardiac death, especially in young athletes. To be able to study the effect of exercise on hearts of mice carrying the most prevalent ARVC-associated mutation in *plakophilin-2 (PKP2)*, we used AAV to express the *R735X* mutant in wild-type mice. Our work shows that injected AAV-*R735X* animals develop an overt ARVC phenotype when subjected to endurance training, supporting the recommendation for exercise cessation in carriers of this mutation.

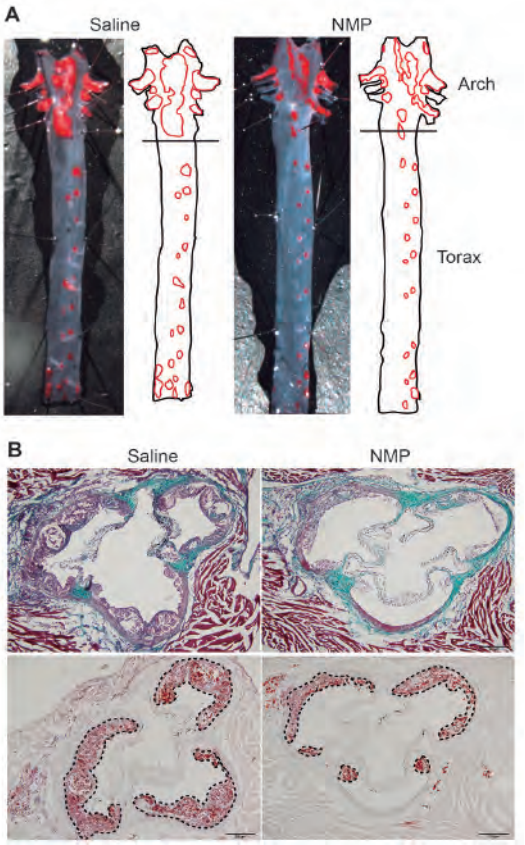
We have applied the same principle to a subtype of familiar hypercholesterolemia induced by a *PCSK9* mutant. We have shown that AAV-*PCSK9*^{DY}-transformed mice develop the disease and could be used as a platform for testing specific PCSK9-targeted therapies. These findings demonstrate that AAV-transfer methodology has the potential to make valuable contributions to the specific understanding of cardiovascular diseases.



General working-model used in the laboratory to understand and test compounds in a specific disease. For example, for ARVC pathology we have already developed a cellular model in human induced pluripotent stem cells (iPS) and a mouse model. In the near future we plan to develop a pig model of ARVC, to take advantage of the pigs's closer similarity to human physiology.



ARVC is considered a desmosomal disease. (A) Desmosomal structure and protein components in which mutation has been linked to ARVC. (B) Representative transmission electron microscopy images showing inter-cardiomyocyte desmosome organization. PM, plasma membrane; DP, dense plaque; ES, extracellular space. Heat-map color code conversions of these images are also shown.



Staining of atheroma plaques in the aortas of mice fed a high-cholesterol diet. The images show en face Oil red O staining in whole aorta (A) and Masson's trichrome and Oil red O staining in transverse sections (B). We have demonstrated the ability of FDA approved compounds, including NMP (shown in the figure), to reduce inflammation and atherosclerosis development in hypercholesterolemic animals.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (BFU2012-35258)*
- *Ministerio de Economía y Competitividad (RYC-2009-04341)*

SELECTED PUBLICATIONS

González-Terán B, López JA, Rodríguez E, Leiva L, Martínez-Martínez S, Bernal JA, Jiménez-Borreguero LJ, Redondo JM, Vázquez J, and Sabio G. **p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation**. *Nat Commun* (accepted)

Cruz FM, Tomé M, Bernal JA*, Bernad A. **miR-300 mediates Bmi1 function and regulates differentiation in primitive cardiac progenitors.** *Cell Death Dis* (2015) 6, e1953
* Co-Corresponding Author

Cruz FM, Sanz-Rosa D, Roche-Molina M, García-Prieto J, García-Ruiz JM, Pizarro G, Jiménez-Borreguero LJ, Torres M, Bernad A, Ruíz-Cabello J, Fuster V, Ibáñez B, Bernal JA.**Exercise triggers ARVC phenotype in mice expressing a disease-causing mutated version of human plakophilin-2.** *J Am Coll Cardiol* (2015) 65: 1438-50

Nakagawa Y, Sedukhina AS, Okamoto N, Nagasawa S, Suzuki N, Ohta T, Hattori H, Roche-Molina M, Narváez AJ, Jeyasekharan AD, Bernal JA, Sato K. **NF-κB signaling mediates acquired resistance after PARP inhibition.** *Oncotarget* (2015) 6: 3825-39

Roche-Molina M, Sanz-Rosa D, Cruz FM, García-Prieto J, López S, Abia R, Muriana FJ, Fuster V, Ibáñez B, Bernal JA. **Induction of sustained hypercholesterolemia by single adeno-associated virus-mediated gene transfer of mutant hPCSK9.** *Arterioscler Thromb Vasc Biol* (2015) 35: 50-9

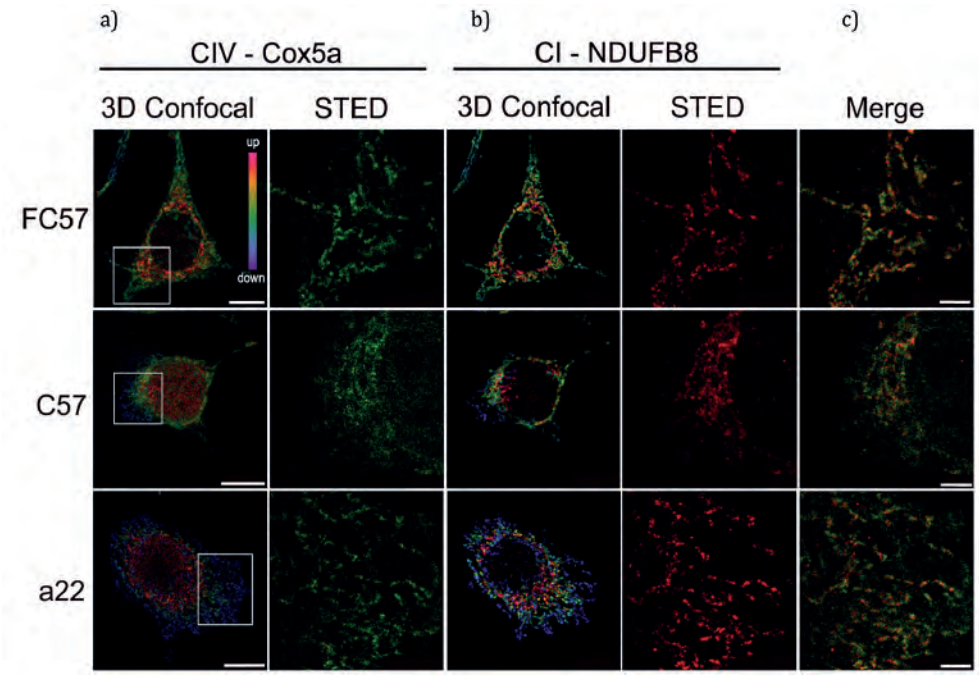
Functional genetics of the oxidative phosphorylation system

Head of Laboratory
José Antonio Enríquez

RESEARCH INTEREST

Our aim is to better understand the role of mammalian oxidative phosphorylation (OXPHOS) in the homeostasic response in health and disease from a variety of perspectives. We cover the whole spectrum of regulation from the molecular structure of the respiratory complexes and supercomplexes to the adaptation to metabolic and cardiac challenging. We are studying the organization of the respiratory complexes by stimulated emission depletion (STED) superresolution microscopy (Fig.1) to identify interaction partners. This goal is complemented with mitochondrial high-throughput proteomics aimed at defining the protein interactome and also identifying posttranslational modifications in healthy, heart-stressed and metabolically altered animals. High-throughput omics are also implemented through transcriptomic, metabolomic and sh-library approaches in order to identify new targets responsible for mitochondrial function and maintenance.

One of the main objectives of the group is to reveal the role of mitochondria in metabolism, cardiac insult, and drug responses. We work with models in which mitochondrial function is mildly or severely affected, and use mice with the same nuclear background but carrying different non-pathological variants of mitochondrial DNA in the same cell (heretoplasmic) or in the whole organism (conplastic) to study the response to metabolic challenges, aging (Fig.2), angiogenesis, and cardiac performance. We also extend those studies to mice in which mitochondrial function has been genetically modified by altering its respiratory subunits, chaperones, mitochondrial ultrastructure or signaling. A new line of research involves the study of the less known structural genes of the mitochondrial ATPase in embryo development, differentiation and function (Fig. 3).



Qualitative colocalization of (a) CIV (Cox5a) and (b) CI (NDUFB8) with FC57, C57, and a22. Columns in the left columns show 3D maximum projections of confocal microscopy images of immunolabeled cultured cell lines. Images in the right columns show high magnification analysis by STED microscopy. c) Overlays of dual-color STED microscopy images.

Research Scientist:
Rebeca Acín Pérez

Support Scientist:
María Concepción Jiménez Gómez

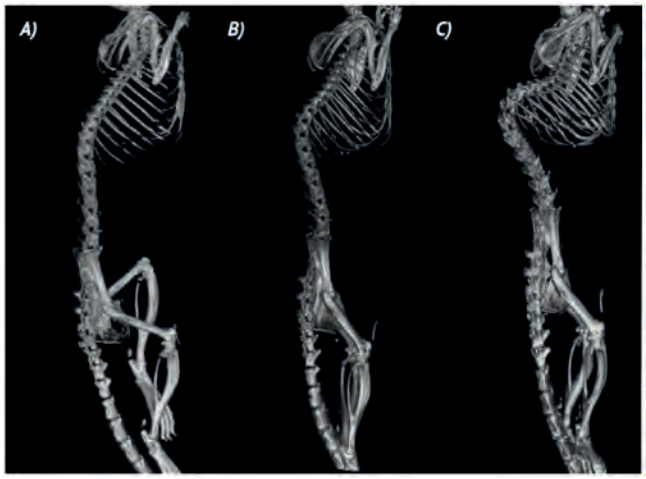
Postdoctoral Researchers:
Umut Cagin
Sergio Caja Galán
Sara Cogliati
Carmen Colás Estéban
Tanja Celic

Predoctoral Researchers:
Adela María Guarás Rubio
Ana Victoria Lechuga Vieco
Elena Martín García
Rocío Nieto Arellano
Carolina García Poyatos

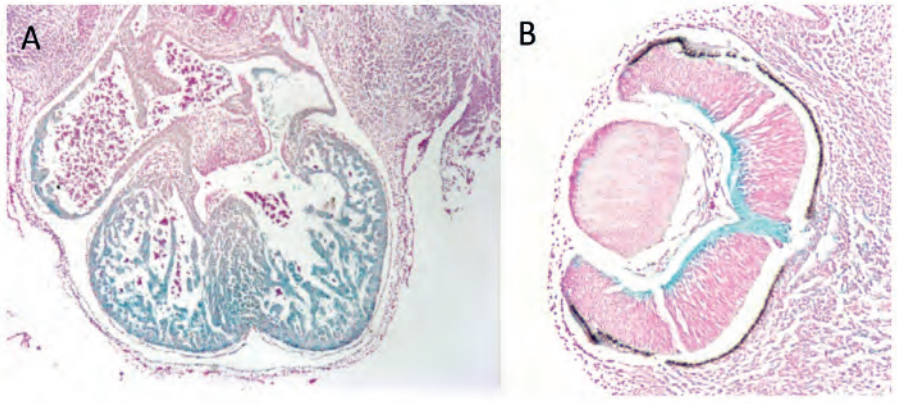
Masters Student:
Alvaro Serrano

Technicians:
Isabel Martínez Carrascoso
María del Mar Muñóz Hernández
Clara López

Visiting Scientists:
Mª Eugenia Soriano
Nerea Ramos
Irene López
Diana Moroni *(Cicerone 2015)*
Marina Mojena
Eligio F. Iannetti



CT analysis of kyphosis in heteroplasmic mice. Damaged skeletal muscle, cardiac muscle and kyphosis are signatures of premature aging in mice with two physiological mtDNA haplotypes. **A)** Skeleton of a control BL/6C57 mouse. **B)** Conplastic BL/6NZB mouse. **C)** Heteroplasmic BL/6C57-NZB mouse.



β-Gal staining of an embryo section. The endogenous expression of the LacZ gene led us to characterize the expression pattern of ATP synthase related gene in the heart (A) and eye ganglion neurons (B).

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2015-71521-REDC)*
- *Ministerio de Economía y Competitividad (BFU2013-50448)*
- *Ministerio de Economía y Competitividad (SAF2012-32776)*
- *Marie Curie Initial Training Networks (ITN). Mitochondrial European Educational Training (GA Nº 317433).*
- *Comunidad de Madrid. Programa de Biomedicina (S2011/BMD-2402).*
- *Ministerio de Economía y Competitividad (RyC 2011-07826). PI: Rebeca Acín*
- *European Commission. Marie Curie Career Integration Grant. PI: Rebeca Acín*

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Enríquez JA. **Supramolecular organization of respiratory complexes**. *Annu Rev Physiol* (doi: 10.1146/annurev-physiol-021115-105031. Epub 2015 Dec 21).

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Cagin U, Enríquez J.A. **The complex crosstalk between mitochondria and the nucleus: What goes in between?** *Int J Biochem Cell Biol* (2015) 63: 10-5

Acín-Pérez R, Carrascoso I, Baixauli F, Roche-Molina M, Latorre-Pellicer A, Fernández-Silva P, Mittelbrunn M, Sanchez-Madrid F, Pérez-Martos A, Lowell CA, Manfredi G, Enríquez JA. **ROS-triggered phosphorylation of complex II by Fgr kinase regulates cellular adaptation to fuel use**. *Cell Metab* (2014) 19: 1020-33

Quirós PM, Español Y, Acín-Pérez R, Rodríguez F, Bárcena C, Watanabe K, Calvo E, Loureiro M, Fernández-García MS, Fueyo A, Vázquez J, Enríquez JA, López-Otín C. **ATP-dependent Lon protease controls tumor bioenergetics by reprogramming mitochondrial activity**. Cell Rep (2014) 8: 542-56

Advanced development in arrhythmia mechanisms and therapy

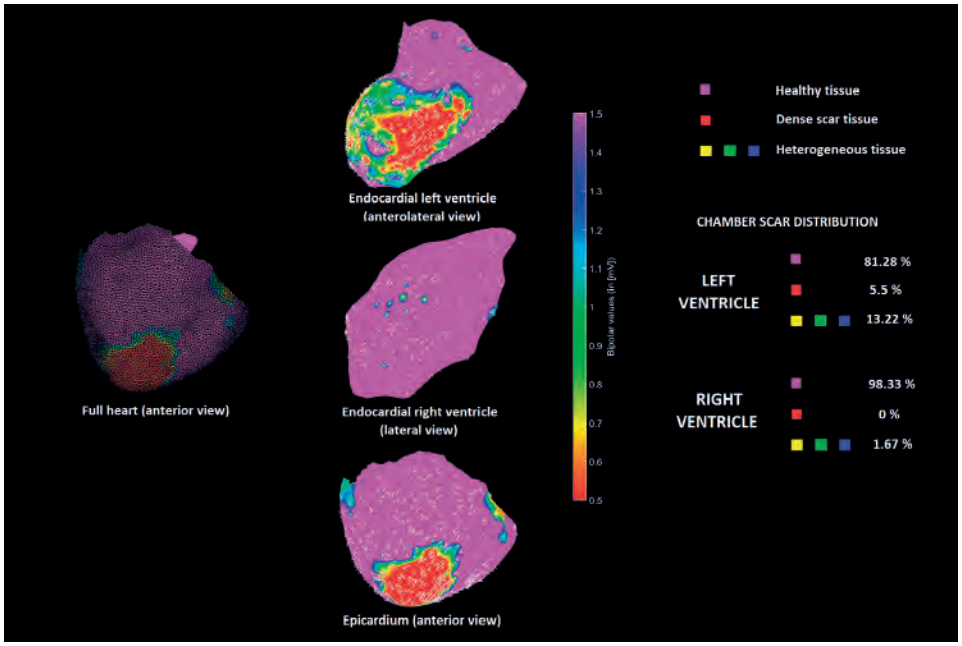
Head of Laboratory
David Filgueiras Rama

RESEARCH INTEREST

The laboratory focuses on the mechanisms underlying complex cardiac arrhythmias found in highly prevalent cardiovascular diseases, as well as in specific population subsets at particular risk of sudden cardiac death. Atrial fibrillation (AF), ventricular fibrillation (VF) and infarct scar-related ventricular tachycardia (VT) are three of the most prevalent cardiac rhythm disorders, in which the capacity of current therapeutic strategies to accurately eliminate or prevent the arrhythmogenic substrate is limited. Our goal is to achieve in-depth insight into the mechanisms of these complex arrhythmias through the use of appropriate experimental and numerical models, and for this insight to be used to improve patient care and develop new and more specific therapies.

We use a translational approach to study infarct scar-related VT in pigs and clinical infarct-related reentrant VT. High-resolution MRI images, both in humans (in vivo) and animals (ex vivo) provide the structural details to construct patient and animal-specific 3D anatomical models of the ventricles. Electrophysiologically realistic numerical simulations can be incorporated in the 3D model to induce and characterize reentrant VTs. Computational simulations are validated and compared with electropysiological data and outcomes obtained during the electrophysiological study and ablation procedure, either in animals or in humans.
Both in-hospital and out-of-hospital cardiac arrest due to VF are associated with high mortality rates and significant cerebral disability. However, early prognosis in comatose survivors after cardiac arrest due to VF is unreliable, especially in patients undergoing mild hypothermia. We have developed a reliable spectral-based risk-score to enable early prediction of cerebral performance and survival in patients undergoing therapeutic hypothermia for VF and comatose status.

In AF, we aim to develop new computational tools for accurate mapping of the propagation dynamics during fibrillation that will enable clinical electrophysiologists to effectively target the main driving sources of the arrhythmia. We use a translational approach in pigs with different stages of AF (paroxysmal, persistent and long-standing persistent AF) that resembles human pathophysiology. The combination of detailed structural characterization of the atria with *in vivo* and *ex vivo* propagation dynamics will provide the most precise data to date about the propagation dynamics underlying AF maintenance.



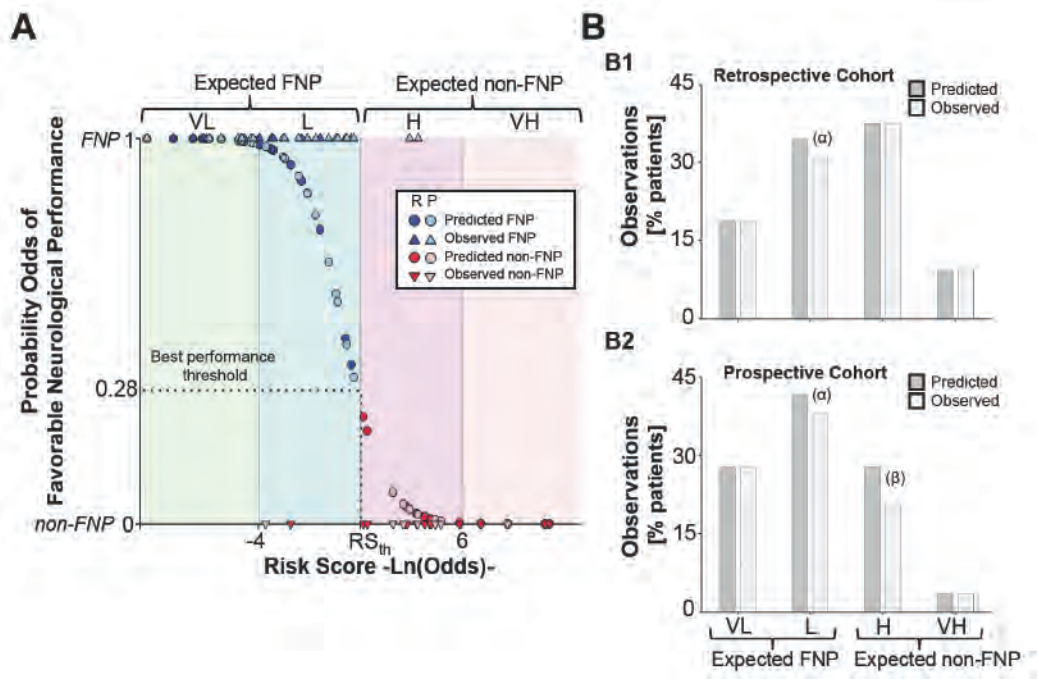
Endocardial and epicardial reconstruction of infarct-related susbtrate. Voltage maps of the endocardium and epicardium of both ventricles. The three structures are superimposed in the left column, and separated in the middle column. The right column shows quantification of the healthy myocardium, dense scar areas, and heterogeneous areas for each ventricle.

Graduate Technician:
Jorge García Quintanilla

Predoctoral Researchers:
Daniel García León
José Manuel Alfonso Almazán

Res@CNIC Fellow:
Alejandro Cruz Utrilla

Visiting Students:
José María Lillo Castellano
Manuel Marina Breysse
Beatriz Domingo García
Conrado Javier Calvo Sainz



VF_Spectral_Based_Score. Risk score based on the predictive performance of the spectral-based model. A. Observed (triangles) and predicted (circles) probability of FNP for the entire population. Blue and red represent FNP (favorable neurological performance) and non-FNP, respectively (dark fill, retrospective; light fill, prospective). We defined four risk groups of non-FNP performance according to their risk scores as follows: expected FNP; very low (VL) and low risk (L) and expected non-FNP; high (H) and very high risk (VH). B. Percentage of patients (predicted, dark gray and observed, light gray) who belong to each of the risk score groups in both the retrospective (B1) and prospective cohorts (B2). (α) and (β) represent false negative and false positive individuals, respectively.

MAJOR GRANTS

- *Spanish Society of Cardiology (Electrophysiology & Arrhythmia Division).*
- *Salud 2000 Foundation.*
- *Jesús Serra Foundation.*
- *Pro-CNIC Foundation.*

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Filgueiras-Rama D, Calvo CJ, Salvador-Montañés Ó, Cádenas R, Ruiz-Cantador J, Armada E, Rey JR, Merino JL, Peinado R, Pérez-Castellano N, Pérez-Villacastín J, Quintanilla JG, Jiménez S, Castells F, Chorro FJ, López-Sendón JL, Berenfeld O, Jalife J, López de Sá E, Millet J. **Spectral analysis-based risk score enables early prediction of mortality and cerebral performance in patients undergoing therapeutic hypothermia for ventricular fibrillation and comatose status**. *Int J Cardiol* (2015) 186: 250-8

Quintanilla JG, Moreno J, Archondo T, Usandizaga E, Molina-Morúa R, Rodríguez-Bobada C, González P, García-Torrent MJ, Filgueiras-Rama D, Pérez-Castellano N, Macaya C, Pérez-Villacastín J. **Increased intraventricular pressures are as harmful as the electrophysiological substrate of heart failure in favoring sustained reentry in the swine heart**. *Heart Rhythm* (2015) 12: 2172-83

Filgueiras-Rama D, de Torres-Alba F, Castrejón-Castrejón S, Estrada A, Figueroa J, Salvador-Montañés O, López T, Moreno-Yanguela M, López Sendón JL, Merino JL. **Utility of intracardiac echocardiography for catheter ablation of complex cardiac arrhythmias in a medium-volume training center**. *Echocardiography* (2015) 32: 660-70

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Epigenetic regulation in cardiac aging and disease

Head of Laboratory
Susana González

Postdoctoral Researcher:
Anne Marie Bleau

Predoctoral Researchers:
Isabel Hidalgo Gavilán
Itziar Cossío Cuartero
Vera Lúcia Ferreira Oliveira
Ileana Beatriz González Valdés
Esmeralda Armando Lewis
María Inmaculada Martos Folgado
Carlos José Martos Rodríguez
Eleni Petra

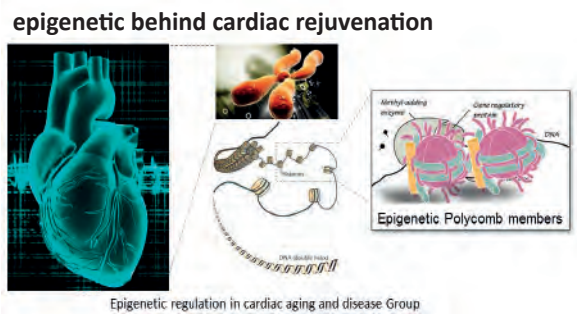
Technician:
Rebeca Diges López

Masters Student
Alicia González Martínez

RESEARCH INTEREST

The regeneration of adult tissues after injury involves tight homeostatic control by adult stem cells through their ability to self-renew and differentiate into multiple lineages. These characteristics are strongly affected with aging, leading to a loss in tissue regeneration capacity. Emerging evidence suggests that polycomb-group (PcG)-mediated alteration of the epigenetic status in hematopoietic stem cells (HSCs) is a major driving force behind many age-related HSC changes. Interestingly, PcG is often misregulated in human malignancies. Protection of the transcriptional "stemness" network is thus essential for the maintenance of a healthy HSC compartment throughout life. Whether the functional decline in adult stem cells is related to reversible chromatin modifications remains a key unanswered question in the field. We propose that changes to the chromatin state can restore the regenerative capacity of stem cells. To investigate this hypothesis, we are exploring the role of the epigenetic polycomb-mediated silencing mechanism in stemness maintenance, with particular emphasis on the self-renewal capacity and the microenvironment of HSCs, an important adult stem cell population with diverse regenerative abilities. Unraveling the molecular mechanisms by which polycomb members control stem cell fate will provide new insights into hematopoietic stem cell biology and increase the understanding of neoplastic transformation.

We have a particularly strong interest in the emerging role of different classes of chromatin regulators and how their dysregulation in the adult heart alters specific gene programs, with subsequent development of major cardiomyopathies. While dilated cardiomyopathy (DCM) is as the third most common cause of heart failure, it is still poorly modeled in nonhuman species. We propose that epigenetic remodeling could provide an important means of modulating the transcriptional reprogramming of cardiac gene expression in this condition. Understanding the action of Polycomb factors will allow the development of strategies to control physiological and pathological gene expression.



The epigenetic basis of cardiac rejuvenation. Aging is the greatest risk factor for cardiovascular disease. The aging process involves chromatin modifications by polycomb group proteins in HSCs, leading to a reduced stemness phenotype. By modulating PcG epigenetic status, we aim to restore the regeneration capacity of stem cells in adult tissues.

MAJOR GRANTS

- *European Commission. European Research Council Consolidator Grant (ERC-CoG-647670)*
- *Ministerio de Economía y Competitividad (SAF2013-42252-R)*

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Lopez-Arribillaga E, Rodilla V, Pellegrinet L, Guiu J, Iglesias M, Roman AC, Gutarra S, Gonzalez S, Munoz-Canoves P, Fernandez-Salguero P, Radtke F, Bigas A, Espinosa LL**. Bmi1 regulates murine intestinal stem cell proliferation and self-renewal downstream of Notch**. *Development* (2015) 142: 41-50

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Translational laboratory for cardiovascular imaging and therapy

Head of Laboratory
Borja Ibáñez
(CNIC, Fund. Jiménez Díaz Hospital)

RESEARCH INTEREST

Our laboratory focuses on the study of myocardial diseases, ranging from ischemia/reperfusion to heart failure. Our studies span the molecular origins of disease and their manifestations at the macro-anatomical and physiological levels, and our group includes experts in molecular biology, clinical cardiology and cardiovascular imaging. Our evaluation of experimental animal models makes use of advanced imaging techniques that can also be applied to humans, strengthening the translational potential of our research. To exploit this potential, we work on multidisciplinary programs in close collaboration with hospitals and clinical researchers.

A major interest of the group is cardioprotection during myocardial infarction (MI). We have established models of MI in rodents and large animals, and use these to study the mechanisms underlying the beneficial effects of various cardioprotective strategies, mainly related to modulation of the adrenergic system. We are pioneering the use state-of-the-art magnetic resonance imaging (MRI) to better characterize post-infarcted myocardial healing by combining studies in large animal models and human study participants. An example of this work is our leadership of the randomized METOCARD-CNIC clinical trial, which used MRI to evaluate the effectiveness of early intravenous metoprolol in patients suffering a myocardial infarction. The primary objective of this trial is already reported and we now are preparing a large multinational clinical trial based on these results (MOVE ON!). MOVE ON! will address the effect of this protective strategy not only on infarct size, but more importantly on long term mortality and morbidity. In parallel with these clinical trials, we study the cellular and molecular mechanisms underlying the observed cardioprotection in in vitro and genetically modified small animal models. We are also opening new fields of research focused on the metabolism of heart failure and the study of nutritional approaches to treat this condition.

We are part of the Spanish network for inherited cardiomyopathies, where our major interest is the development of better imaging-based strategies for improved risk stratification of patients carrying malignant mutations. This clinical work is combined with preclinical and basic studies to better understand the genotype-phenotype correlations of these mutations.

We are also interested in the myocardial response to pulmonary hypertension. We have developed small and large animal models of pulmonary hypertension and use imaging technology to evaluate the response to different therapies. We have identified a novel therapeutic approach for the treatment of pulmonary hypertension in preclinical studies and we have been funded to bring this therapy into a pilot clinical trial that will start during the coming year.

Postdoctoral Researchers:
Rodrigo Fernández-Jiménez *(CNIC, Hospital Clínico San Carlos)*
Gonzalo Pizarro *(CNIC, Hospital Universitario Quirón)*
José Manuel García Ruíz *(CNIC, Hospital Universitario Central de Asturias)*

Predoctoral Researchers:
Jaime García-Prieto Cuesta
Andrés Pun García
Jaume Agüero Ramón-Llin
Federico Sierra Rodríguez de la Rubia
Carlos Galán Arriola
Piotr Waldemar Wilczynski
Robert Austin Bruce Benn

Research Coordinator:
Noemí Escalera Biendicho

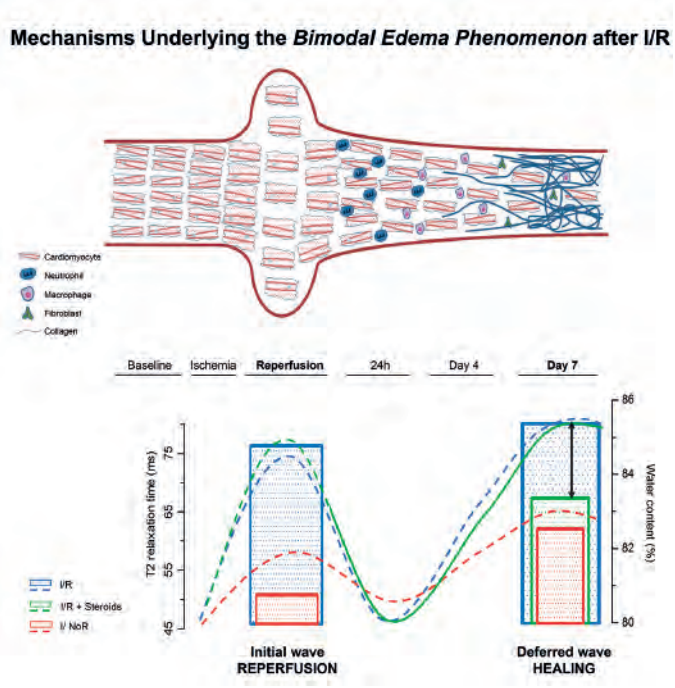
Technicians:
Mónica Gómez Parrizas
Parvin Rupa Khaton

Res@CNIC Fellows:
Julio C. García Rubio
Isabel Valandrón Sucasas

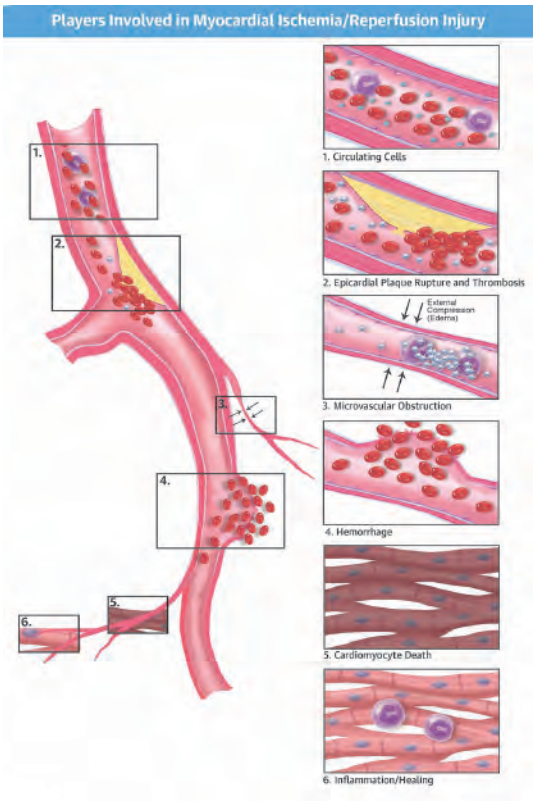
Invesmir Fellows:
Ali Ayaon Albarrán
Manuel Lobo González

Visiting Students:
Rocío Villena Gutiérrez
Ester Jiménez Arroyo
Sulayman Lazaar Soler
Blanca Sanz Magallón
Andrés Escudero Díaz
Constanza Ballesteros Martínez
Andrés Escudero Díaz

Visiting Scientists:
Jesús González Mirelis
Alonso Mateos Rodríguez
Daniel Pereda Arnau
Jorge Solís Martín
Mauro Echavarría
Montserrat Rigol Muxart
Núria Solanes Batlló
Santiago Roura Ferrer
Joaquim Bobi i Gibert
Iker Rodríguez Arabaolaza
Evelyn Santiago Vacas
Mónica García Bouza
Bunty Kishore Ramchandani Ramchandani



Mechanisms underlying the Bimodal Edema Phenomenon after myocardial I/R. (from Fernández-Jiménez R et al., *J Am Coll Cardiol*, 2015 66(7): 816-28).



Players involved in ischemia/reperfusion injury. (from Ibanez B et al., *J Am Coll Cardiol*, 2015 65(14): 1454-71).

MAJOR GRANTS

- *Ministerio de Economía y Competitividad - EXPLORA CIENCIA (SAF2013-49663-EXP)*
- *Ministerio de Economía y Competitividad - Acciones de Dinamización Europa investigación (EUIN2013-50881)*
- *Ministerio de Economía y Competitividad. ISCIII-FIS (PI13/01979)*
- *Ministerio de Economía y Competitividad. ISCIII-RETICS (RiC, RD12/0042/0054) - European Commision FP7-ICT-2011-8 (LIPHOS-317916)*
- *Marató, Fundación TV3 (REF: 70/C/2012)*
- *European Commision FP7-PEOPLE-2013-ITN (CARDIONEXT).*

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Wai T†, García-Prieto J†, Baker MJ, Merkwirth C, Benit P, Rustin P, Rupérez FJ, Barbas C, Ibañez B*, Langer T*. **Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice.** *Science* (2015) 350: aad0116-1-11
†Equal contribution; **co-corresponding authors*

Fernández-Jiménez R, García-Prieto J, Sánchez-González J, Agüero J, López-Martín GJ, Galán-Arriola C, Molina-Iracheta A, Doohan R, Fuster V, Ibanez B. **Pathophysiology underlying the bimodal edema phenomenon after myocardial ischemia/reperfusion.** *J Am Coll Cardiol* (2015) 66:816-28

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Fernández-Jiménez R, Sánchez-González J, Aguero J, García-Prieto J, López-Martín GJ, García-Ruiz JM, Molina-Iracheta A, Rosselló X, Fernández-Friera L, Pizarro G, García-Álvarez A, Dall'Armellina E, Macaya C, Choudhury R, V Fuster, B Ibanez. **Myocardial edema after ischemia/reperfusion is not stable and follows a bimodal pattern: advanced imaging and histological tissue characterization.** *J Am Coll Cardiol* (2015) 65: 315-23

Cardiac arrhythmia

Head of Laboratory
José Jalife

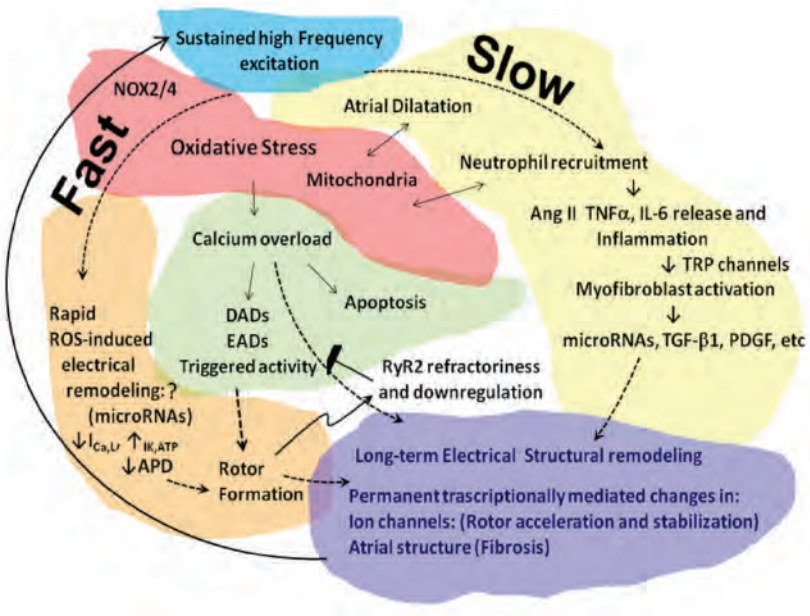
Visiting Student:
Sandeep V. Pandit

RESEARCH INTEREST

The laboratory centers its research interest on understanding the causes of cardiovascular disease at the molecular, cellular and electrophysiological levels. Our specific research objectives are focused on 1) the mechanisms of atrial and ventricular fibrillation at the structural and functional level; 2) the molecular genetics of cardiac fibrillation; and 3) the cellular basis of cardiac arrhythmia in genetic and rare diseases that can lead to sudden death.

The laboratory has well-established collaborations with expert engineers, biologists and clinicians around the world, as well as collaborations with other CNIC groups. These partnerships provide a unique research environment in which to generate new and clinically relevant breakthroughs on arrhythmia mechanisms that will benefit the medical and basic science communities, and ultimately the patient.

An ongoing multidisciplinary project is the whole genome characterization of large animal models of atrial fibrillation with a clear translational impact. The project aims to define transcriptomic changes in a sheep model of induced atrial fibrillation. Bioinformatic analysis of changes in gene expression and its correlation with proteomic data generated by the group will enable mapping of the networks and pathways altered in paroxysmal and persistent states of atrial fibrillation. These results are also being validated in a pig atrial fibrillation model that has recently been established at the CNIC. The use of these models will allow us to better understand the molecular determinants and consequences of atrial fibrillation and offer new insights into therapeutic targets of this disease.



Atrial fibrillation-induced remodeling and the substrate for atrial fibrillation persistence.

MAJOR GRANTS

- *Leducq Foundation Transatlantic Networks of Excellence Program (not CNIC). Co – Investigator*
- *NIH / NHLBI - R01 (HL122352) (not CNIC). Co-Investigator*
- *NIH / NHLBI - T32 (HL125242) (not CNIC). Co-Investigator*
- *The University of Michigan Health Sciences-Peking University Health Science Center Joint Institute. (not CNIC). Co-Investigator.*

SELECTED PUBLICATIONS

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Molecular regulation of heart development and disease

Head of Laboratory

Enrique Lara-Pezzi

RESEARCH INTEREST

Our laboratory investigates the molecular processes underlying heart remodeling, which are still poorly understood. In collaboration with Dr Fernando Rodriguez-Pascual's group (Centro de Biología Molecular Severo-Ochoa, Madrid) we have unveiled an unexpected beneficial role of lysyl oxidase (Lox) in post-infarction heart remodeling. Lox facilitates the cross-linking of extracellular matrix and thereby contributes to the development of fibrosis. We found that inhibition of Lox activity results in decreased infarct expansion and improved cardiac function one month after myocardial infarction. This effect is similar to that of the calcineurin splicing variant CnAβ1. Expression of this isoform promotes the vascularisation of the infarct region, reinforcing the structure in the infarcted area and preventing infarct expansion and therefore heart remodeling.

The beneficial activity of CnAβ1 in the adult heart stands in stark contrast to the detrimental effects of other calcineurin isoforms, which promote maladaptive cardiac hypertrophy and heart failure by activating the transcription factor NFAT, among other targets. Due to retention of an intron in its mRNA, CnAβ1 has a unique C-terminal domain that has no similarity with any other known protein. This unique domain drives CnAβ1 to the Golgi apparatus and facilitates activation of the Akt signaling pathway. In embryonic stem cells, CnAβ1 is necessary for mesodermal differentiation in a parallel pathway to that activated by the calcineurin isoform CnAβ2 via NFAT for this same purpose. We are now exploring the therapeutic potential of CnAβ1 in a swine model of myocardial infarction using gene therapy based on adeno-associated vectors.

Postdoctoral Researcher:
Laura Padrón

Predoctoral Researchers:
Jesús Gómez Salinero
Alberto Gatto
Enda Clinton
Girolamo Giudice
Paula Ortiz Sánchez
Jose Javier Larrasa Alonso

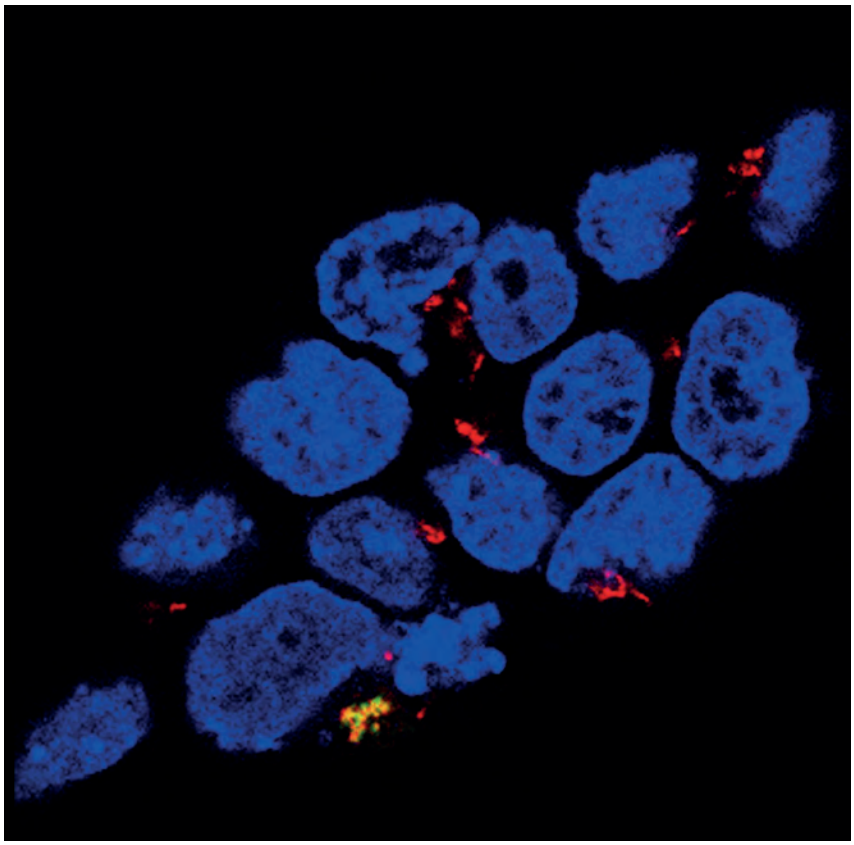
Graduate Technician:
María Villalba Orero

Technician:
Marina López Olañeta

Res@CNIC Fellow:
Juan M. Monteagudo Ruiz

Masters Student:
Carlos Martí Gómez-Aldaraví

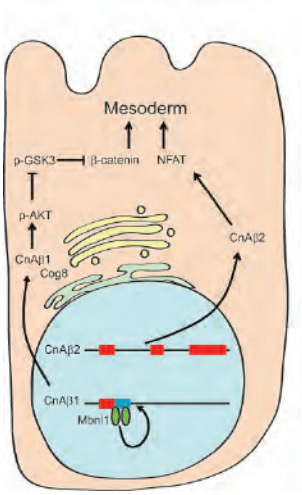
Visiting Scientists:
Pablo García Pavía
Raquel San José Martín-Albo



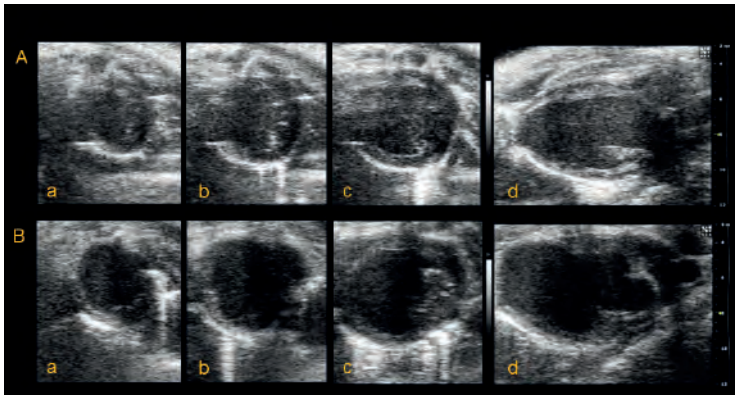
CnAβ1 is localized in the Golgi apparatus. Unlike other calcineurin isoforms, such as CnAβ2, which is freely distributed throughout the cytoplasm, CnAβ1 is compartmentalized to the Golgi apparatus. This localization is necessary for activation of the Akt signaling pathway, a major regulator of cell growth and survival. P19 cells were transfected with a chimeric GFP-CnAβ1 construct and immunostained with antibodies against GFP (green) and the Golgi marker GM130 (red). Nuclei were counterstained with DAPI (blue). Scale bar 25 µm.

RESEARCH AREAS

1. Myocardial Pathophysiology



Regulation of mesoderm differentiation in mESCs through CnAβ isoforms. The splicing factor muscle blind like 1 (Mbnl1) promotes CnAβ1 isoform production from the CnAβ locus. CnAβ1 is located at the Golgi apparatus, where it interacts with Cog8. CnAβ1 regulates the phosphorylation levels of AKT from the Golgi. The active form of AKT inhibits GSK3 activation, leading to an increase in the levels of β-catenin that promotes mesoderm specification. CnAβ2 is localized in the cytoplasm and activates NFAT to promote mesoderm specification.



Echocardiographic analysis of an infarcted mouse heart. **A**, Short axis apical, medium and basal echocardiographic views (a, b and c, respectively) combined with the long axis view (d) for accurate estimation of left ventricle motion and remodeling in a untreated C5/BL6 mice. **B**, The same analysis as in (A) carried out on a C5/BL6 mouse 28 days after occluding the left anterior descending coronary artery.

MAJOR GRANTS

- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2013-ITN, "CardioNext" 608027)*
- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600)*
- *Comunidad de Madrid (GRUPOSCAM10, "Fibroteam" S2010/BMD-2321)*
- *Ministerio de Economía y Competitividad (SAF2012-31451)*
- *Instituto de Salud Carlos III (MSII14/00027)*

SELECTED PUBLICATIONS

González-Santamaría J, Villalba M, Busnadiego O, López-Olañeta MM, Sandoval P, Snabel J, López-Cabrera M, Erler JT, Hanemaaijer R, Lara-Pezzi E*, Rodríguez-Pascual F*. **Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction**. *Cardiovasc Res* (doi: 10.1093/cvr/cvv214. Epub 2015 Aug 10)
*Co-corresponding authors

Lara-Pezzi E, Menasché P, Trouvin JH, Badimón L, Ioannidis JP, Wu JC, Hill JA, Koch WJ, De Felice AF, de Waele P, Steenwinckel V, Hajjar RJ, Zeiher AM. **Guidelines for translational research in heart failure.** *J Cardiovasc Transl Res* (2015) 8: 3-22

López-Olañeta MM, Villalba M, Gómez-Salinero JM, Jiménez-Borreguero LJ, Breckenridge R, Ortiz-Sánchez P, García-Pavía P, Ibáñez B, Lara-Pezzi E. **The calcineurin variant CnAβ1 improves post-infarction ventricular remodelling by promoting infarct vascularisation**. *Cardiovasc Res* (2014) 102: 396-406

Gatto A, Torroja-Fungairiño C, Mazzarotto F, Cook SA, Barton PJ, Sánchez-Cabo F, Lara-Pezzi E. **FineSplice, enhanced splice junction detection and quantification: a novel pipeline based on the assessment of diverse RNA-Seq alignment solutions**. *Nucleic Acids Res* (2014) 42:e71.

Nuclear receptor signaling

Head of Laboratory
Mercedes Ricote

RESEARCH INTEREST

Macrophages are hematopoietic cells of the myeloid lineage with important functions in development, homeostasis, tissue repair and immunity. Macrophages can be found in practically all tissues, making important contributions to homeostasis and protection against injury. Projects in our group focus on elucidating the transcriptional control of macrophages in different tissues, especially in the heart, adipose tissue and bone marrow, with special emphasis on their possible medical utility in the treatment of metabolic and cardiovascular diseases.

A special interest of our group is the transcriptional regulation of macrophage functions by nuclear hormone receptors. Our laboratory has shown that nuclear receptors play a major regulatory role in homeostasis, inflammation and immunity. Our recent studies indicate that retinoid X receptors (RXRs) play a key role in orchestrating macrophage transcriptional programs necessary for debris clearance, proliferation, polarization and lipid metabolism. Moreover, genetic deletion of these receptors in macrophages severely compromises macrophage homeostatic responses, leading to autoimmunity, osteopetrosis and insulin resistance. Based on these findings, we hypothesize that nuclear receptors play important roles in orchestrating hematopoietic stem cell and macrophage transcriptional programs necessary for tissue repair and regeneration. To test this hypothesis we are currently conducting complementary loss-of-function and drug-mediated gain-of-function mouse studies, and also genome-wide studies using transcriptomic (RNA-seq and GRO-seq) and cistromic (ChIP-seq) technologies. We will examine mice lacking RXR in hematopoietic stem cells, macrophages, endothelial, and cardiomyoctes, allowing us to examine the specific role of these receptors in tissue homeostasis and injury.

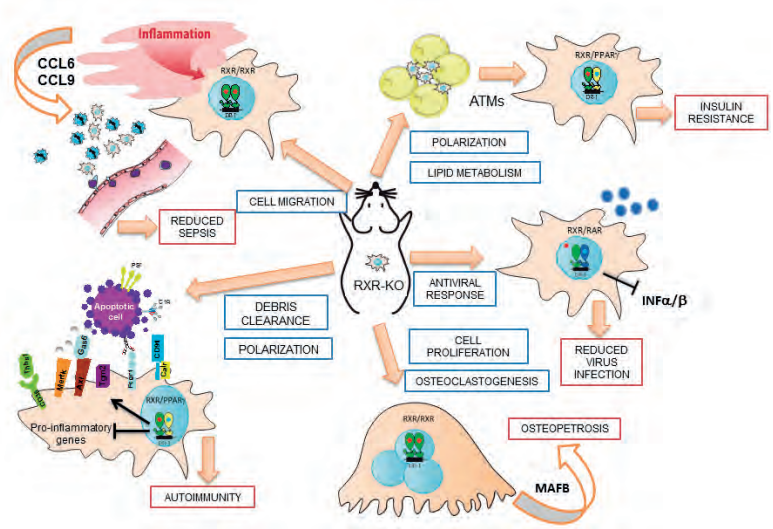
Research Scientist:
María Piedad Menéndez Gutiérrez

Postdoctoral Researchers:
Lorenzo Veschini

Predoctoral Researchers:
Anna Kwasniewska
Wencke Walter
Angel Núñez Buiza
Laura Alonso Herranz

Masters Students:
Ana Paredes
José Juan Aparicio

Technician:
Vanessa Núñez González



Defining RXR functions in macrophage biology. Macrophages express RXRα and RXRβ. RXRs play key roles in macrophage homeostasis and disease by controlling transcriptional programs necessary for inflammation, apoptotic cell uptake, proliferation, antiviral response, polarization and lipid metabolism. Macrophage-specific RXRα/β-delition results in sepsis, autoimmunity, osteopetrosis and insulin resistance.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2012-31483)*
- *Fundación TV3 Marató*
- *European Commission, 7th Frame Program (FP7-PEOPLE-2013-ITN) (PITN-GA-2013-608027)*
- *Ministerio de Economía y Competitividad (SAF2015-71878-REDT)*

SELECTED PUBLICATIONS

Natrajan MS, de la Fuente AG, Crawford AH, Linehan E, Nuñez V, Johnson KR, Wu T, Fitzgerald DC, Ricote M, Bielekova B, Franklin RJ. **Retinoid X receptor activation reverses age-related deficiencies in myelin debris phagocytosis and remyelination.** *Brain* (2015) 138: 3581-97

Walter W, Sánchez-Cabo F, Ricote M. **GOplot: an R package for visually combining expression data with functional analysis.** *Bioinformatics* (2015) 31: 2912-4

Menéndez-Gutiérrez MP, Rőszer T, Fuentes L, Núñez V, Escolano A, Redondo JM, De Clerck N, Metzger D, Valledor AF, Ricote M. **Retinoid X receptors orchestrate osteoclast differentiation and postnatal bone remodeling.** J Clin Invest (2015) 125: 809-23

Ballesteros I, Cuartero MI, Pradillo JM, de la Parra J, Perez-Ruiz A, Corbi A, Ricote M, Hamilton JA, Sobrado M, Vivancos J, Nombela F, Lizasoain I and Moro MA. **Rosiglitazone-induced CD36 up-regulation resolves inflammation by PPARgamma and 5-LO-dependent pathways.** *J Leukoc Biol* (2014) 95: 587-98

Ma F, Liu SY, Razani B, Arora N, Li B, Kagechika H, Tontonoz P, Nunez V, Ricote M and Cheng G. **Retinoid X receptor alpha attenuates host antiviral response by suppressing type I interferon.** *Nat Commun* (2014) 5: 5494

Stress kinases in diabetes, cancer and cardiovascular disease

RESEARCH INTEREST

Metabolic syndrome is a medical disorder defined by the co-occurrence of obesity, impaired glucose tolerance, dyslipidemia and hypertension. The condition is associated with proinflammatory and prothrombotic states, and clinical outcomes include cardiovascular disease and type 2 diabetes. Moreover, metabolic syndrome may be a predisposing factor for the development of some types of cancer, such us hepatocellular carcinoma.

The high cardiovascular risk associated with metabolic syndrome and type 2 diabetes suggests that common mechanisms are involved in the etiology of these conditions, and that agents acting on the same therapeutic targets might improve disease parameters in both. Research suggests that one such target might be the stress activated protein kinases (SAPKs), an important family of kinases implicated in the transduction of stress signals into the cell.

Our group investigates the involvement of SAPKs in the development of cancer, diabetes, cardiac hypertrophy and atherosclerosis induced by obesity. Our research is conducted with a number of disease models in combination with whole-body and tissue-specific knockout mice, and has shown that the p38γ/δ isoforms control IL6 and TNF production in myeloid cells. We are now studying how the regulation of inflammation by these kinases affects the development of metabolic syndrome. We are also studying the function of these kinases in other tissues, such us muscle, heart, the central nervous system and adipose tissue, in order to elucidate the role of these kinases in the development of different diseases associated with obesity (steatosis, diabetes, cardiovascular diseases and some types of cancer).

Head of Laboratory

Guadalupe Sabio

Postdoctoral Researchers:
Nuria Matesanz
Antonia Tomás Loba
Ivana Nikolic

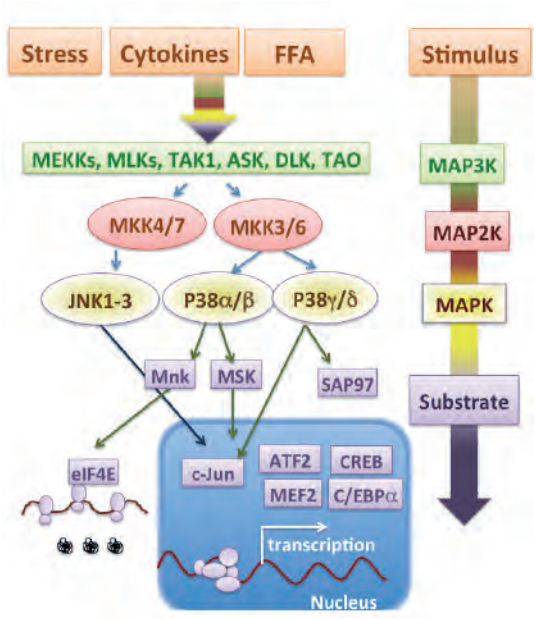
Predoctoral Researchers:
Edgar Bernardo
Bárbara González
Elisa Manieri
María del Valle Montalvo

Technicians:
Elena González
Luis Leiva
Alfonso Mora
(since July)
Victor Emilio Bondia
(since September)

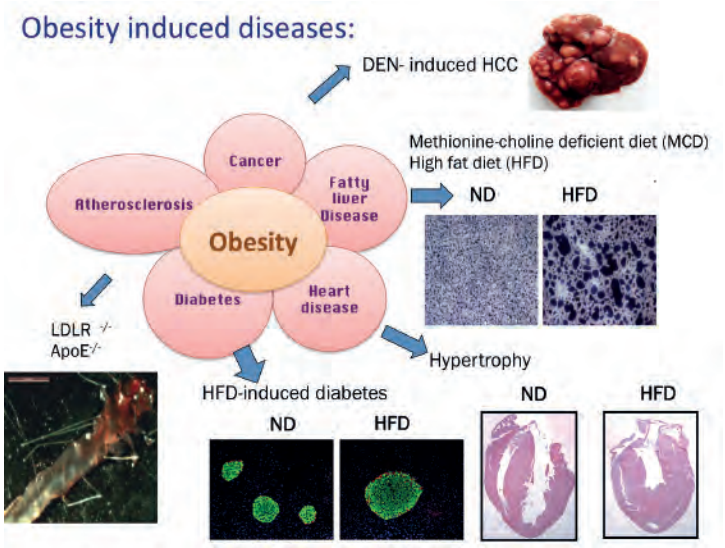
Res@CNIC Fellow:
Ana Pardo Sanz

Masters Student:
Leticia Herrera

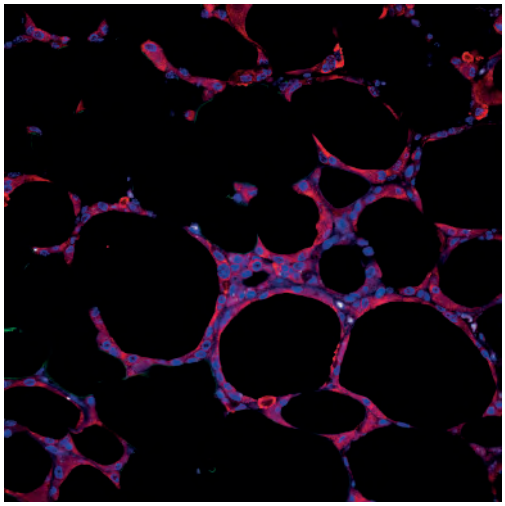
Group photo: Dani Pozo *(El Español)*



MAPK pathway
A general feature of MAPK pathways is a canonical cascade consisting of MAPK kinase kinases (MAP3K), a MAPK kinase (MAP2K) and a MAPK. The MAPK family can be divided into three main pathways: ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38. Numerous stimuli, including growth factors, inflammatory cytokines and a wide spectrum of cellular stresses, can activate MAPK signaling pathways. Once activated, MAPKs can phosphorylate several downstream targets, including other protein kinases, cytosolic substrates, and transcription factors.



Obesity related dieseases
Obesity is one of the leading causes of life-threatening diseases and can compromise health and shorten life expectancy. In our group we study several of themes such as diabetes, cancer, and heart disease.



Obesity-induced iInflamation of white adipose tissue

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2013-43506-R)*
- *European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)*
- *Comunidad de Madrid. INMUNOTHERCAN (S2011/BMD-2326)*

SELECTED PUBLICATIONS

González-Terán B, López JA, Rodríguez E, Leiva L, Martínez Martínez S. Jiménez Borreguero LJ, Redondo JM, Vázquez J, Sabio G. **p38 and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation**. *Nat Commun* (accepted)

González-Terán B, Matesanz N, Verdugo MA, Sreeramkumar V, Hernández-Cosido L, Bernardo E, Leiva-Vega L, Rodríguez E, Torres JL, Perez S., Ortega L, Cuenda A, Nogueira R, Hidalgo A, Miguel Marcos M, Sabio G. **p38γ and p38δ reprogram liver metabolism by modulating neutrophil infiltration** *EMBO Journal* (accepted)

Manieri E, Sabio G. **Stress kinases in the modulation of metabolism and energy balance.** *J Mol Endocrinol* (2015) 55: R11-22

Vernia S, Cavanagh-Kyros J, Garcia-Haro L, Sabio G, Barrett T, Jung DY, Kim JK, Xu J, Shulha HP, Garber M, Gao G, Davis RJ. **The PPARα-FGF21 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway.** *Cell Metab* (2014) 20: 512-25.

Sabio G, Davis RJ. **TNF and MAP kinase signalling pathways**. *Seminars in Immunology* (2014) 26: 237-45

Immunobiology of inflammation

Head of Laboratory
David Sancho Madrid

RESEARCH INTEREST

Our main interest is in how dendritic cells (DCs) and macrophages modulate immunity and inflammation. We are interested in the analysis of DC subsets, their specific functions and plasticity. We have found that Batf3-dependent DCs are crucial for generation of Th1 immunity through the production of IL-12 (Fig. 1 and Martínez-López et al. 2015). Some of our work is conducted with the Th1-immunity model of infection by the eukaryotic parasite *Leishmania major*; this organism induces tissue damage and mimics many tissue-derived danger signals. We have also found that Batf3-dependent DCs do not play a major role in the development of atherosclerosis. In contrast, cross-presenting DCs are crucial for the generation of a basal immune response that can be rescued by immunostimulatory antibodies for cancer immunotherapy (Sánchez-Paulete et al., in press).

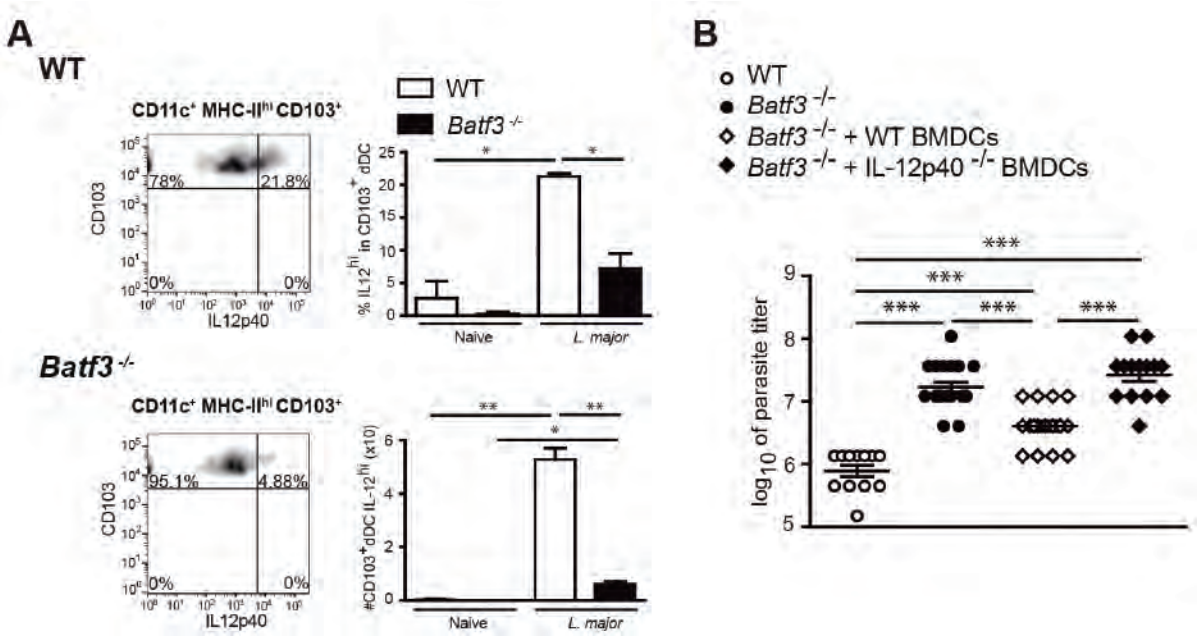
We have also analyzed the modulation of signals through C-type lectin receptors and have found that SHIP-1 associates with the intracellular hemITAM motif in Dectin-1 and selectively modulates its ability to induce reactive oxygen species in DCs (Fig. 2 and Blanco-Menéndez et al. 2015). In addition, we are working on DNGR-1 as a model C-type lectin that detects tissue damage during infection and we have characterized an impact on the CD8+ T cell memory response. We are also analyzing the effects of sensing non-self and damaged-self on the metabolism of myeloid cells. We believe that this research has potential for the development of new vaccines and immunotherapy strategies.

Postdoctoral Researchers:
Salvador Iborra Martín
Johan J.B. Garaude
Carlos del Fresno Sánchez
Laura Conejero Hall

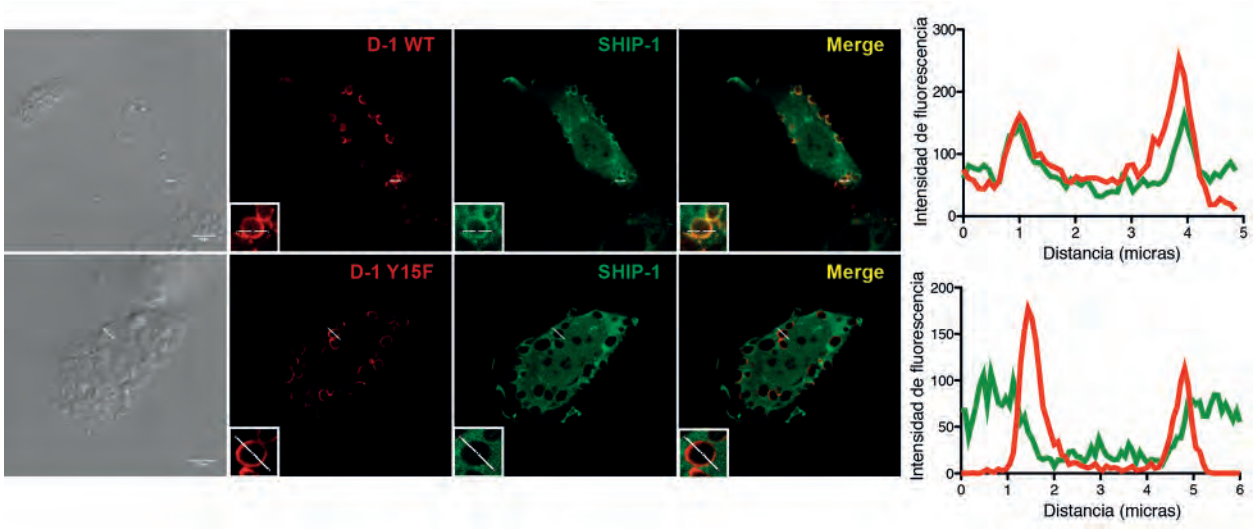
Predoctoral Researchers:
Noelia Blanco Menéndez
Helena M. Izquierdo Fernández
María Martínez López
Neris M. Enamorado Escalona
Paola Brandi
Francisco J. Cueto Rodríguez
Paula Saz

Masters Student:
Sofía Chayeb

Technicians:
Ruth Conde Garrosa
Sarai Martínez Cano
Jesús Sánchez



Batf-3 dependent CD103+ DC are major IL-12 producers (A) Production of IL-12p40 in ear dermal CD103⁺ DCs. Left: representative plots. Right: arithmetic mean + SEM of frequency (upper panel) and absolute numbers (lower panel) from naive mice or mice infected with IL-12p40 producing DCs. (B) Transfer of Batf3-dependent DCs rescues impaired Th1 immunity in Batf3KO mice in a IL12p40-dependent fashion.



SHIP-1 colocalizes with Dectin-1 in the phagosome in a Tyr15-dependent fashion. CHO cells expressing either mCherry-tagged wild type Dectin-1 (WT D-1) or mCherry-tagged Y15F-mutated Dectin-1 (Y15F D-1) were cotransfected with EGFP-SHIP-1 fusion protein. Cells were then exposed to 10 µg/ml zymosan for 20 minutes. Colocalization of WT D-1 and Y15F D-1 with SHIP-1 was examined by confocal microscopy. White lines indicate transverse sections of illustrative phagosomes. Fluorescence intensity profiles for green and red channels are plotted as histograms.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (EUIN2015-62652)*
- *Ministerio de Economía y Competitividad. Programa Redes de Excelencia 2014. (SAF2014-53563- REDT).*
- *EU Framework Programme for Research and Innovation H2020. Call: H2020-PERSONALISING HEALTH AND CARE (GA635122-PROCROP).*
- *Ministerio de Economía y Competitividad (SAF2013-42920-R)*
- *European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260414)*
- *Research cooperation agreement with MedImmune (Cambridge, UK)*
- *ERS/EU Marie Curie Post-doctoral Research Fellowships (RESPIRE 2 - 3708-2013).*

SELECTED PUBLICATIONS

Sánchez-Paulete AR, Cueto FJ, Martínez-López M, Labiano S, Morales-Kastresana A, Rodríguez-Ruiz ME, Jure-Kunkel M, Azpilikueta A, Aznar MA, Quetglas JI, Sancho D*, Melero I*. **Cancer Immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal antibodies requires BATF3-dependent dendritic cells.** *Cancer Discov* (doi: 10.1158/2159-8290.CD-15-0510. Epub 2015 Oct 22)
**Co-corresponding authors*

Blanco-Menéndez N, Del Fresno C, Fernandes S, Calvo E, Conde-Garrosa R, Kerr WG, Sancho D. **SHIP-1 couples to the dectin-1 hemITAM and selectively modulates reactive oxygen species production in dendritic cells in response to candida albicans**. *J Immunol* (2015) 195: 4466-78

Hanč P, Fujii T, Iborra S, Yamada Y, Huotari J, Schulz O, Ahrens S, Kjær S, Way M, Sancho D, Namba K, Reis e Sousa C. **Structure of the complex of F-actin and DNGR-1, a C-type lectin receptor involved in dendritic cell cross-presentation of dead cell-associated antigens**. *Immunity* (2015) 42: 839-49

Martínez-López M, Iborra S, Conde-Garrosa R, Sancho D. **Batf3-dependent CD103$^+$ dendritic cells are major producers of IL-12 that drive local Th1 immunity against Leishmania major infection in mice.** *Eur J Immunol* (2015) 45: 119-29

Iborra S, Sancho D. **Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors.** *Immunobiology* (2015) 220: 175-84

RESEARCH AREAS

1. Myocardial Pathophysiology
2. Vascular Pathophysiology
3. Cell and Developmental Biology

2

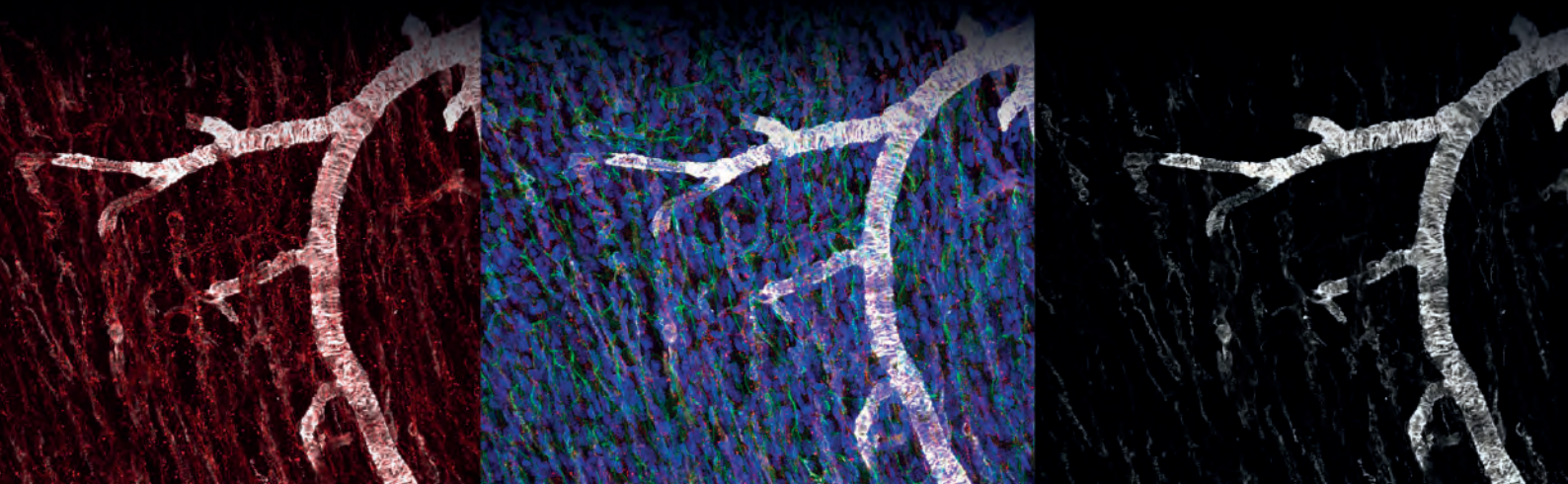
RESEARCH AREAS

Vascular Pathophysiology

Area coordinators:

Almudena Ramiro *Antonio Fernández-Ortiz*

Research in the Vascular Pathophysiology Area (VPA) focuses on the biology of the cardiovascular system in health and disease, using a multidisciplinary and transverse approach, embracing molecular and cellular biology as well as translational and clinical research. The work in the VPA is broadly divided into 2 programs: Cardiovascular Biology and Signaling & Inflammation. VPA research groups use a wide variety of techniques, including animal, tissue, cell and molecular models, to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases. VPA groups work on translational and clinical research through several research projects, including Secure and PESA. We also have a major interest in cardiovascular proteomics. The VPA hosts three technical units: Genomics, Proteomics/Metabolomics, and Bioinformatics.



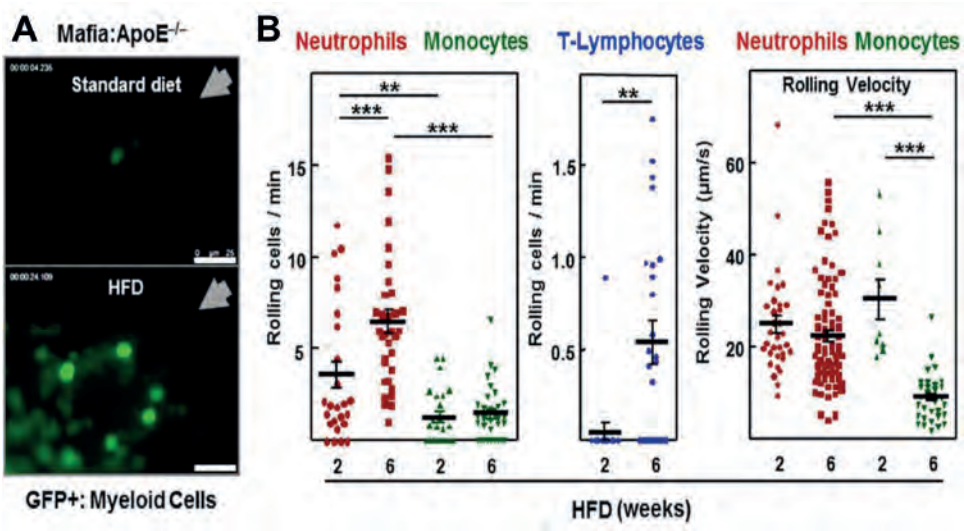
Molecular and genetic cardiovascular pathophysiology

Head of Laboratory
Vicente Andrés García

RESEARCH INTEREST

The World Health Organization has estimated that cardiovascular disease (CVD) will by 2020 be the main health and socio-economic problem worldwide, in part due to the progressive aging that the world population is experiencing. Atherosclerosis and heart failure contribute significantly to CVD-related morbimortality in the elderly. These anomalies and the aging process are greatly accelerated in Hutchinson-Gilford progeria syndrome (HGPS), a rare genetic disorder caused by the expression of progerin, a mutant form of lamin A. The most serious aspect of HGPS is extensive atherosclerosis and cardiac electrophysiological alterations that are associated with early death (average lifespan: 13.5 yr, range: 8-21 yr), predominantly from myocardial infarction or stroke. Progerin is also expressed at low level in aged tissues of non-HGPS individuals, suggesting a role in normal aging. Understanding how this mutant form of lamin A causes CVD and premature aging may therefore shed light on normal aging.

Our research currently focuses on: 1) Identifying mechanisms through which wild-type lamin A/C regulates CVD; 2) Identifying tissue-specific and systemic mechanisms through which progerin promotes atherosclerosis and aging, and developing novel therapeutic strategies; 3) Generating a porcine model of HGPS using CRISPR/Cas9 technology to accelerate translational research in HGPS; and 4) Unraveling molecular mechanisms common to premature and physiological aging and specific to each process.



New method for high-resolution imaging of intravascular atherogenic inflammation in live mice allows simultaneous tracking of inflammatory leukocytes and platelets within the carotid artery of atherosusceptible Mafia:ApoE$^{-/-}$ mice. **A)** Myeloid leukocytes (green) rolling at the bifurcation of the carotid artery in mice fed standard chow or high-fat diet (HFD) for 10 days. Scale bars, 25 µm. Arrows show the direction of blood flow. B) Number of rolling cells (left and middle) and rolling velocity (right) of neutrophils (red), monocytes (green), and T lymphocytes (blue). Data from 18 to 94 fields from 4 mice. Lines show mean ± SEM. ***P*<0.01 and ****P*<0.001 (From: Chèvre et al. Circ Res. 2014;114:770-779).

Postdoctoral Researchers:
Lara del Campo Milán
José María González Granado *(Miguel Servet Programme)*
Álvaro Macías Martínez
Cristina Rius Leiva
José Rivera Torres

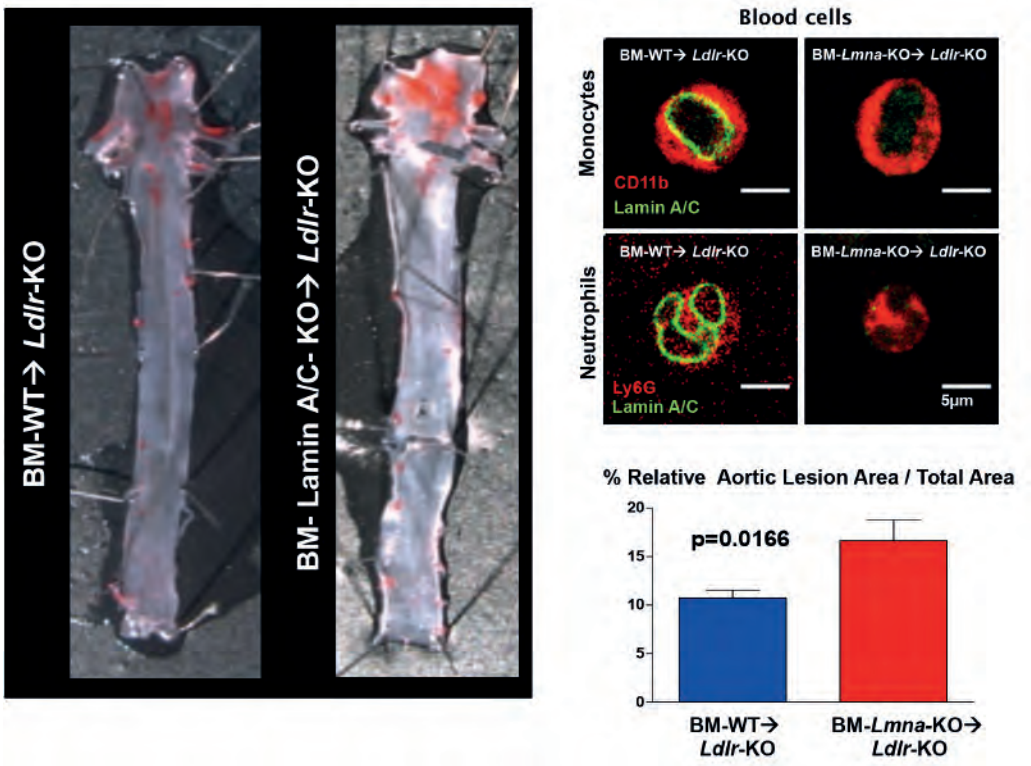
Predoctoral Researchers:
Alberto del Monte Monge
Victor Fanjul Hevia
Pedro Molina Sánchez
Magda Rita Hamczyk
Amanda Sánchez López
Raquel Toribio Fernández

Visiting Students:
María del Carmen Ortuño Costela
Dunia Asensio Cobo
Javier Gómez Ortega

Lab Manager:
Beatriz Julia Dorado de la Corte

Technicians:
María Jesús Andrés Manzano
Marta Blanco Berrocal
Cristina González Gómez

Visiting Scientists:
Immacolata Giordano
Giovanna Granata
Claudia Gucciardi
Andreu Llobera Adán
Ricardo Villa Bellosta



Lamin A/C deficiency in immune cells aggravates atherosclerosis. Ldlr-KO mice transplanted with wild-type bone marrow have perinuclear lamin A/C (green) in circulating monocytes and neutrophils (red), which is absent in mice reconstituted with Lmna-KO bone marrow. Oil-red-O staining reveals significantly increased atherosclerosis in aortas of BM-Lmna-KO->Ldlr-KO mice.

MAJOR GRANTS

- *European Commission FP7-ICT-2011-8 (LIPHOS-317916)*
- *Progeria Research Foundation (Established Investigator Award PRF 2014)*
-*Ministerio de Economía y Competitividad. Modalidad Retos Investigación (SAF2013-46663-R)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RiC, RD12/0042/0028)*
- *Ministerio de Economía y Competitividad. FIS (CP11/00145) PI: J.M. González Granado*
- *Fundación Ramón Areces (XVII Concurso Nacional para la Adjudicación de Ayudas a la Investigación en Ciencias de la Vida y de la Materia). PI: J.M. González Granado*

SELECTED PUBLICATIONS

Arroyo, AG, Andrés, V. **ADAMTS7 in cardiovascular disease: From bedside to bench and back again?** *Circulation* (2015) 131:1156-9

Rivera-Torres, J, Guzmán-Martínez, G, Villa-Bellosta, R, Orbe, J, González-Gómez, C, Serrano, M, Díez, J, Andrés, V*, Maraver, A*. **Targeting γ-secretases protects against angiotensin II-induced cardiac hypertrophy**. *J Hypertension* (2015) 33:843–50
* Co-corresponding authors

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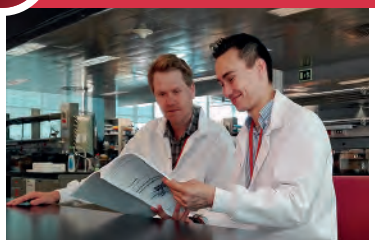
* Co-corresponding authors

Experimental pathology of atherosclerosis

Head of Laboratory
Jacob Fog Bentzon



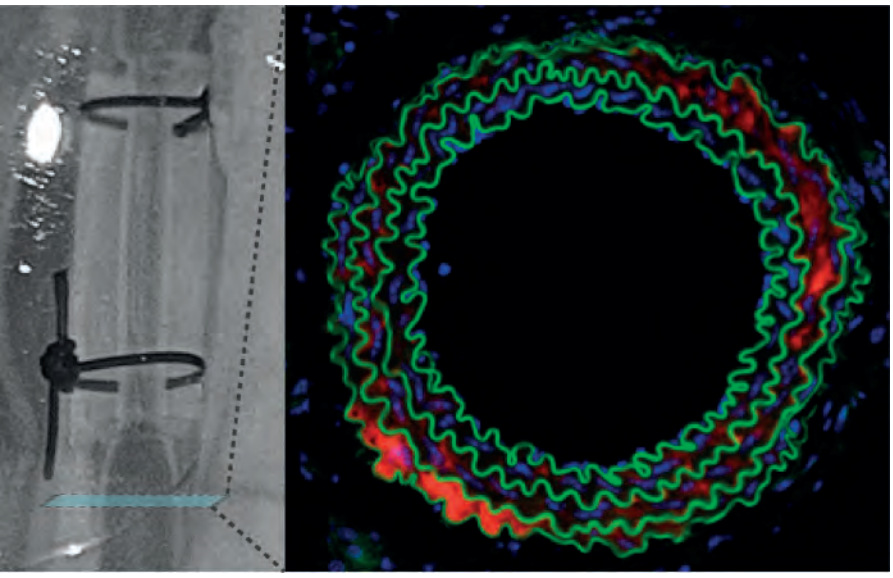
Visiting Scientist:
Kevin Jacobsen

RESEARCH INTEREST

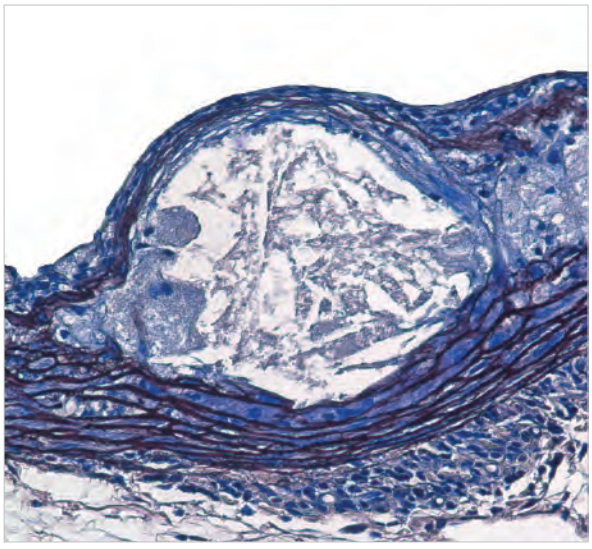
The development of atherosclerosis and its complications heart attack and stroke is a major constraint to living long and heatlhy lives around the world. Our mission is to explore the mechanisms leading to atherosclerosis and finding ways to prevent its progression. The group joined CNIC from Aarhus Universty in September 2015 and the work involves extensive collaboration between Spain and Denmark.

An important element of our strategy has been the development of new tools for atherosclerosis research. Gene modified minipigs with atherosclerosis, created by animal cloning and now established at CNIC, offer human-like dimensions and pathology for studies with a direct translational outlook. As a complementary method for basic research, we have devised a virus-mediated gene transfer technique to induce atherosclerosis in mice, circumventing the need for complicated breeding programmes and offering greater flexibility in the design of experiments. This method is currently being implemented in many research groups around the world.

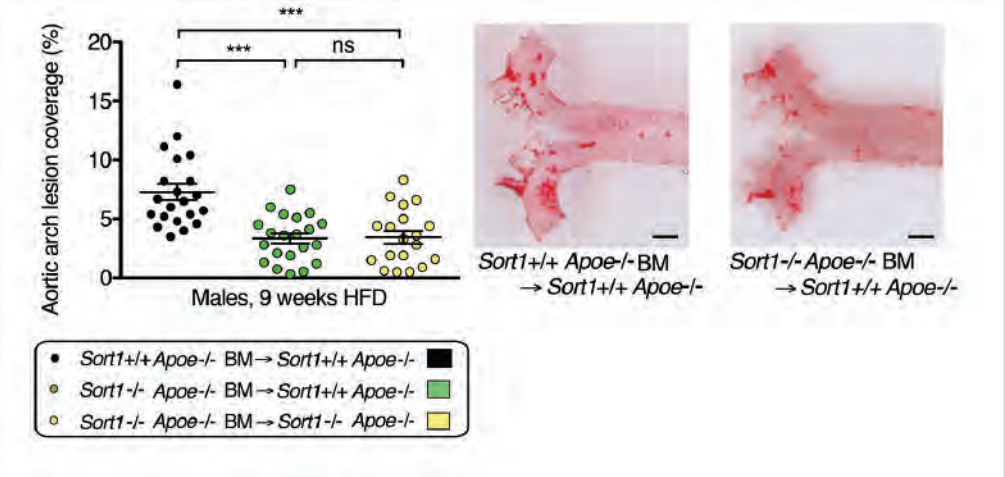
Applying these and other techniques, our group has recently described how blood flow controls atherosclerosis susceptibility of arteries by regulating the composition of the arterial matrix and its ability to sequester cholesterol-rich lipoproteins. Furthermore, we have identied a sorting receptor for proinflammatory cytokines that facilitates the development of atherosclerosis in mice.



Retention of red fluorescencent lipoproteins in an area exposed to experimentally induced atherogenic blood flow. The plane (left) indicates the location of the section shown (right).



Fibroatheromatous atherosclerosis induced by injecting a PCSK9-encoding recombinant virus in a mouse.



Lack of sortilin in circulating immune cells impairs the development of atherosclerosis in hypercholesterolemic Apoe-deficient mice.

MAJOR GRANTS

- Det Frie Forskningsråd, Sapere Aude Level II grant (DFF – 4004-00459). Funds held at Aarhus University.
- Novo Nordisk Fonden, Interdisciplinary Synergy grant (PI: Søren Moestrup). Funds held at University of Southern Denmark.

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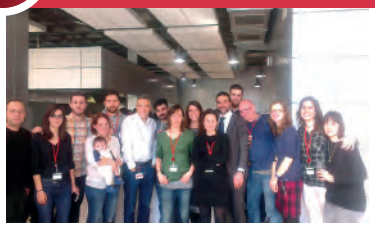
Bentzon JF, Otsuka F, Virmani R, Falk E. **Compendium review: Mechanisms of plaque formation and rupture.** *Circ Res* (2014) 114: 1852-66

Intercellular signalling in cardiac development & disease

Head of Laboratory
José Luis de la Pompa



RESEARCH INTEREST

We study the molecular mechanisms that regulate heart development, as we believe that this is an essential step toward understanding congenital heart disease and the eventual design of therapies to treat it. In the last year we have studied the role of various intercellular signals in chamber and valve development and cardiac regeneration, with the ultimate goal of identifying new molecular markers of cardiac disease or processes eventually amenable to therapeutic intervention.

During ventricular chamber development, the Notch signaling pathway first connects chamber endocardium and myocardium to sustain trabeculation. Notch signalinng later coordinates ventricular patterning and compaction with coronary vessel development to generate the mature chamber, via a temporal sequence of ligand signaling determined by the glycosyltransferase Manic Fringe (MFng). Early endocardial expression of MFng promotes Dll4-Notch1 signaling, which induces trabeculation in the developing ventricle. Ventricular maturation and compaction require MFng and Dll4 downregulation in the endocardium, which allows myocardial Jag1 and Jag2 signaling to Notch1 in this tissue. Perturbation of this signaling equilibrium severely disrupts heart chamber formation (Figure 1).

During cardiac valve development, endocardial Dll4-Notch1 signaling leads to epithelial-mesenchyme transition (EMT) and formation of the valve primordia. Later, Jag1-Notch1 signaling restrains Bmp-mediated valve mesenchyme proliferation by sustaining Hbegf-EGF receptor signaling. Our studies identify a mechanism of signaling crosstalk during valve morphogenesis implicated in the origin of congenital heart defects associated with reduced NOTCH function (Figure 2).

We also investigate the behavior, morphology and role of the endocardium during zebrafish cardiac regeneration. Time-lapse 3D-whole mount imaging in adult zebrafish hearts reveals a highly dynamic endocardium: spared endocardial cells remain after cryoinjury and proliferate shortly after cryoinjury, showing strongly upregulated Notch signaling (Figure 3). Endocardial cells expand within the injury site and form a structure that persists throughout regeneration. Examination of cardiomyocyte dynamics in Notch gain- and loss-of-function models reveals that Notch promotes cardiomyocyte proliferation. Notch signaling is thus a key regulator of endocardial gene expression and morphology.

Research Scientist:
Donal MacGrogan

Postdoctoral Researchers:
Beatriz Martínez Poveda
Belén Prados
Tania Papoutsi

Predoctoral Researchers:
Juliane Münch
Gaetano D'Amato
Dimitrios Grivas
Stanislao Igor Travisano
Paula Gómez Apiñaniz
Marcos Siguero Álvarez
Rebeca Torregrosa Carrión

Masters Student:
Alejandro Salguero

Technicians:
Patricia Martínez Martín
Vanessa Bou Pérez
Abel Galicia Martín
Sara Perruca Magro

Visiting Scientists:
José María Pérez-Pomares

Res@CNIC Fellow:
Adolfo Sobrino Balandrón

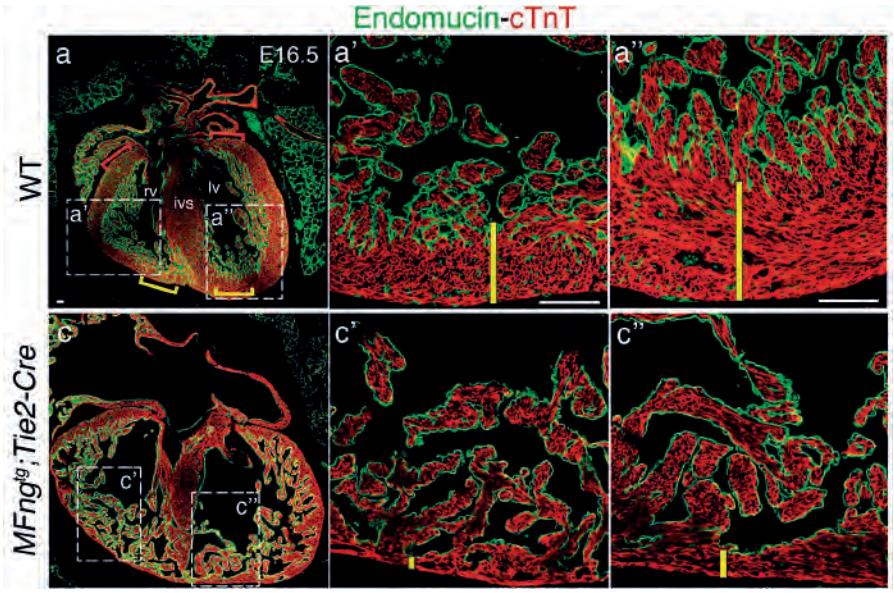


Figure 1.
Notch signaling abrogation disrupts compaction. E16.5 heart sections stained with endomucin (green) and cTnT antibodies (red) to delineate chamber endocardium and myocardium. The WT heart (**a-a''**) has a thick, cTnT-positive compact myocardium in both ventricles, with compacting trabeculae covered by endomucin-positive endocardium. The *MFng^{tg};Tie2-Cre* heart (**c-c''**) has a very thin compact myocardium, uncompacted trabeculae and a disrupted ventricular septum. The yellow bar indicates the thickness of compact myocardium. Scale bar=100µm.

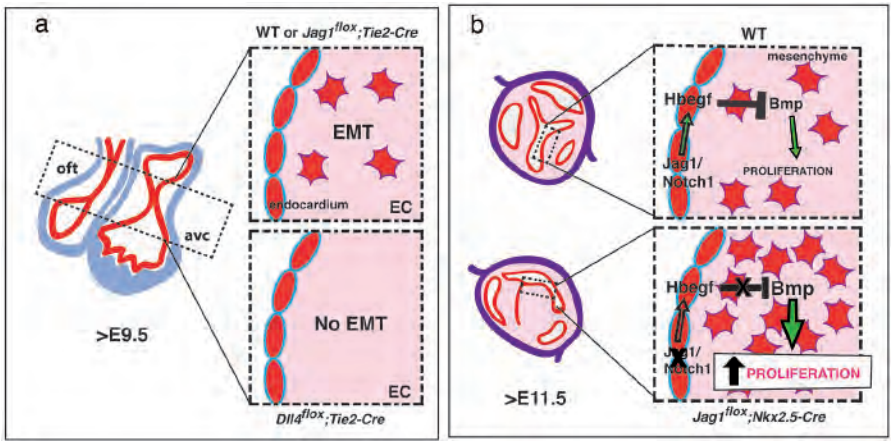


Figure 2.
Regulation of valve primordium formation and morphogenesis by sequential ligand-dependent Notch activation. **(a)** Endocardial Dll4, but not Jag1, is required for EMT. **(b)** Endocardial Jag1 is required for post-EMT valve morphogenesis. In WT valves, Jag1-Notch1 signaling restricts mesenchyme cell proliferation by downregulating Bmp signaling via Hbegf. *Jag1^{flox};Nkx2.5-Cre* mutants have reduced *Hbegf* expression, resulting in increased Bmp signaling and uncontrolled proliferation.

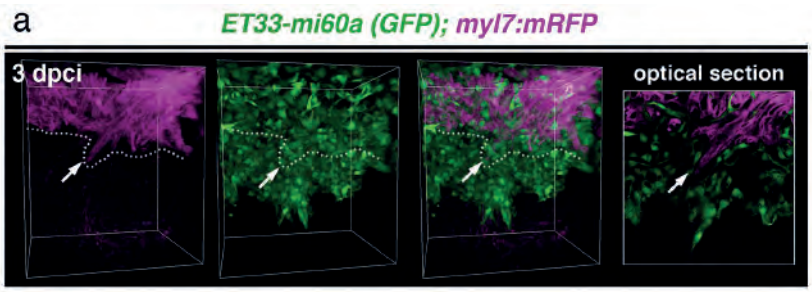


Figure 3.
In the regenerating zebrafish heart the endocardium expands within the injury site and precedes regeneration of the myocardium. **(a)** Volume rendering and an optical section of a region of the inner injury border in an injured *ET33mi-60A; myl7mRFP* ventricle at 3 dpci, with endocardium labeled green and myocardium magenta. Dense endocardial cells (green) surround migrating cardiomyocytes (magenta, white arrowhead) and precede them into the injury site. The dotted line demarcates the regenerating myocardium.

MAJOR GRANTS

- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600) (Coordinador E. Lara)*
- *Ministerio de Economía y Competitividad. Red de excelencia Temática (SAF2015-71863-REDT)*
- *Ministerio de Economía y Competitividad. FIS RETICS (TERCEL: RD12/0019/0003 and RIC: RD12/0042/0005)*
- *Ministerio de Economía y Competitividad (SAF2013-45543-R)*
- *Fundación BBVA (2015-2017)*
- *Fundación La Marató (2016-2018)*

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MacGrogan D, Luxán G, Driessen-Mol A, Bouten C, Baaijens F, de La Pompa JL **How to Make a Heart Valve: From Embryonic Development to Bioengineering of Living Valve Substitutes**. *Cold Spring Harb Perspect Med* (2014) 4: a013912

Matrix metalloproteinases in angiogenesis and inflammation

Head of Laboratory
Alicia G. Arroyo

RESEARCH INTEREST

In order for the vasculature to optimally deliver nutrients and oxygen throughout the body, endothelial cells must adapt to varying tissue needs, and this results in a high degree of vascular heterogeneity. However, we still know relatively little about the mechanisms that govern capillary patterning in homeostasis and upon injury. Our group is dedicated to elucidating how the microvascular network responds to inflammation and contributes to tissue repair. Our research focuses on membrane-type matrix metalloproteinases, endopeptidases able to perform proteolytic and also non-proteolytic actions. Our previous work showed that the protease MT1-MMP plays a key role in angiogenesis and inflammation by processing extracellular matrix components or regulating intracellular signals, such as Rac1 pathway components. We have also shown that the protease MT4-MMP is essential for proper arterial vascular function through its cleavage of osteopontin. Our recent in vivo data suggest that these proteases have different actions in endothelial cells and macrophages and that targeting MT1-MMP versus MT4-MMP has distinct tissue- and context-dependent impacts on the microvasculature during inflammation. Our laboratory is currently investigating: i) MT1-MMP and MT4-MMP actions in the cardiac microvasculature; and ii) the role of macrophage-secreted MT1-MMP or MT4-MMP substrates in the vascular response during cardiac repair.

We are pursuing these goals using 2D and 3D angiogenic models, high- and super-resolution microscopy, 3D image analysis, proteomics, bioinformatics, protein modeling, lentiviral strategies and genetically modified mouse lines. We ultimately intend to apply this knowledge to develop novel angiotherapies aimed at enhancing capillary perfusion and tissue performance in several pathophysiological contexts.

Research Scientist:
Pilar Gonzalo

Postdoctoral Researchers:
Vanessa Moreno
Susana Rocha
Lorenzo Veschini
(until March 2015)

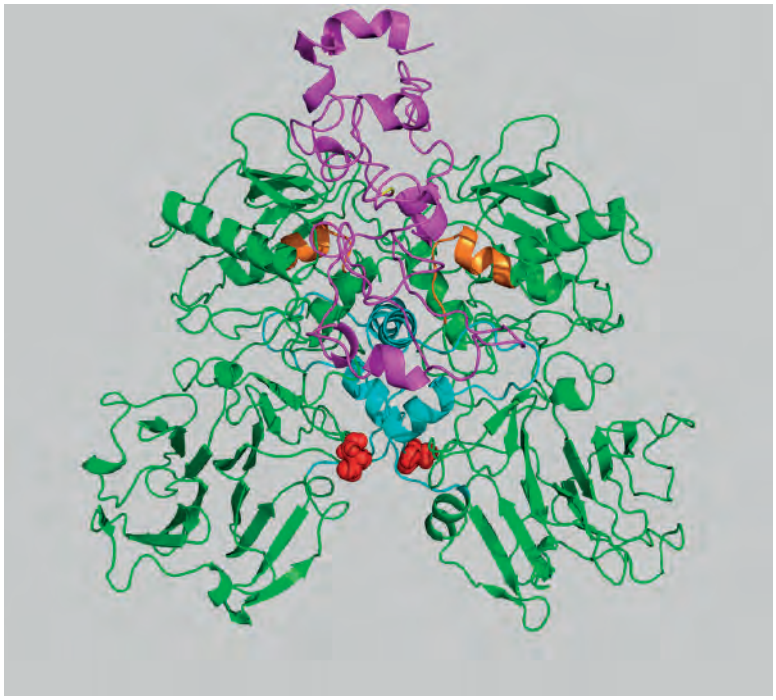
Predoctoral Researchers:
Cristina Clemente
Sergio Esteban
Polyxeni Gkontra
Jesús Gómez Escudero
Mara Martín Alonso
Magdalena Maria Zak
Álvaro Sahún

Technicians:
Laura Balonga
Ángel Colmenar

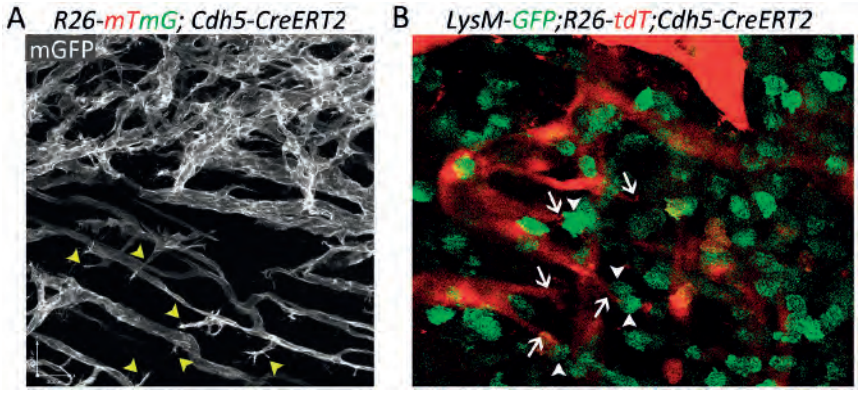
Visiting Scientist:
Cristina Sánchez-Camacho

Masters Student:
Ricardo Santamaría

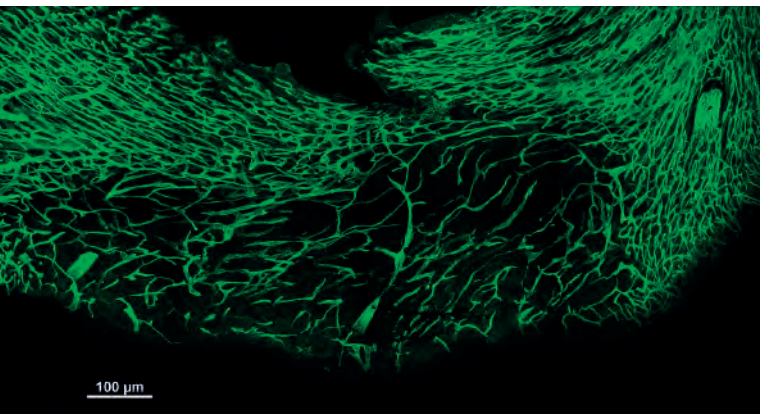
Project Manager:
Lilit Manukyan
(CardioNext ITN)



In silico modeling of substrate/protease docking. In collaboration with the Bioinformatics Unit, we use in silico modeling to explore the accessibility of potential novel substrates to the catalytic sites of membrane type-matrix metalloproteinases. The image shows the docking model of osteopontin (purple) with the human protease MT4-MMP homodimer (green); note that osteopontin is located close to the MT4-MMP catalytic center (orange; see Martín et al., 2015, for more details). In silico modeled cleavage sites are then experimentally validated.



Exploring macrophage/vascular communication in vivo. The dorsal skinfold chamber model allows direct visualization of macrophages and endothelial cells during inflammatory angiogenesis. Confocal imaging of genetically-labeled mice shows in vivo capillary sprouting (arrowheads in A), and macrophages labeled with GFP (arrowheads in B) interacting with Tomato-expressing endothelial sprouts (arrows in B) in response to TNFα in the skin vasculature.



3D confocal microscopy image analysis of the cardiac microvasculature. 3D-volumetric composition of confocal microscopy images from thick heart sections allows the visualization and analysis of the cardiac microvasculature with unprecedented resolution. The image shows the maximal projection of multiple images acquired from thick heart sections (60 µm) and stained for the endothelial cell marker ICAM-2 (green). The heart in the image is from a newborn mouse 5 days after cryoinjury; note that the affected area has a reduced vascular density.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2014-52050-R)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0023)*
- *Comunidad Autónoma de Madrid. Redes de Excelencia. ANGIOBODIES 2.0 (S2010/BMD-2312)*
- *Fundació La Marató TV3 (165/C/2012)*
- *European Union (PITN-GA-2013-608027) (CardioNext) (Coordinator)*

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Regulatory molecules of inflammatory processes

Head of Laboratory
Pilar Martín Fernández

RESEARCH INTEREST

Our group studies the control of inflammation in autoimmune and cardiovascular diseases. Recently, our attention has focused on the potential of miRNAs derived from Th17 or regulatory T (Treg) cells in the design of strategies to combat and diagnose these diseases. Much of our work is conducted in mouse models of myocarditis and peripheral post-ischemic neovascularization, a model of peripheral artery disease (PAD). The true incidence of myocarditis is unknown because it is frequently first diagnosed as non-ischemic dilated cardiomyopathy; PAD affects 1 in 3 people aged 70 years or above. These diseases can easily become chronic and life-threatening, and are associated with devastating long-term side effects and high medical costs. Inadequate understanding of Th17 and Treg biology is an obstacle to the development of immunotherapy protocols for these diseases. Our recent work characterized the role of Th17 cells and Tregs in heart, skin and lung inflammatory diseases. Our current work focuses on these antagonistic T cell subsets, expressing Rorγt^{+} and Foxp3^{+}, and their central role in the control of inflammation. Defects in the development or function of these cells exacerbates autoimmune and cardiovascular disorders.

We are also interested in the role of Th17 cells and Tregs in the rejection of allogenic grafts and heart transplants. Several clinical trials are currently using Tregs to ameliorate the effects of graft-versus-host disease (GvHD) in hematologic cancer and leukemia patients. However, the therapeutic use of Tregs in transplant recipients is still under development, and new markers and protocols are needed for their correct identification, purification and expansion.

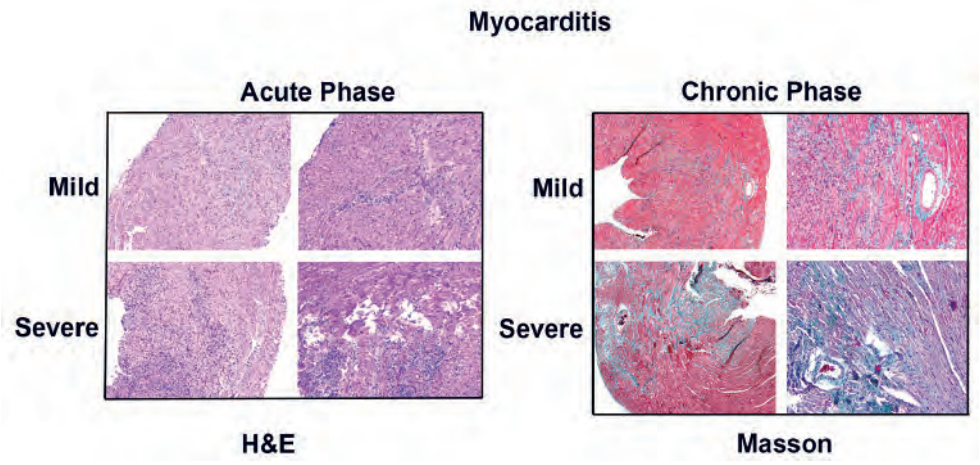
Postdoctoral Researcher:
Aikaterini Tsilingiri

Predoctoral Researchers:
Raquel Sánchez Díaz
Marta Relaño Orasio

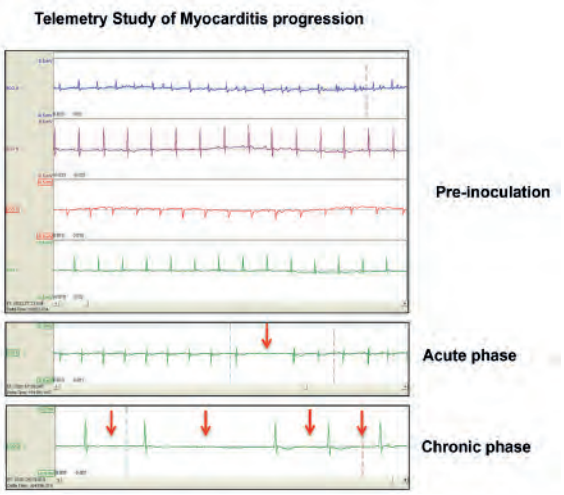
Masters Students:
Rafael Blanco Domínguez
Avinash Londhe

Technicians:
Juan José Lazcano Duque
Sandra Lasarte Ramiro
Irene García Fernández

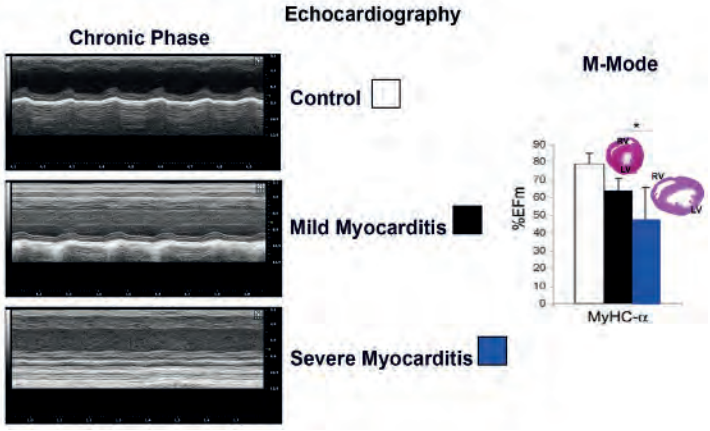
Visiting Scientists:
Beatriz Linillos
Miguel Fernández de la Torre
Daniel García Rivas



Histological analysis of myocarditis. *Left*. Hematoxylin and Eosin staining of heart sections from mice in the acute phase of experimental autoimmune myocarditis (EAM), 21 days after MyHC-α peptide immunization. Small and large infiltrates are shown in mice with mild or severe EAM. *Right*. Masson's trichrome staining reveals enhanced fibrosis in mice with severe manifestation of myocarditis in the chronic phase (56 days after MyHC-α peptide immunization). Right panels; high-power views of infiltrates and collagen deposition in the left ventricle (magnification x 100).



Electrophysiological study with implantable telemetry. Representative telemetrically recorded ECGs from mice during the progression of myocarditis, analogous to Holter monitoring in humans. The ECGs were obtained from mice fitted with implanted wireless radiofrequency transmitters implanted into the abdominal cavity. Recordings were performed before inoculation with MyHC-α peptide and during the acute and chronic phases of EAM. Telemetry reveals a progressive increase in the number of sinus pauses (indicated by red arrows) in parallel with disease progression.



Cardiac function in the chronic phase of myocarditis. M-mode transthoracic echocardiography of left ventricular function reveals a reduction in cardiac contractility in mice with mild or acute myocarditis. Left ventricular fractional shortening (not shown) and ejection fraction (EF) were both significantly smaller than in controls (nonimmunized mice). RV; right ventricle, LV; left ventricle.

MAJOR GRANTS

- Comunidad de Madrid. Redes de Excelencia. INDISNET (S2010/BMD-2332)
- Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)

SELECTED PUBLICATIONS

Fabbiano S, Menacho-Márquez M, Robles-Valero J, Pericacho M, Matesanz-Marín A, García-Macías C, Sevilla MA, Montero MJ, Alarcón B, López-Novoa JM, Martín P, Bustelo XR. **Immunosuppression-Independent Role of Regulatory T Cells against Hypertension-Driven Renal Dysfunctions.** *Mol Cell Biol* (2015) 35 :3528-46

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B lymphocyte biology

Head of Laboratory
Almudena R Ramiro

RESEARCH INTEREST

B cells lead the humoral immune response thanks to the generation of a vast collection of antibodies that can specifically recognize pathogens and tag them for removal. The very same mechanisms responsible for antibody diversity have profound biomedical implications in autoimmunity, immunodeficiency, and cancer.

Our lab is focused on the molecular and cellular events that take place in germinal centers—microstructures generated by B cells during immune responses. Our interests cover basic aspects of B cell biology, including the DNA remodeling associated with antibody diversification by the enzyme AID in germinal centers, the regulatory programs driven by microRNAs in germinal centers, and the generation of animal models to explore the impact of these events on the etiology of disease, most notably in inflammation and cancer.

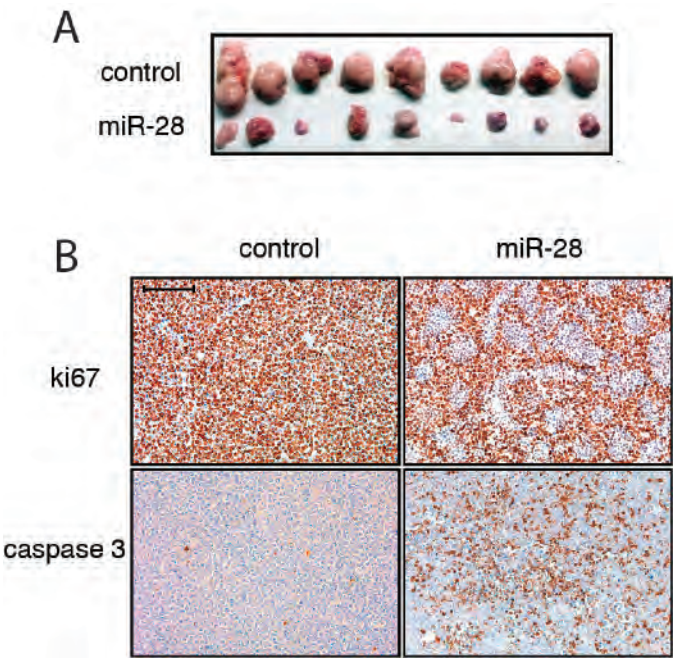
Our recent work has shown that microRNAs contribute to immune tolerance, and that individual microRNAs play critical roles in the regulation of germinal centers and can act as oncogenes (miR217) or tumor suppressors (miR28). In addition, we have developed mouse models to study different regulatory aspects of AID activity in vivo. Finally, we are characterizing the functional contribution of antibodies and their diversification to atherogenesis.

Research Scientists:
Virginia G de Yébenes
María Pilar Delgado

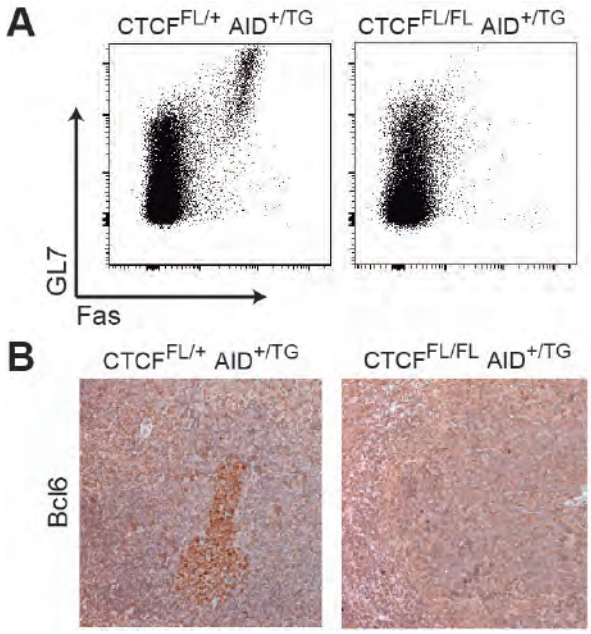
Predoctoral Researchers:
Nahikari Bartolomé
Arantxa Pérez-García
Ángel F. Álvarez
Cristina Lorenzo
Ester Marina

Technician:
Sonia Mur
Dobromira Veselinova

Student:
Pablo Garzón



miR28 impairs B cell lymphoma growth. A) Xenograft tumors were generated with Burkitt lymphoma cells lentivirally transduced with either miR-28 or a scramble construct; cells were injected in the flank of immunodeficient recipient mice. The picture shows representative images of tumors after 21 days of growth. B) miR-28 impairs proliferation and survival of lymphoma cells. Xenograft tumors were stained with Ki67 to monitor proliferation (upper panels) and caspase-3 (lower panels) to monitor apoptosis.



CTCF is required for germinal center generation. A) CTCF-deficient germinal center B cells were generated by crossing mice bearing CTCF-floxed alleles ($CTCF^{FL/FL}$) with mice expressing an AID-driven Cre recombinase ($AIDCre^{+/TG}$). Germinal centers were generated by promoting a T dependent response in mice and analyzed by flow cytometry of Fas+GL7+ cells from spleen. Representative plots are shown of control mice ($CTCF^{FL/FL}$ $AIDCre^{+/TG}$, left) and CTCF-deficient mice ($CTCF^{FL/FL}$ $AIDCre^{+/TG}$, right). B) Representative immunoghistochemical staining with the germinal center marker BCL6 in the same spleens as in A.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2013-42767-R)*

SELECTED PUBLICATIONS

Pérez-García A, Pérez-Durán P, Wossning T, Sernandez IV, Mur SM, Cañamero M, Real FX, Ramiro AR. **AID-expressing epithelium is protected from oncogenic transformation by an NKG2D surveillance pathway**. *EMBO Mol Med* (2015) 7: 1327-36.

Ramiro AR, Barreto VM. **Activation-induced cytidine deaminase and active cytidine demethylation**. *Trends Biochem Sci*. (2015) 40: 172-81

de Yébenes VG, Bartolomé-Izquierdo N, Nogales-Cadenas R, Pérez-Durán P, Mur SM, Martínez N, Di Lisio L, Robbiani DF, Pascual-Montano A, Cañamero M, Piris MA, Ramiro AR. **miR-217 is an oncogene that enhances the germinal center reaction.** *Blood* (2014) 124: 229-39

Gene regulation in cardiovascular remodelling and inflammation

Head of Laboratory
Juan Miguel Redondo Moya

RESEARCH INTEREST

Much of our work centers on the regulation of calcineurin (CN) signaling in angiogenesis and inflammation. In recent years, we have characterized the mechanisms and sequences involved in the interactions of CN with NFAT and other substrates and with immunosuppressive drugs, and we have characterized how specific CN targeting modulates inflammatory responses. More recently, we have studied mediators in vascular and cardiac remodeling related to Angiotensin II and CN pathways. We are currently elucidating the mechanisms that mediate this remodeling and have generated conditional mice deficient for CN and Rcan1 isoforms in the endothelial, vascular smooth muscle, and cardiomyocyte compartments. We have already identified genes regulated by CN in different mouse models of cardiac hypertrophy (CH), and are characterizing their roles in CH using conditional cardiac CN and Rcan1mice. We are also elucidating the role of Chd4/NuRD in cardiac homeostasis and have found that the NuRD complex determines skeletal muscle identity by silencing the skeletal muscle program in cardiomyocytes and the cardiac program in skeletal muscle. Another major area of interest is the mechanisms that mediate aortic diseases such as familial forms of thoracic aortic aneurysm and dissection (TAAD), including Marfan syndrome. We have identified a number of mediators that play a major role in the pathogenesis of these diseases, and we are now characterizing the underlying mechanisms.

Research Scientists:
Sara Martínez Martínez
Pablo Gómez del Arco

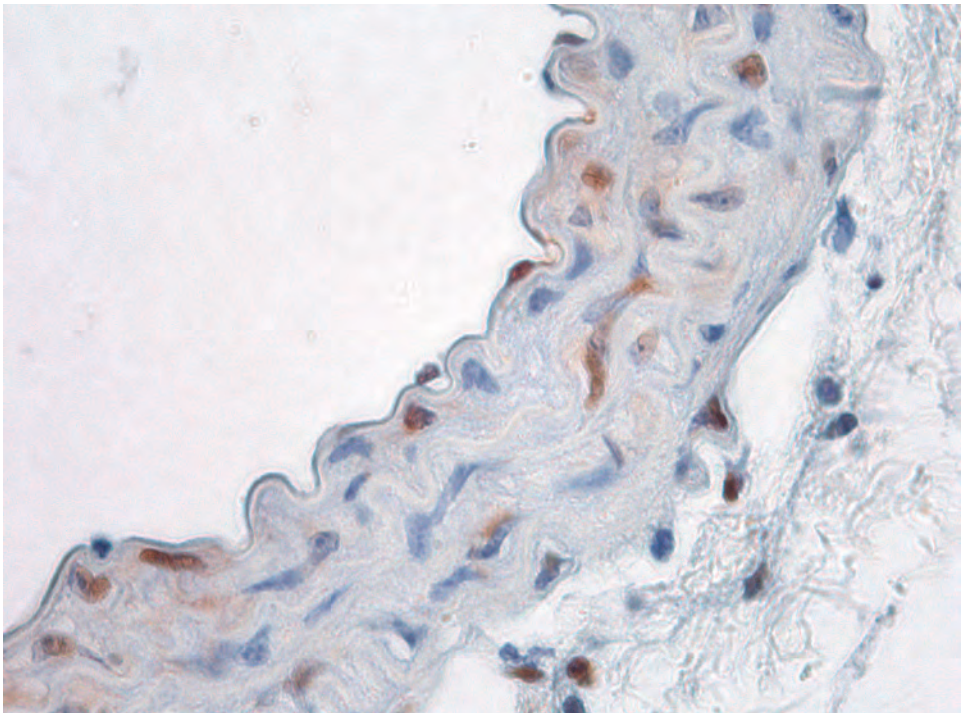
Postdoctoral Researchers:
Nerea Méndez Barbero

Predoctoral Researchers:
Yuri Chiodo
Jorge Oller Pedrosa
Silvia Villahoz
Paula Sofía Yunes Leites

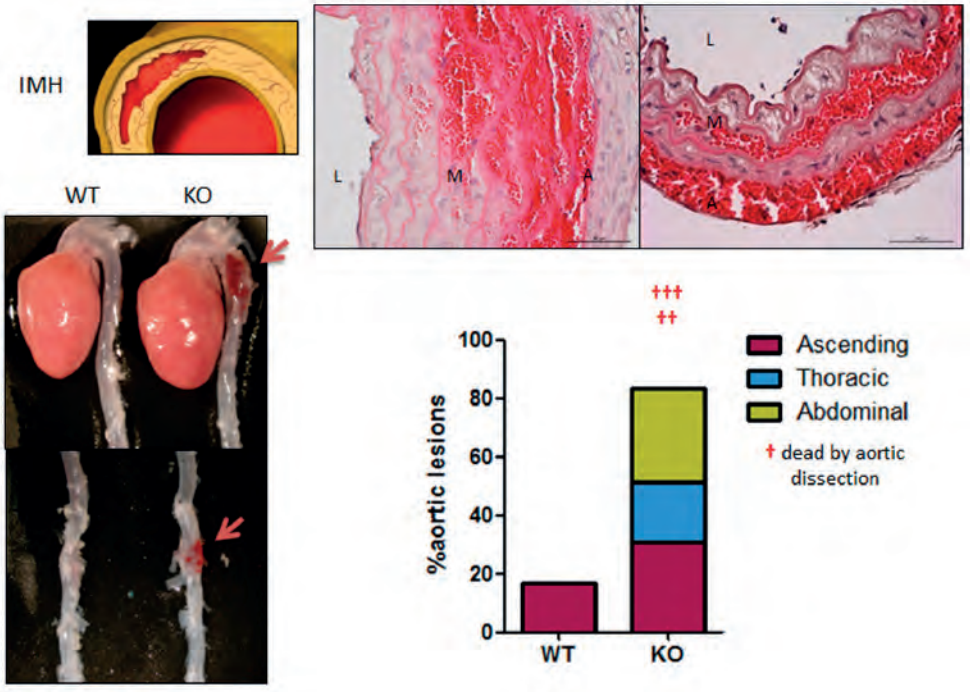
Student:
Lizet Sandra Iturri Canelas

Technicians:
Dolores López Maderuelo
Rut Alberca Rodríguez
Beatriz Carolina Ornés Poleo
Alicia Peral Rodríguez

Visiting Scientists:
Ángel Luis Armesilla Arpa
Miguel Ramón Campanero García



Ang-II induces C/EBPβ activation in the aorta. Inmunostaining of phosphorylated C/EBPβ (brown) in aortic tissue of a mouse infused with Ang-II. Nuclei are counterstained in blue.



Mouse model of aortic intramural hematoma (IMH) induced by vascular pathological stimuli. This phenotype has been identified in several mouse models of deficiency for recently identified targets in vascular wall remodeling. Intramural hematomas can develop into life-threatening aortic dissections.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2012-34296)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0022)*
- *Fundació La Marató TV3 (264/C/2012) (PI: Sara Martínez)*

SELECTED PUBLICATIONS

Oller J, Alfranca A, Méndez-Barbero N, Villahoz S, Lozano-Vidal N, Martín-Alonso M, Arroyo AG, Escolano A, Armesilla AL, Campanero MR*, Redondo JM*. **C/EBPβ and Nuclear Factor of Activated T Cells Differentially Regulate Adamts-1 Induction by Stimuli Associated with Vascular Remodeling.** *Mol Cell Biol* (2015) 35:3409-22
* Co-corresponding authors

Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Alfranca A, Méndez-Barbero N, Pollán Á, Sánchez-Camacho C, Denhardt DT, Seiki M, Vázquez J, Salaices M, Redondo JM, Milewicz D, Arroyo AG. **Deficiency of MMP17/MT4-MMP proteolytic activity predisposes to aortic aneurysm in mice**. *Circ Res* (2015) 117:e13-26

Baggott RR, Alfranca A, López-Maderuelo D, Mohamed TM, Escolano A, Oller J, Ornes BC, Kurusamy S, Rowther FB, Brown JE, Oceandy D, Cartwright EJ, Wang W, Gómez-del Arco P, Martínez-Martínez S, Neyses L, Redondo JM*, Armesilla AL*. **Plasma membrane calcium ATPase isoform 4 inhibits vascular endothelial growth factor-mediated angiogenesis through interaction with calcineurin.** *Arterioscler Thromb Vasc Biol* (2014) 34: 2310-20

Sánchez SA, Méndez-Barbero N, Santos-Beneit AM, Esteban V, Jiménez-Borreguero LJ, Campanero MR*, Redondo JM*. **Nonlinear optical 3-dimensional method for quantifying atherosclerosis burden.** *Circ Cardiovasc Imaging* (2014) 7: 566-9
* Co-corresponding authors

Escolano A, Martínez-Martínez S, Alfranca A, Urso K, Izquierdo HM, Delgado M, Martín F, Sabio G, Sancho D, Gómez-del Arco P and Redondo JM. **Specific calcineurin-targeting in macrophages confers resistance to inflammation via MKP-1 and p38**. *EMBO J* (2014) 33:1117-33

CNIC-UAM COLLABORATIVE PROGRAM Intercellular communication in the inflammatory response

Head of Laboratory

Francisco Sánchez Madrid

RESEARCH INTEREST

The group pursues three main lines of research.

1) **Regulation of immune synapse formation and function**. We are exploring protein multiplexing at the MTOC, specifically the role of MTOC folding complexes, and the post-translational modifications of Ser/Thr kinases and the tubulin deacetylase HDAC6. We address the molecular mechanisms that control mitochondria transport during leukocyte-endothelial adhesion and extravasation and maturation of the IS. We are also analyzing the role of mitochondrial components in the biogenesis and secretion of exosomes and their impact on macrophage and dendritic cell function.

2) **Fine tuning of T cell biology by miRNAs and exosomes**. The production of exosomes by different T cell subsets is being examined with the aim of identifying and characterizing specific miRNAs delivered to target cells. We also investigate the molecular mechanisms underlying the specific sorting of proteins and miRNAs to exosomes. This information may allow engineering of immune cells to produce exosomes able to specifically modulate the immune response.

3) **Immunoregulatory molecules and miRNAs in inflammatory diseases**. We are analyzing the role of immunoregulatory molecules such as CD69, galectins, aminoacid transporters and HDAC6 in animal models of atherosclerosis and psoriasis in humans in order to identify the molecular basis of these inflammatory diseases.

Research Scientists:
Gloria Martínez del Hoyo
María Mittelbrunn

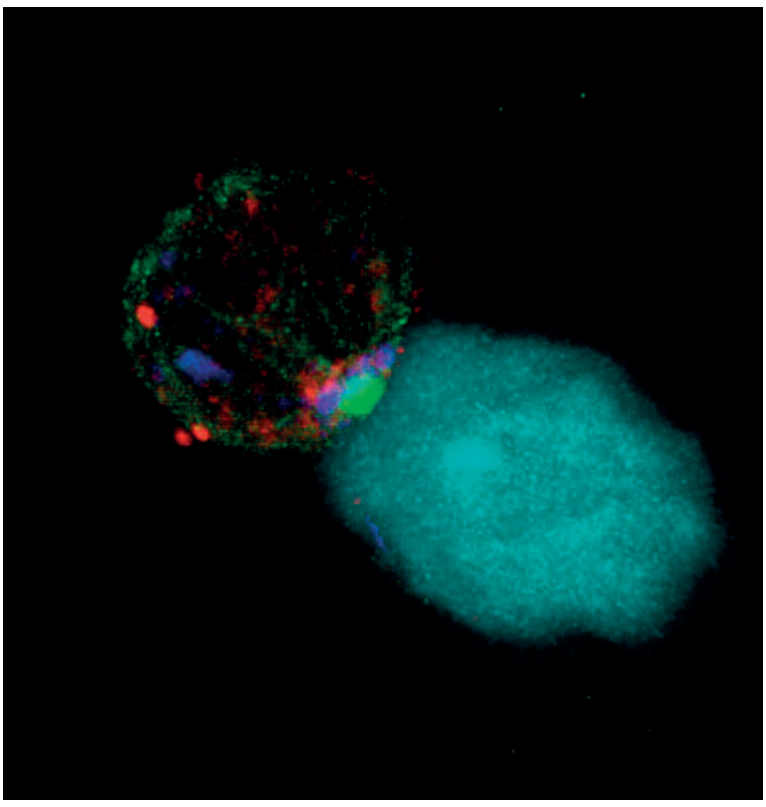
Postdoctoral Researchers:
Hortensia de la Fuente
Noa B. Martín
Vera Rocha
Danay Cibrián
Lola Fernández Messina

Predoctoral Researchers:
Francesc Baixauli
Cristina Gutiérrez
Giulia Morlino
Norman Núñez
Mª Laura Saiz
Carolina Villarroya
Olga Moreno
Noelia Blas
Eugenio Bustos
Daniel Torralba
José Pintor

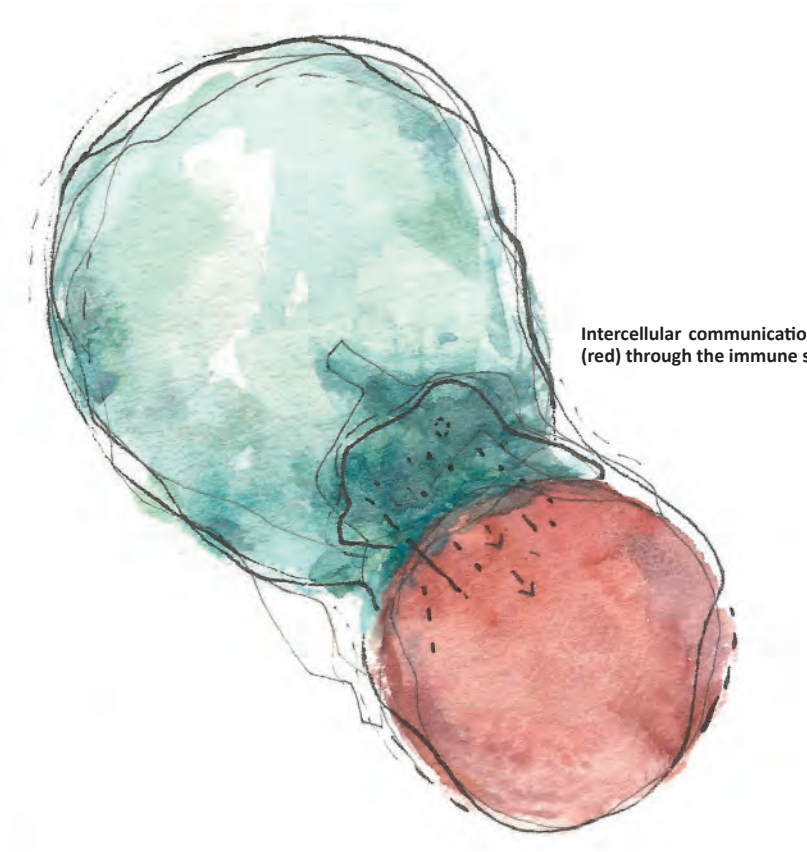
Technicians:
Marta Esther Ramírez
María José López

Visiting Scientists:
María Navarro
Laura Martínez Muñoz
Aránzazu Cruz

Student:
Irene Fernández Delgado



HDAC6 regulates lytic granule localization at the immune synapse.



Intercellular communication between a T cell (blue) and an antigen-presenting cell (red) through the immune synapse. Illustration by Lucía Gutiérrez Vázquez.

MAJOR GRANTS

- *European Commission. ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)*
- *Ministerio de Economía y Competitividad (SAF2014-55579-R)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)*
- *Comunidad de Madrid. Redes de Excelencia. INDISNET (P2010/BMD-2332)*
- *Ministerio de Economía y Competitividad. FIS (PI11/00939) PI: Gloria Martínez del Hoyo*

SELECTED PUBLICATIONS

Mittelbrunn M, Vicente-Manzanares M, Sánchez-Madrid F. **Organizing polarized delivery of exosomes at synapses.** *Traffic* (2015) 16: 327-37

Baixauli F, Acín-Pérez R, Villarroya-Beltrí C, Mazzeo C, Nuñez-Andrade N, Gabandé-Rodriguez E, Ledesma MD, Blázquez A, Martin MA, Falcón-Pérez JM, Redondo JM, Enríquez JA, Mittelbrunn M. **Mitochondrial Respiration Controls Lysosomal Function during Inflammatory T Cell Responses**. *Cell Metab* (2015) 22:485-98

Martínez del Hoyo G, Ramírez-Huesca M, Levy S, Boucheix C, Rubinstein E, Minguito de la Escalera M, González-Cintado L, Ardavín C, Veiga E, Yáñez-Mó M, Sánchez-Madrid F. **CD81 controls immunity to Listeria infection through rac-dependent inhibition of proinflammatory mediator release and activation of cytotoxic T cells.** *J Immunol* (2015) 194:6090-101

Morlino G, Barreiro O, Baixauli F, Robles-Valero J, González-Granado JM, Villa-Bellosta R, Cuenca J, Sánchez-Sorzano CO, Veiga E, Martín-Cófreces NB, Sánchez-Madrid F. **Miro-1 links mitochondria and microtubule dynein motors to control lymphocyte migration and polarity**. *Mol Cell Biol* (2014) 34:1412-26

de la Fuente H, Cruz-Adalia A, Martinez Del Hoyo G, Cibrian-Vera D, Bonay P, Perez-Hernandez D, Vazquez J, Navarro P, Gutierrez-Gallego R, Ramirez-Huesca M, Martin P, Sanchez-Madrid F. **The Leukocyte Activation Receptor CD69 Controls T Cell Differentiation through Its Interaction with Galectin-1.** *Mol Cell Biol* (2014) 34: 2479-87

Cardiovascular proteomics

Head of Laboratory
Jesús María Vázquez Cobos

RESEARCH INTEREST

Our group works on the development of high-throughput quantitative approaches for the dynamic analysis of the deep proteome, which are being applied to basic and translational projects in the cardiovascular field. We are developing novel bioinformatics algorithms for the analysis of very large numbers of samples, including protein identification and systems biology interpretation of quantitative data, and for the study of posttranslational modifications (PTM).

Among other projects, we are using these approaches to explore the molecular mechanisms underlying the byphasic pattern of edema in the pig heart after infarction, and the preconditioning effect of treatments that ameliorate the heart damage produced by ischemia/reperfusion.

We have developed a novel data-independent mass spectrometry scanning technique (DiS) that improves on the performance of conventional shotgun approaches and also allows in-silico-targeted quantification of any suspected peptide, including PTMs. We are using an extension of this technique (Blue-DiS) to generate an extremely detailed structural map of components of mitochondrial oxidative phosphorylation supercomplexes in several models, which include characterization of novel factors and PTMs that modulate complex and supercomplex assembly.

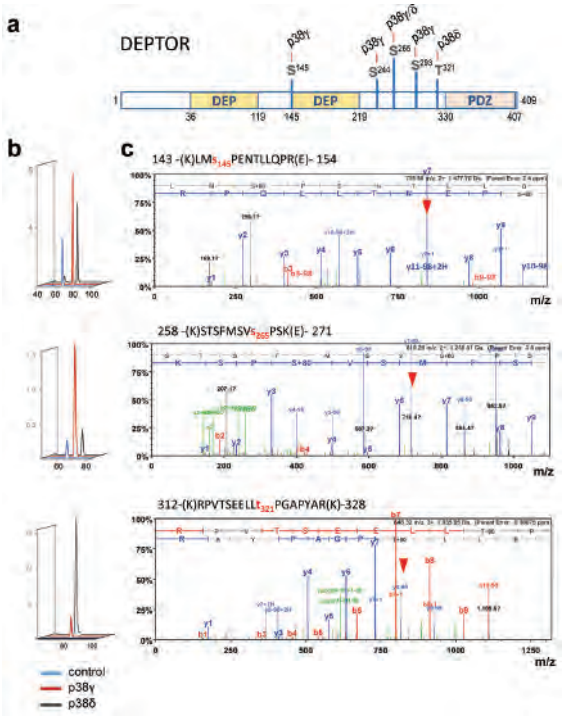
We are also performing translational studies in large cohorts of human samples to uncover molecular mechanisms and biomarkers of cardiovascular disease. We are currently undertaking a high-throughput proteomics analysis of plasma from participants in the PESA study, in the search for factors that correlate with the extent of subclinical atherosclerotic events such as calcium deposition and plaque formation. We are also setting up a mass spectrometry-based platform for targeted and hypothesis-free analysis of lipids and small metabolites.

Postdoctoral Researchers:
Estefanía Núñez Sánchez
Elena Bonzón Kulichenko
Inmaculada Jorge Cerrudo
Alessia Ferranini
Spyridon Michalakopoulos

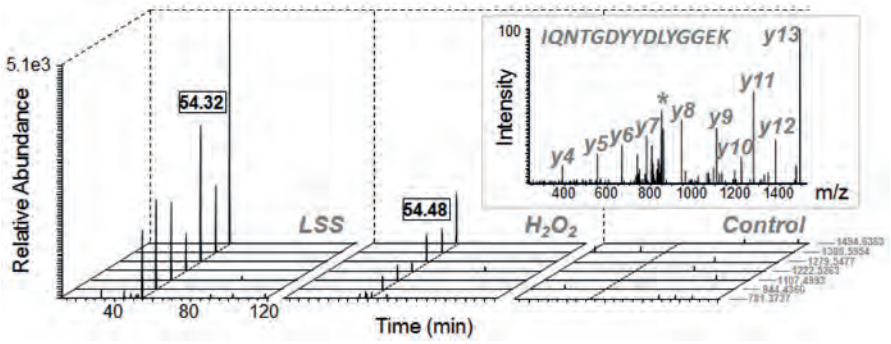
Predoctoral Researchers:
Fernando García Marqués
Marco Trevisan Herraz
Marta Loureiro
Navratan Bagwan
Aleksandra Ronja

Masters Students:
Celia Castañs García
Jesús Lavado García

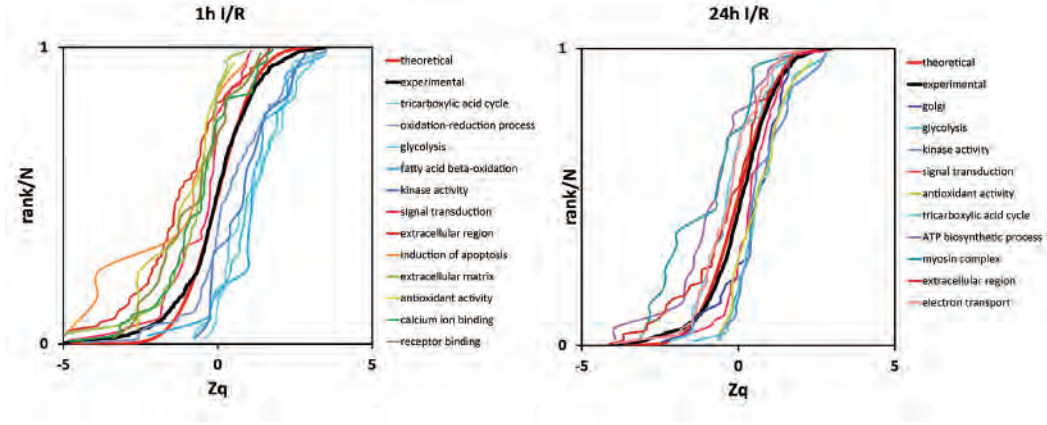
Visiting Scientists:
Elena Burillo
Diego Martínez López
Montserrat Baldán



Analysis of DEPTOR phosphorylation by p38γ and p38δ kinases *in vivo* by mass spectrometry. (a) Scheme of DEPTOR phosphorylation sites. (b) Quantitative analysis of phosphorylation. (c) MS/MS spectra of each phosphopeptide, showing its sequence and assignation of the modified site.



Phosphatase SHP2 is sulfenylated in conditions of laminar shear stress (LSS). Sulfenylated SHP2 was immunoprecipitated using a specific antibody and the amount of SHP2 was quantified by mass spectrometry. Seven of the fragments of the SHP2 peptide indicated in the inset were quantified, showing that sulfenylation of SHP2 takes place when the samples were treated with H_2O_2 or subjected to LSS.



Systems biology analysis of protein abundance changes in a pig infarct model. A high-throughput quantitative proteomics analysis was performed to compare heart tissue from infarcted and remote areas after 1h ischemia and 1h or 24 hreperfusion. The analysis revealed a clearly coordinated behavior of proteins belonging to the indicated functional categories.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (BIO2012-37926)*
- *Ministerio de Economía y Competitividad. FIS Proteored (PT13/0001/0017)*
- *Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)*
- *European Commission: 7th Framework Programme for Research (FP7-PEOPLE-ITN-2013)*
- *Progeria Research Fund Specialty Award (USA)*
- *Fundació La Marató de TV3*

SELECTED PUBLICATIONS

González-Terán B, López JA, Rodríguez E, Leiva L, Martínez-Martínez S, Bernal JA, Jiménez-Borreguero LJ, Redondo JM, Vazquez J, Sabio G. **p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation.** *Nat Commun* (accepted)

Osorio FG, Soria-Valles C, Santiago-Fernández O, Bernal T, Mittelbrunn M, Colado E, Rodríguez F, Bonzon-Kulichenko E, Vázquez J, Porta-de-la-Riva M, Cerón J, Fueyo A, Li J, Green AR, Freije JMP, López-Otín C. **Loss of the proteostasis modulator AIRAPL causes myeloid transformation by deregulating IGF-1 signaling.** *Nat Med* (doi:10.1038/nm.4013. Epub 2015 Dec 21)

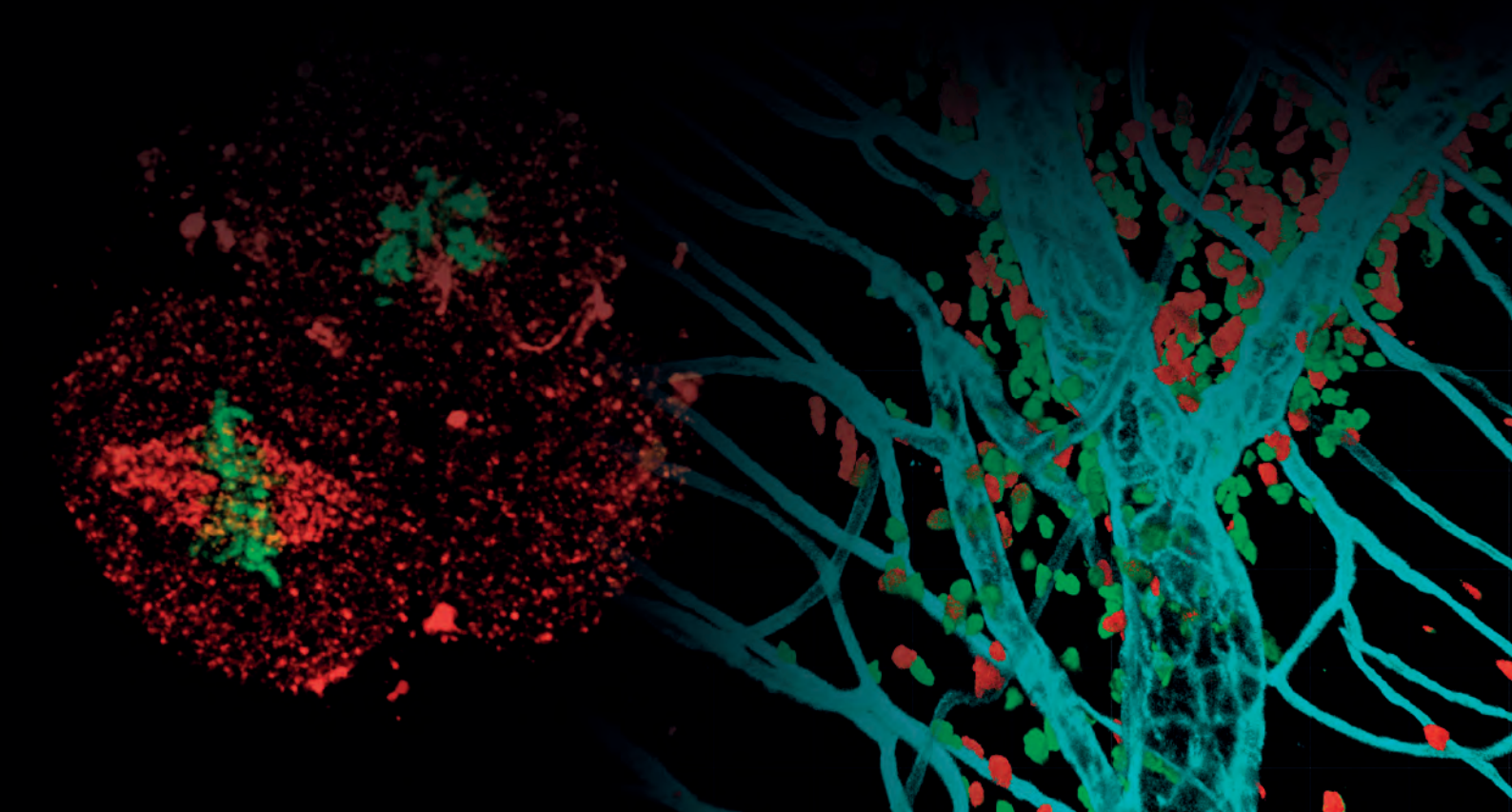
Mara Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Sánchez-Camacho C, Pollán Á, Alfranca A, Seiki M, Redondo JM, Vázquez J, Salaices M, Milewicz D, Arroyo AG. **Deficiency of MT4-MMP proteolytic activity causes a dilative arterial disorder.** *Circ Res* (2015) 117: e13-26

Bonzon-Kulichenko E, Garcia-Marques F, Trevisan-Herraz M, Vázquez J. **Revisiting peptide identification by high-accuracy mass spectrometry: problems associated to the use of narrow mass precursor windows.** *J Proteome Res* (2015) 14: 700-10

Navarro P, Trevisan-Herraz M, Bonzon-Kulichenko E, Núñez E, Martínez-Acedo P, Pérez-Hernández D, Jorge I, Mesa R, Calvo E, Carrascal M, Hernáez ML, García F, Bárcena JA, Ashman K, Abian J, Gil C, Redondo JM, Vázquez J. **General statistical framework for quantitative proteomics by stable isotope labeling.** *J Proteome Res* (2014) 13: 1234-47

RESEARCH AREAS

1. Myocardial Pathophysiology
2. Vascular Pathophysiology
3. **Cell and Developmental Biology**



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RESEARCH AREAS

Cell and Developmental Biology

Area coordinators:

Miguel Manzanares *Miguel Ángel del Pozo*

RESEARCH INTEREST

The Cell and Developmental Biology Area comprises 10 laboratories that conduct basic and translational research, ranging from mechanistic aspects of cell signaling and behavior to the principles of cardiovascular development. Research topics include the molecular and cellular embryology of the heart, mechanisms of tissue repair, the underpinnings of heart and vascular homeostasis, and how these aspects relate to disease. Specific research lines are aimed at understanding how temporally and spatially regulated transcriptional networks determine the very first cell fate decisions in the early embryo, as well as the different stages of heart development. Laboratories in the CDB Area also investigate processes important for cardiovascular homeostasis such as angiogenesis, inflammation, and regeneration. Finally, a number of research lines are aimed at elucidating key cell signaling pathways and molecular principles underlying the mechanical properties, function and adaptability of the cardiovascular system, using state-of-the-art cell biophysics and single-molecule techniques.

Single-molecule mechanobiochemistry

Head of Laboratory
Jorge Alegre-Cebollada

RESEARCH INTEREST

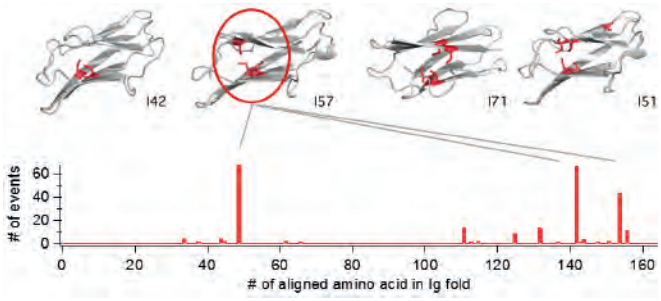
The elastic properties of the myocardium determine cardiac performance, and disruptions to these properties underlie diseases such as heart failure and cardiomyopathies, through mechanisms that we are just beginning to understand. In our group, we explore how the mechanics of specific structural proteins determines the macroscopic elasticity of the heart in health and disease. We follow a multidisciplinary approach to measure the mechanical properties of key cardiac proteins such as titin, and investigate how these mechanical properties are affected by posttranslational modifications and mutations that cause cardiomyopathies. During 2015, we set up methods based on mass spectrometry and fluorescent polyacrylamide gels that allow us to monitor redox posttranslational modifications that target titin and other cardiac proteins. We have detected a strong oxidation signal coming from 3 conserved cysteines in the immunoglobulin domains of titin that can engage in disulfide bonds (Figure 1). We also brought the CNIC's first single-molecule atomic force microscope (AFM) into service. This instrument is designed to examine the mechanical properties of proteins, and we have used it to measure the mechanics of protein domains whose mutation gives rise to hypertrophic cardiomyopathy (Figure 2). Also in 2015, we started a new line of research to engineer protein biomaterials with tailored and regulatable mechanical properties that can be predicted from the molecular mechanics of the constituent proteins. These novel materials could find application in tissue engineering and repair.

Postdoctoral Researcher:
Elías Herrero-Galán

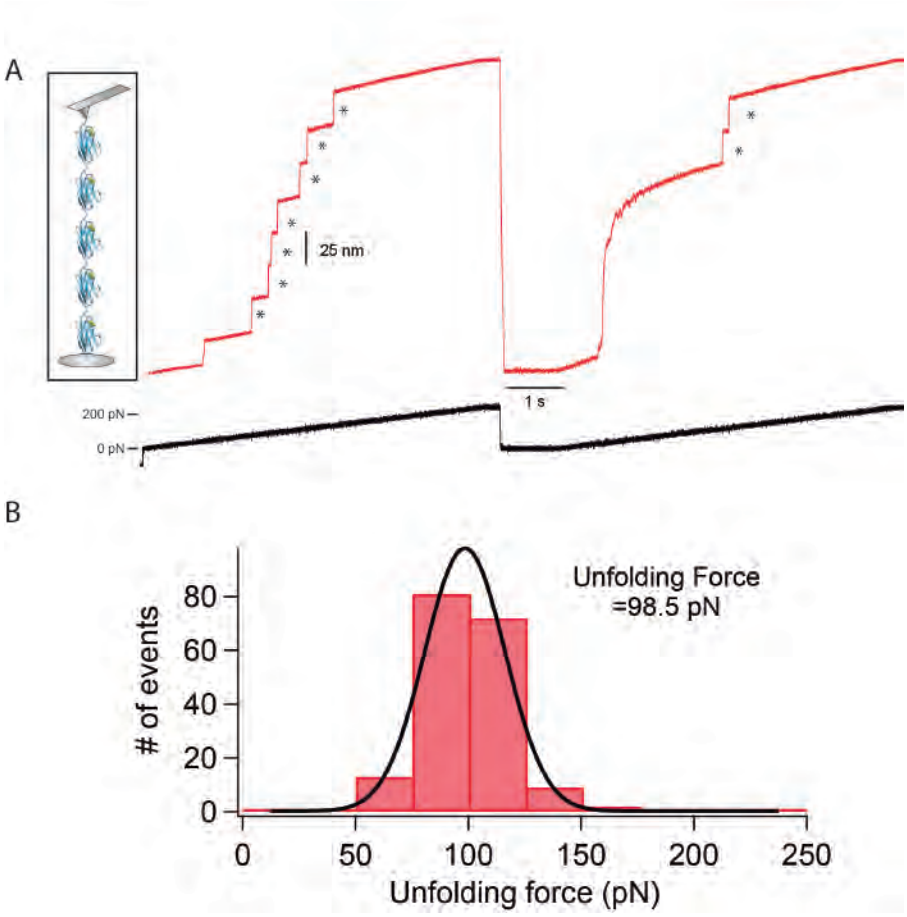
Predoctoral Researcher:
Carla Huerta

Masters Students:
Cristina Sánchez-González
Carmen Suay

Technician:
Diana Velázquez-Carreras



Top: Four different titin Ig domains. Cysteine residues have been highlighted. Domain I57 contains the three most conserved cysteines in titin. ***Bottom***: Frequency of cysteine residues along the sequence of the more than 200 titin Ig domains.



A. A polyprotein made from the repetition of a single domain is pulled using an AFM tip (inset). The force is linearly increased (black) and protein length is monitored (red). We detected the mechanical unfolding of 7 domains (asterisks). Force is then relaxed to 0 pN, allowing refolding of two domains (detected in a second force ramp). **B**. We compiled several traces to determine the mechanical stability of the domain.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (BIO2014-54768-P)
- Ministerio de Economía y Competitividad (RYC-2014-16604)

SELECTED PUBLICATIONS

Saqlain F, Popa I, Fernández JM, Alegre-Cebollada J. **A novel strategy for utilizing voice coil servoactuators in tensile tests of low volume protein hydrogels.** *Macromol Mater Eng* (2015) 300: 369-76

Rivas-Pardo JA, Alegre-Cebollada J, Ramírez-Sarmiento CA, Fernandez JM, Guixé V. **Identifying sequential substrate binding at the single-molecule level by enzyme mechanical stabilization.** *ACS Nano* (2015) 9: 3996-4005

Alegre-Cebollada J, Kosuri P, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernández JM. **S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding.** *Cell* (2014) 156: 1235-46

Solsona C, Kahn TB, Badilla CL, Álvarez-Zaldiernas C, Blasi J, Fernandez JM, Alegre-Cebollada J. **Altered thiol chemistry in human amyotrophic lateral sclerosis-linked mutants of superoxide dismutase 1.** *J Biol Chem* (2014) 289: 26722-32

Molecular genetics of angiogenesis

Head of Laboratory
Rui Benedito

RESEARCH INTEREST

Our group is interested in understanding the cellular and molecular mechanisms involved in the formation and homeostasis of blood vessels, which are an important therapeutic target in cardiovascular disease and cancer. Modulation of vascular structure and function in disease is still a major challenge, in part due to our inability to block or induce the exact mechanisms that vessels use to grow under normal physiologic conditions.

We are currently revisiting and challenging some existing concepts in vascular biology by using new genetic tools that enable us to study the function of genes at higher cellular resolution. We aim to identify and characterize new mechanisms involved in vascular differentiation and growth, but also study their importance in the vasculature paracrine function in diverse phisiological or disease situations. Some of these mechanisms are highly conserved, whereas others seem to be active or important only in some vascular beds or pathological contexts.

In the last year we continued to investigate the regulation of endothelial differentiation, proliferation and aging by the Notch, VEGF and FGFR signaling pathways. We began the study of specific genes in the development and homeostasis of the heart coronary vessels, and we also investigated how endothelial cells transdifferentiate into hematopoietic stem cells early in life and how they later modulate their biology in the bone marrow.

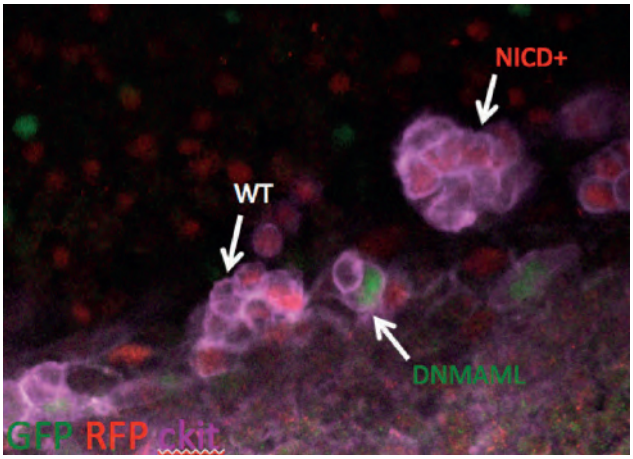
Postdoctoral Researchers:
Elena M Doménech
Wen Luo
Tania Sánchez Pérez
Sarita Saraswati

Predoctoral Researchers:
Mayank Bansal
Macarena Fernández Chacón
Briane D Laruy
Carlos López Fernández de Castillejo
Samuel Pontes Querol

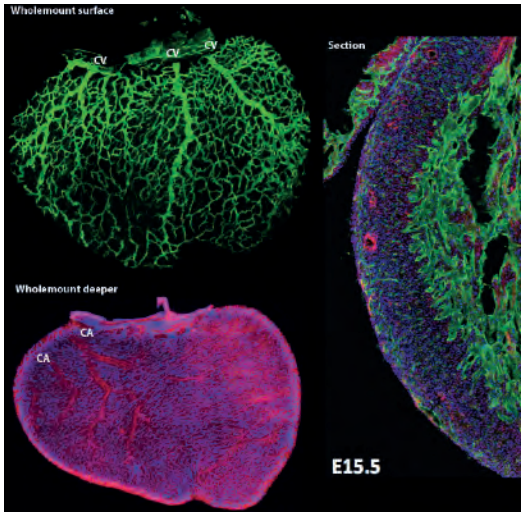
Masters Student:
Irene García González

Graduate Technicians:
Verónica Casquero García
Luis Heredia
Juan Ramón Perea Úbeda-Portugués

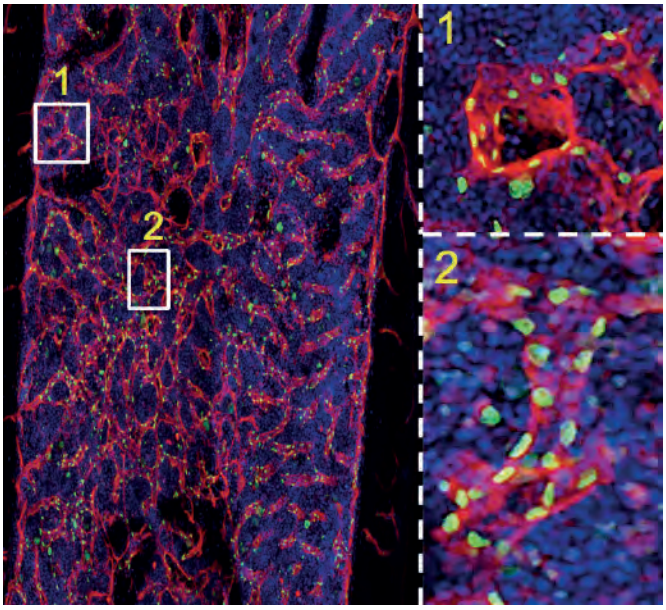
Technician:
Mª Sofía Sánchez Muñoz



Confocal 3D picture showing hematopoietic stem cell clusters (pink/c-Kit+) arising from endothelial cells on the adjacent dorsal floor of the mouse aorta. Notch signaling is higher in cells with red nuclei and lower in cells with green nuclei.



Confocal 3D images of a microdissected embryo heart showing the coronary vasculature. The images show the surface capillaries and veins (green) and the deeper arteries (red). The image to the right shows a heart section.



Confocal 3D images showing intact bone vascular architecture (red) surrounded by blood cells (blue). Cells with green nuclei have high Notch activity.

MAJOR GRANTS

- *European Research Council Starting Grant 2014. (ERC-2014-StG 638028_AngioGenesHD).*
- *Ministerio de Economía y Competitividad (SAF2013-44329-P)*
- *Ministerio de Economía y Competitividad. Contrato Ramón y Cajal (RYC-2013-13209)*
- *Ministerio de Economía y Competitividad. Contrato Posdoctoral PI: Tania Sánchez (FPDI-2013-18049)*
- *Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship. PI: Samuel Pontes*
- *Fundación La Caixa. Predoctoral Fellowship. PI: Macarena Fernández*
- *Boheringer Ingelheim Fons. Predoctoral Fellowship. PI: Carlos López Fernández de Castillejo*
- *European Commission. International IPP. PI: Wen Luo*
- *European Commission. International IPP. PI: Sarita Saraswati*

SELECTED PUBLICATIONS

D'Amato G, Luxán G, Del Monte-Nieto G, Martínez-Poveda B, Torroja C, Walter W, Bochter MS, Benedito R, Cole S, Martinez F, Hadjantonakis AK, Uemura A, Jiménez-Borreguero LJ, de la Pompa JL. **Sequential Notch activation regulates ventricular chamber development (2015)** *Nat Cell Biol* (doi: 10.1038/ncb3280. Epub 2015 Dec 7))

Bernier-Latmani J, Cisarovsky C, Demir CS, Bruand M, Jaquet M, Davanture S, Ragusa S, Siegert S, Dormond O, Benedito R, Radtke F, Luther SA, Petrova TV **DLL4 promotes continuous adult intestinal lacteal regeneration and dietary fat transport** *J Clin Invest* (2015) 125: 4572-86

Rocha SF, Schiller M, Jing D, Li H, Butz S, Vestweber D, Biljes D, Drexler HC, Nieminen-Kelha M, Vajkoczy P, Adams S, Benedito R, Adams RH. **Esm1 Modulates Endothelial tip cell behavior and vascular permeability by enhancing VEGF bioavailability** *Circ Res* (2014) 115: 581-90

Integrin signaling

Head of Laboratory

Miguel Ángel del Pozo

RESEARCH INTEREST

We have shown that crucial cell functions are affected by key mechanoregulatory molecules: integrins (which mediate cell adhesion to the extracellular matrix), Rac/Rho GTPases (which regulate actin cytoskeleton functions & mechanical contractility), and caveolae-resident proteins. Caveolae are actin-linked plasma membrane invaginations abundant in mechanically stressed tissues (including heart, vessels, muscle & fat) and are involved in signaling, viral entry, membrane trafficking & lipid metabolism. The precise functions of caveolae and their main constituent proteins caveolins (Cav 1-3) and cavins (1-4) remain unresolved. Cav-deficient mice show tissue abnormalities, and caveolar disorders are associated with lipodystrophy, muscular dystrophy, osteoporosis, CVD and cancer. We and others have shown that caveolae can sense and transduce mechanical cues. We found that Cav1 can modulate cell shape and responses via force-dependent remodeling of the 3D microenvironment. Elongated cancer associated fibroblasts (CAFs) form stiff, parallel-fiber networks through which cancer cells move rapidly, invading and metastasizing.

Our work shows that stromal-Cav1 drives not only pathological remodeling of the tumor microenvironment, but also physiological remodeling, for example in the mammary gland and the skin. We are now addressing the role of Cav1 in cardiac remodeling after acute myocardial infarction using the LAD (left anterior descending) artery permanent ligation model. Abnormal cardiac remodeling and fibrosis after acute myocardial infarction can lead to heart failure and death.

Rac1 had been detected in the nucleus, and our work has provided insight into the molecular mechanism of Rac1 nucleocytoplasmic shuttling. Rac1-driven nuclear actin polymerization controls nuclear membrane organization and shape. Dysregulation of this mechanism in cancer leads to Rac1 nuclear accumulation, promoting nuclear deformation and cell invasion through narrow spaces.

In 2015 we also established Cav1 as a major checkpoint in the transition from an epithelial to a mesenchymal identity in the peritoneum, through the suppression of MEK-ERK1/2-Snail1 signaling. The efficacy of a MEK pharmacological inhibitor in counteracting the EMT/fibrosis developed in Cav1-/- mice during peritoneal dialysis warrants further translational studies in other chronic inflammatory diseases.

Research Scientists:
Asier Echarri
Inés Martín Padura

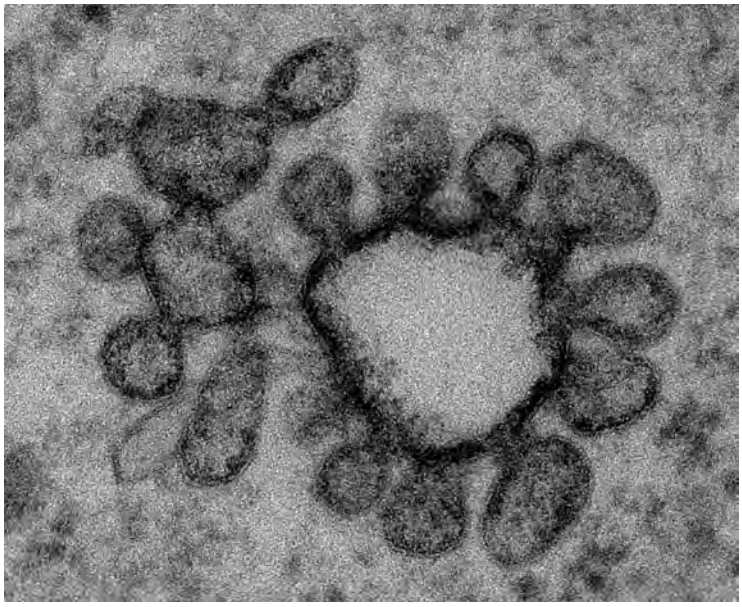
Postdoctoral Researchers:
Inmaculada Navarro
Fidel Lolo Romero
Silvia Fernández-Soriano
Sarah Francoz
Miguel Sánchez Álvarez

Predoctoral Researchers:
Roberto Moreno Vicente
Lucas Albacete
Alberto Díez
Mª del Carmen Aboy
Giulio Fulgoni

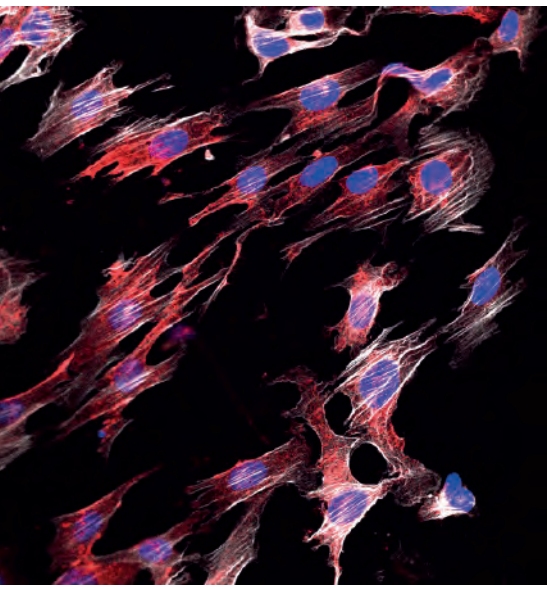
Masters Students:
María García
Olga Boix (since October)

Technicians:
Sara Sánchez Perales
Teresa Osteso Ibáñez
Dácil M. Pavón
Mauro Catalá

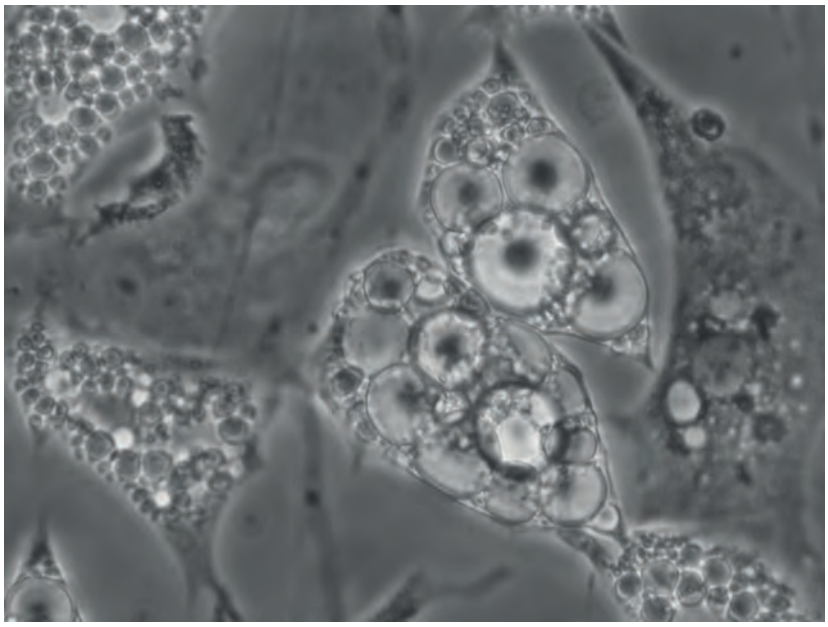
Visiting Scientist:
Raffaele Strippoli



Caveolar Rosettes. A) Surface-connected invaginations of the plasma membrane decorated with caveolae observed by electron microscopy. B) GSD super-resolution image of a cell stained for Cav1. The arrow shows a rosette-like structure decorated with Cav1-positive aggregates.



Cells stretched cyclically over 2 hours, fixed and then stained for Cav1 (red), nuclei (blue) and actin cytoskeleton (gray). Stretching at high amplitude leads to actin fiber alignment.



Adypocytes differentitated in vitro from adipocyte precursors isolated from adult white adipose tissue.

MAJOR GRANTS

- *European Commission. Marie Curie Actions Initial Training Network (ITN) (Horizon 2020, "BIOPOL")*
- *WorldWide Cancer Research (UK) (formerly known as AICR) (AICR 15 – 0404)*
- *Ministerio de Economía y Competitividad (SAF2014-51876-R)*
- *Ministerio de Economía y Competitividad. Consolider COAT (CSD2009-00016)*
- *Ministerio de Economía y Competitividad. Red de Excelencia en Mecanobiología (BFU2014-52586-REDT)*
- *Fundació La Marató TV3 (674/C/2013)*

SELECTED PUBLICATIONS

Echarri A, Del Pozo MA. **Caveolae - mechanosensitive membrane invaginations linked to actin filaments.** *J Cell Sci.* (2015) 128: 2747-58

Kosmalska AJ, Casares L, Elosegui-Artola A, Thottacherry JJ, Moreno-Vicente R, González-Tarragó V, del Pozo MA, Mayor S, Arroyo M, Navajas D, Trepat X, Gauthier NC, Roca-Cusachs P. **Physical principles of membrane remodelling during cell mechanoadaptation.** *Nat Commun* (2015) 6: 7292

Navarro-Lérida I, Pellinen T, Sánchez SA, Guadamillas MC, Wang Y, Mirtti T, Calvo E, Del Pozo M.A. **Rac1 nucleocytoplasmic shuttling drives nuclear shape changes and tumor invasion.** *Dev Cell* (2015) 32: 318-34

Strippoli R, Loureiro J, Benedicto I, Pérez-Lozano ML, Moreno V, Barreiro O, Pellinen T, Minguet S, Foronda M, Osteso MT, Calvo E, Vázquez J, López-Cabrera M, Del Pozo MA. **Caveolin-1 deficiency induces MEK-ERK1/2-Snail1-dependent epithelial-mesenchymal transition and fibrosis during peritoneal dialysis.** *EMBO Mol Med* (2015) 7: 102-23

Shi Y, Tan SH, Ng S, Zhou J, Yang ND, Khoo GB, McMahon KA, Parton RG, Hill MM, Del Pozo MA, Kim YS, Shen HM. **Critical role of CAV1/caveolin-1 in cell stress responses in human breast cancer cells via modulation of lysosomal function and autophagy**. *Autophagy* (2015) 11: 769-84

Regeneration and aging

Head of Laboratory
Ignacio Flores

Posdoctoral Researcher:
Tania Aguado

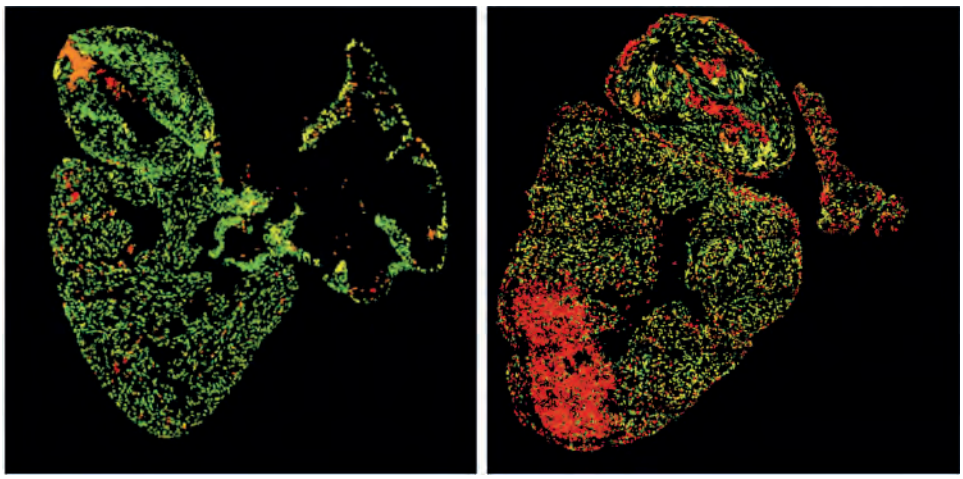
Predoctoral Researchers:
Esther Aix
Dorotha Bednarek
Carlota Sánchez Ferrer

Technician:
Irene de Diego

RESEARCH INTEREST

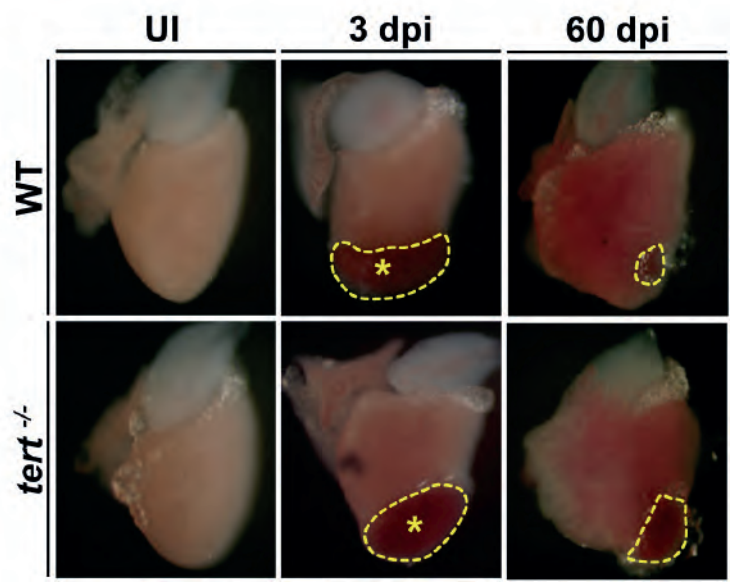
Although recent advances have overturned the old view of the human heart as an inert postmitotic organ, it is clear that the heart's capacity to proliferate, rejuvenate and regenerate is very limited. This presents a problem for strategies to treat damaged hearts after infarction, one of the leading causes of death worldwide.

Our group works on strategies to enhance cardiac regeneration. Toward this goal, we are characterizing the subpopulation of cardiac cells capable of regeneration. Based on this knowledge, we are currently exploring strategies to promote the repair of injured hearts. We have eliminated and reactivated telomerase, an anti-aging enzyme, in adult cardiac cells in order to assess the role of this enzyme in the re-expression of cardiac embryonic genes after infarction and in heart regeneration. A key element of our strategy is the comparison of animal models that differ greatly in their regeneration capacity, from the zebrafish, which can restore up to 20% its heart after injury, through the newborn mouse, whose heart possesses transient regenerative potential, to the adult mouse, in which heart regeneration capacity is very limited. In the zebrafish model, we found that telomerase is essential for zebrafish heart regeneration. The inability to regenerate is mainly due to a strong inhibition of the proliferation response, associated with accumulation of cardiac cells with DNA damage and senescence characteristics. Through these efforts, we hope to achieve a more complete knowledge of the role of endogenous cardiac progenitor cells and telomerase in heart rejuvenation and regeneration, which could eventually lead to the development of improved regeneration therapies.



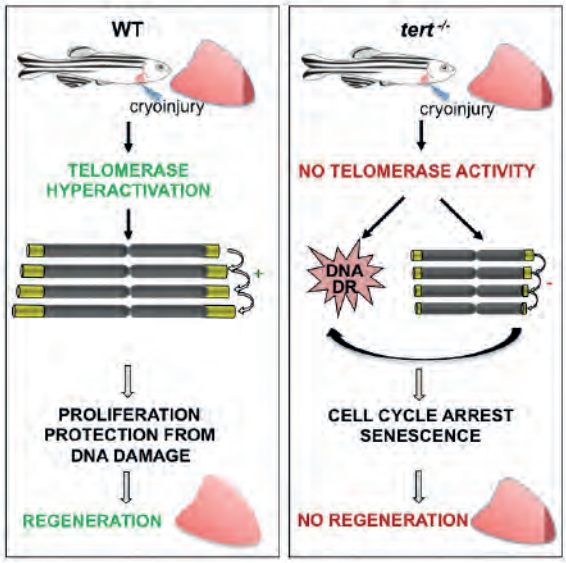
Heart cryoinjury elongates telomeres
Representative telomap images of zebrafish hearts with no injury (left) and 3 days post-injury (right). Nuclei are assigned to a four-color code according to their average telomere fluorescence in arbitrary units. The cells with the longest telomeres are shown in red, and those with the shortest telomeres are shown in green.

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064



Heart regeneration is strongly inhibited in tert$^{-/-}$ animals
Whole-mount views of uninjured and cryoinjured WT and tert$^{-/-}$ zebrafish hearts dissected at the indicated times post-injury. Dotted lines outline the injured area. Asterisks mark the initial injury site.

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064



Model of telomerase function in zebrafish heart regeneration

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2012-38449)*
- *Ministerio de Economía y Competitividad. FIS. RETICS (Red de Investigación Cardiovascular RD12/0042/0045)*
- *Asociación Española contra el Cáncer PI: Tania Aguado*

SELECTED PUBLICATIONS

Bednarek D, Gonzalez-Rosa JM, Guzman-Martinez G, Gutierrez-Gutierrez O, Aguado T, Sanchez-Ferrer C, Marques IJ, Galardi-Castilla M, de Diego I, Gomez MJ, Cortes A, Zapata A, Jimenez-Borreguero LJ, Mercader N*, Flores I*. **Telomerase is essential for zebrafish heart regeneration**. *Cell Rep* (2015) 12: 1691-703
*Co-corresponding authors

Imaging cardiovascular inflammation and the immune response

Head of Laboratory
Andrés Hidalgo Alonso

RESEARCH INTEREST

We are interested in multiple aspects of the biology of myeloid leukocytes and hematopoietic stem and progenitor cells (HSPC), the source of all blood cells. In the past year we have identified the regulatory function of E-selectin ligand 1 (ESL-1) in regulating the hematopoietic microenvironment, or niche, in which these HSPCs reside. We have discovered that through this glycoprotein, HSPCs are able to maintain normal proliferation within the bone marrow. In a completely different organ, the heart, we are working to identify how a group of myeloid cells, cardiac-resident macrophages, prevent fibrosis and maintain normal heart function. We are currently deciphering the mechanisms by which macrophages carry out this important function. Another major interest of the lab lies in understanding the biology of neutrophils. These leukocytes are important because they keep the organism free of pathogenic microorganisms but can also cause major inflammatory injury to organs, for example during sepsis or myocardial infarction. We have found that under healthy conditions these cells recirculate throughout the organism to support basic homeostatic functions, even in distant tissues.

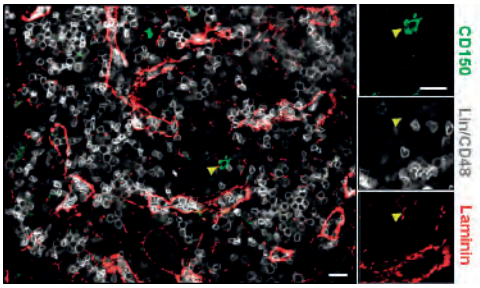
Postdoctoral Researchers:
Noelia Alonso González
Magdalena Leiva Arjona

Predoctoral Researchers:
José María Adrover Montemayor
José Ángel Nicolás Ávila

Technicians:
Juan Antonio Quintana Fernández
Georgiana Crainiciuc
Patricia Castro Hernanz

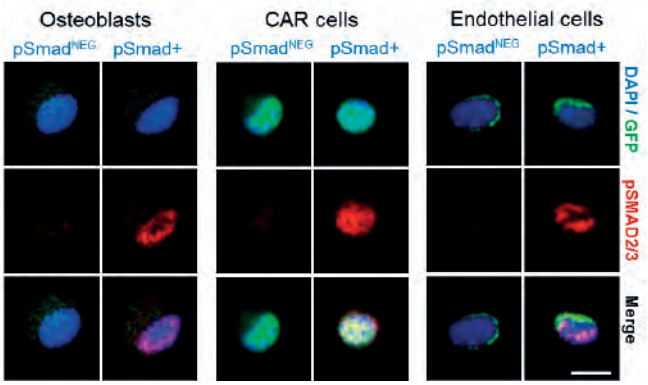
Master Student:
Arturo González de la Aleja Molina

Visiting Scientist:
Linnea A. Weiss



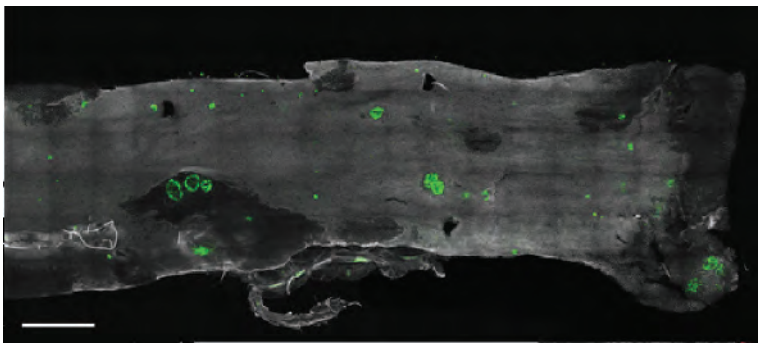
Hematopoietic stem cells in their niche
Confocal image of the bone marrow of a mouse, showing a blood stem cell (green) surrounded by vessels (red) and mature leukocytes (white). Both the proliferation and the status of the niche are controlled by the glycoprotein ESL-1 in stem cells.

Leiva M et al., Nat Commun, 2016 7:10222. doi: 10.1038/ncomms10222



Regulated signaling in bone marrow "niche" cells
Different types of stromal cells that form the hematopoietic niche (osteoblasts, CAR or reticular cells, and endothelial cells) expressing varying levels of green fluorescent protein (GFP). These cells display different signailing properties marked by the phosphorylation of Smad2/3 proteins (red) in the nucleus (blue). This signaling pathway is regulated by ESL-1 in the bone marrow.

Leiva M et al., Nat Commun, 2016 7:10222. doi: 10.1038/ncomms10222



Neutrophils cluster in the intestinal mucosa
Whole-mount imaging of the large intestine of a mouse, showing groups, or "clusters", of neutrophils. These intestinal neutrophils can be identified by the expression of a green fluorescent protein (GFP).

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2013-49662-EXP)*
- *Ministerio de Economía y Competitividad (ERA-NET Infect-ERA 2014 #143 BActInfectERA)*
- *Comunidad de Madrid (P2010-BMD-2314)*
- *Ministerio de Economía y Competitividad (SAF2012-31142)*
- *Fundación La Marató-TV3 (120/C/2015)*

SELECTED PUBLICATIONS

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Martinez-Moreno M*, Leiva M*, Aguilera-Montilla N, Sevilla-Movilla S, Isern de Val S, Arellano-Sánchez N, Gutierrez N, Maldonado R, Martinez-Lopez J, Buño I, Garcia-Marco J, Sánchez-Mateos P, Hidalgo A, Garcia-Pardo A and Teixidó J. **In vivo adhesion of malignant cells to bone marrow microvasculature is regulated by a4b1 cytoplasmic-binding proteins**. *Leukemia* (doi: 10.1038/leu.2015.332 Epub 2015 Dec 10)
* Equal contribution

Lasarte S, Samaniego R, Salinas-Muñoz L, Guía-Gonzalez MA, Weiss LA, Mercader E, Ceballos-Garcia E, Navarro-González T, Moreno-Ochoa L, Perez Millan F, Pion M, Sanchez-Mateos P, Hidalgo A, Muñoz-Fernandez MA and Relloso M. **Sex hormones coordinate neutrophil immunity in the vagina by controlling chemokine gradients**. *J Infect Dis* (doi: 10.1093/infdis/jiv402. Epub 2015 Aug 3)

Gonzalez-Valdes I, Hidalgo I, Bujabarral A, Lara-Pezzi E, Padron L, Garcia-Pavia P, Gomez P, Redondo JM, Ruiz-Cabello JM, Jimenez-Borreguero LJ, Enriquez JA, de la Pompa JL, Hidalgo A and Gonzalez S. **Heart senescence surveillance by Bmi1 limits dilated cardiomyopathy in heart failure**. *Nat Commun* (2015) 6: 6473

Scheiermann C, Frenette PS, Hidalgo A. **Regulation of leukocyte homeostasis in the circulation**. *Cardiovasc Res* (2015) 107: 340

Functional genomics

Head of Laboratory
Miguel Manzanares

RESEARCH INTEREST

In our lab we are interested in the gene regulatory networks that control the early stages of mammalian development and underlie cardiovascular disease. Our research focuses on understanding how cis-regulatory elements located in the non-coding portion of the genome influence the spatial and temporal expression of nearby genes, as well as how their activity is modulated by chromatin structure. We are also exploring how variation in these elements influences disease risk.

With these goals in mind, we have explored how three-dimensional genome structure relates to gene expression in the cardiovascular system. By using high-resolution deep-sequencing-based chromatin conformation techniques, together with CRISP/R genome editing tools, we have described how a gene-specific regulatory loop is established and is essential for proper expression of the ventricle-specific regulatory gene *Irx4*. We further show that this loop is dependent on the architectural chromatin factor CTCF during embryonic development. At present we are using similar approaches to explore the regulatory basis of atrial fibrillation, the most common type of cardiac arrhythmia and a serious health burden worldwide.

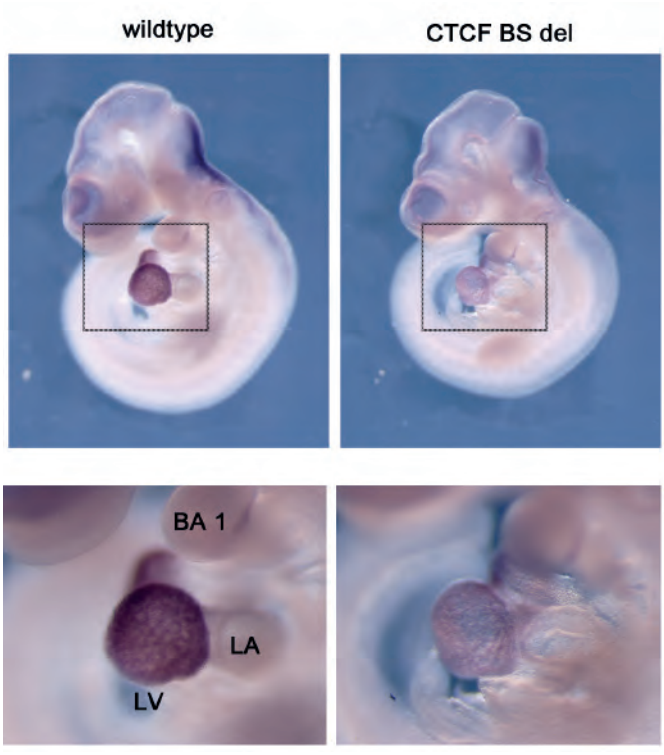
We are also exploring novel ways of using genome editing to interrogate the regulatory genome. We have validated the use of the CRISP/R system to generate transient knock-out mouse embryos, without the need to establish mouse lines. And we have used these tools to deliver a reporter cassette to a genomic location at will, in order to "read" the regulatory environment at specific genomic locations. Further development of these tools will allow us to address the nature and role of genomic regions in detail in developmental processes and cardiovascular disease.

Postdoctoral Researchers:
Luis Augusto Aguirre Pérez
María José Andreu Sauqué
Mª Elena López Jiménez

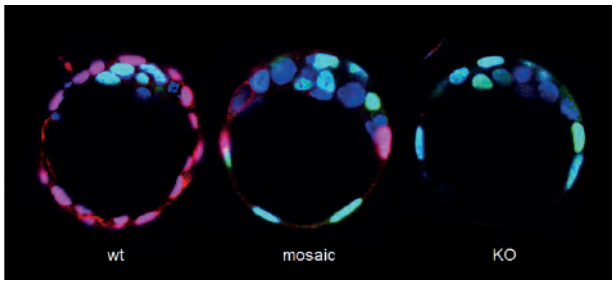
Predoctoral Researchers:
Melisa Gómez Velázquez
Julio González Sainz de Aja
Sergio Menchero Fernández
Teresa Rayón
Raquel Rouco García
Jesús Victorino Santos

Technicians:
Isabel Rollán Delgado
Claudio Badía Careaga

Visiting Scientist:
Gonzalo Carreño Gómez-Tarragona

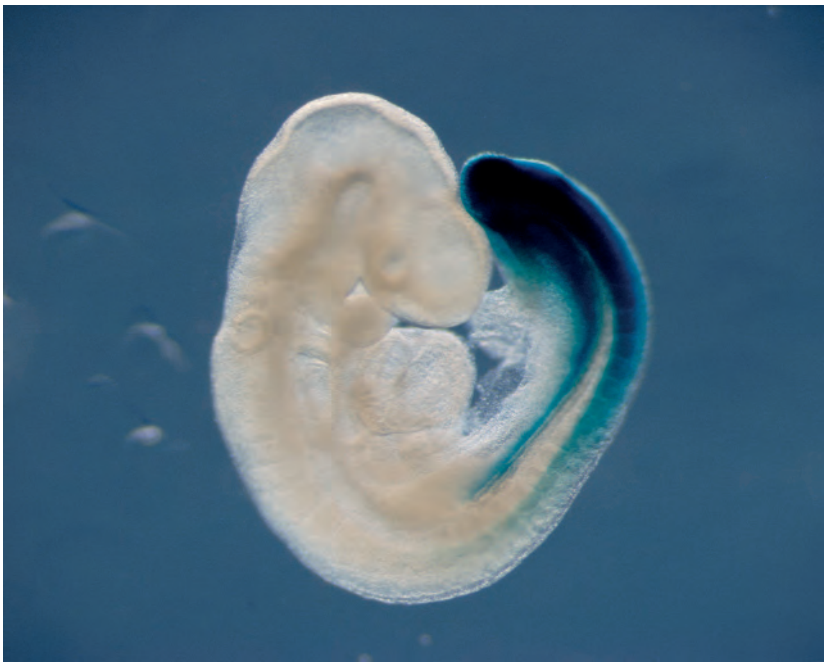


Deletion by CRISP/R-based genome editing of a specific CTCF binding site in a mouse embryo results in a strong reduction of the expression of the cardiac-specific *Irx4* gene.



Genome-editing of the mouse embryo. Direct injection of Cas9 and specific guide-RNAs targeting the first coding exon of *Cdx2* into the one-cell mouse embryo leads in 3 days to blastocysts in which CDX2 expression (red) is lost partially (mosaic, middle panel) or completely (KO, right panel). Expression of the pluripotency marker NANOG (light blue) is unaffected. Nuclei are shown in dark blue.

Genome-editing of the mouse embryo. CRISP/R-mediated integration of a regulatory reporter cassette in the *Cdx2* locus reproduces the expression of this gene in the posterior part of the E9.5 embryo.



MAJOR GRANTS

- *Ministerio de Economía y Competitividad BFU2014-57703-REDC*
- *Comunidad Autónoma de Madrid. S2010/BMD-2315 (CELLDD-CM).*
- *Fundación Centro Nacional de Investigaciones Cardiovasculares. CNIC Translational Projects (CNIC-08-2009)*

SELECTED PUBLICATIONS

Aguirre LA, Alonso ME, Badia-Careaga C, Arias C, Fernandez-Miñan A, López-Jiménez E, Aránega A, Gomez-Skarmeta JL, Franco D, Manzanares M. **Long-range regulatory at the 4q25 atrial fibrillation risk locus involves *PITX2c* and *ENPEP*.** *BMC Biol* (2015) 13: 26

Rayon T, Menchero S, Nieto A, Xenopoulos P, Crespo M, Cockburn K, Cañon S, Sasaki H, Hadjantonakis AK, de la Pompa JL, Rossant J, Manzanares M. **Notch and Hippo converge on *Cdx2* to specify the trophectoderm lineage in the mouse blastocyst.** *Dev Cell* (2014) 30: 410-22

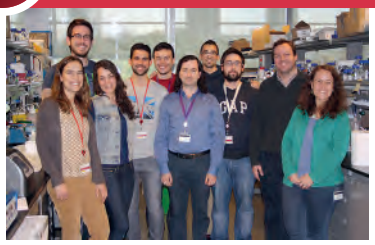
Manzanares M. **Functional genomics of cardiovascular development and disease.** *Brief Funct Genomics* (2014) 13: 1-2

van Weerd H, Badi I, van den Boogaard M, Stefanovic S, van de Werken HJG, Gomez-Velazquez M, Badia-Careaga C, Manzanares M, de Laat W, Barnett P, Christoffels VM. **A large permissive regulatory domain exclusively controls *Tbx3* expression in the cardiac conduction system.** *Circ Res* (2014) 115: 432-41

Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gómez-Marín C, Aneas I, Credidio FL, Sobreira DR, Wasserman NF, Lee JH, Puviindran V, Tam D, Shen M, Son JE, Vakili NA, Sung HK, Naranjo S, Acemel RD, Manzanares M, Nagy A, Cox NJ, Hui CC, Gomez-Skarmeta JL, Nobrega MA. **Obesity-associated variants within *FTO* form long-range functional connections with *IRX3*.** *Nature* (2014) 507: 371-5

Stem cell niche pathophysiology

Head of Laboratory
Simón Méndez Ferrer



RESEARCH INTEREST

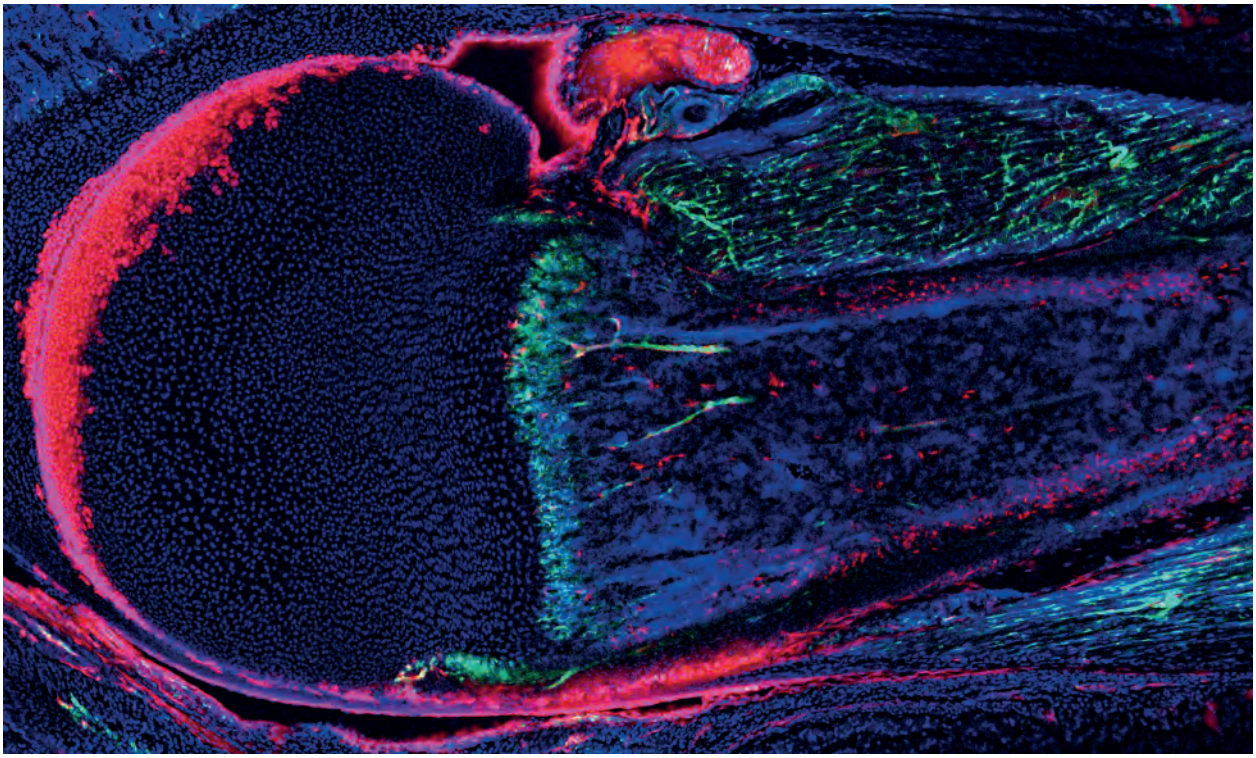
Our group studies how the niche maintains and regulates stem cells and how its dysregulation can contribute to disease. Hematopoietic stem cells (HSCs) traffic between bone marrow and circulating blood, which is the basis of for lifesaving clinical transplantation. Our previous work showed that HSC numbers in blood are regulated by the brain, which regulates bone marrow nestin+ mesenchymal stem cells through peripheral nerves. We recently found that HSC-niche mesenchymal stem cells might be different from those that form the skeleton, instead sharing a common origin with peripheral nerves and supporting glial cells (Figure 1). Thus, tight regulation of peripheral stem-cell niches in vertebrates might build upon the developmental relationships among its cellular components. Moreover, we have shown that damage to this regulatory network is essential for the appearance of myeloproliferative neoplasms, diseases that were previously thought to be driven solely by mutated HSCs (Figure 2). Our recent data has also uncovered a selective regulation by sex hormones of the maintenance, survival and proliferation of normal and leukaemic hematopoietic progenitors. These results might explain gender differences in blood cancer incidence and also offer a new way of targeting leukemic stem cells with clinically approved drugs (Figure 3).

Postdoctoral Researchers:
Raquel del Toro Estévez
María García Fernández
Joan Isern Marín
Daniel Martín Pérez
Abel Sánchez-Aguilera Peño

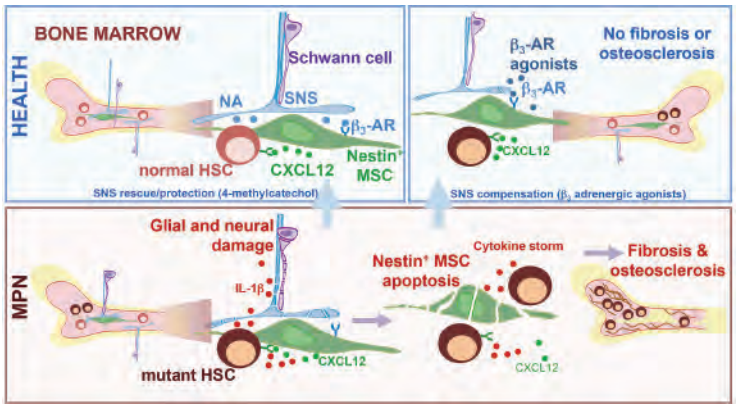
Predoctoral Researchers:
Andrés García García
Sara González Hernández
Carlos López Fernández de Castillejo

Master Student:
Oliver Pérez Howell

Technicians:
Javier Langa Oliva
Sandra Martín Salamanca



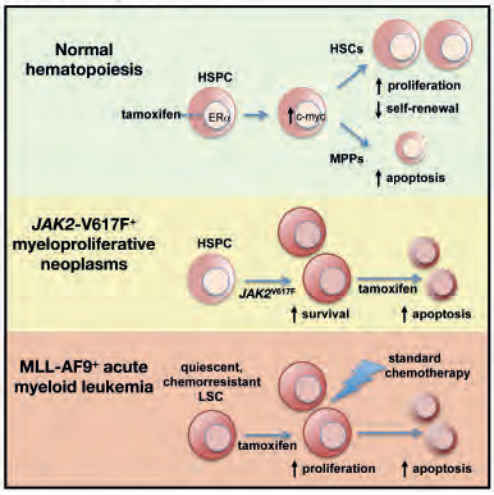
The neural crest is a source of mesenchymal stem cells with specialized functions in the hematopoietic stem cell niche. Neonatal bone marrow section from triple transgenic mouse in which neural crest-derived cells are labeled in red and nestin+ cells in green. Blue signal corresponds to cell nuclei (Isern J et al. eLife 2014).



Neuropathy of the hematopoietic stem-cell niche is essential for myeloproliferative neoplasms. Model illustrating HSC niche alterations and rescue in myeloproliferative neoplasms (MPN). HSC, hematopoietic stem cell; SNS, sympathetic nervous system; MSC, mesenchymal stem cell; NA, noradrenaline; AR, adrenergic receptor; C, control (disease-free mice). (Arranz L et al. Nature 2014)

Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. Treatment of leukemic mice with the selective estrogen receptor modulator tamoxifen can block the development of myeloproliferative neoplasms and sensitize acute myeloid leukemia to conventional chemotherapy (Sánchez-Aguilera A et al. Cell Stem Cell 2014)

Graphical Abstract



MAJOR GRANTS

- *Howard Hughes Medical Institute. International Early Career Scientist.*
- *Comunidad de Madrid. Convocatoria de Programas de I+D en Biomedicina. (S2011/BMD-2542)*
- *Ministerio de Economía y Competividad (RYC-2011-09209)*
- *Ministerio de Ciencia e Innovación (RYC-2009-04703)*
- *Ministerio de Economia y Competitividad (RYC-2011-09726) PI: Abel Sánchez-Aguilera*
- *Ministerio de Economia y Competitividad (SAF-2011-30308)*
- *European Commission FP7. Marie Curie Career Integration Grant (294262)*
- *European Commission FP7. Marie Curie Career Integration Grant (294096) PI: Abel Sánchez-Aguilera*
- *Ministerio de Economía y Competividad (BFU2012-35892) PI: Joan Isern*

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Arranz L, Sánchez-Aguilera A, Martín-Pérez D, Isern J, Langa X, Tzankov A, Lundberg P, Muntión S, Tzeng YS, Lai DM, Schwaller J, Skoda RC, Méndez-Ferrer S. **Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms.** *Nature* (2014) 512:78-81

Isern J, García-García A, Martín AM, Arranz L, Martín-Pérez D, Torroja C, Sánchez-Cabo F, Méndez-Ferrer S. **The neural crest is a source of mesenchymal stem cells with specialized hematopoietic stem-cell-niche function**. *eLife* (2014) 2014:03696

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Development of the epicardium and its role during regeneration

Head of Laboratory
Nadia Mercader

RESEARCH INTEREST

Unlike adult mammals, zebrafish have the capacity to regenerate their hearts upon several types of injury. In the laboratory, we use cryoinjury to induce cardiac tissue damage, with the aim of mimicking the consequences of tissue loss upon myocardial infarction. Our results show that cardiac fibrosis is reversible and occurs as an intermediate step during regeneration. We aim to unravel the endogenous mechanisms of myofibroblast and extracelular matrix regression, as this might have implications for the design of antifibrotic strategies. We recently examined in detail if the tissue regeneration we observe is accompanied by functional recovery. For this, we set up echocardiography to study ventricular pumping efficiency in the zebrafish. Our results reveal that cryoinjury transiently impairs ventricular fractional volume shortening, but that pumping efficiency recovers completely at later postinjury stages. However, many operated fish show long-term alterations in ventricular wall contraction. Echocardiography thus allows a deeper understanding of the mechanisms of cardiac regeneration. One of the first layers to reestablish during regeneration is the epicardium, the outer layer covering the myocardium. We are interested in how the epicardium forms during embryonic development. Using live imaging in zebrafish embryos we are studying the mechanisms through which the proepicardial cells emerge from the pericardial wall and attach to the myocardium. We found that proepicardium formation is dependent on the beating heart. Our current effort s are dedicated to understanding the underlying mechanosensory pathways.

Postdoctoral Researchers:
Laura Andrés Delgado
Inés Marqués

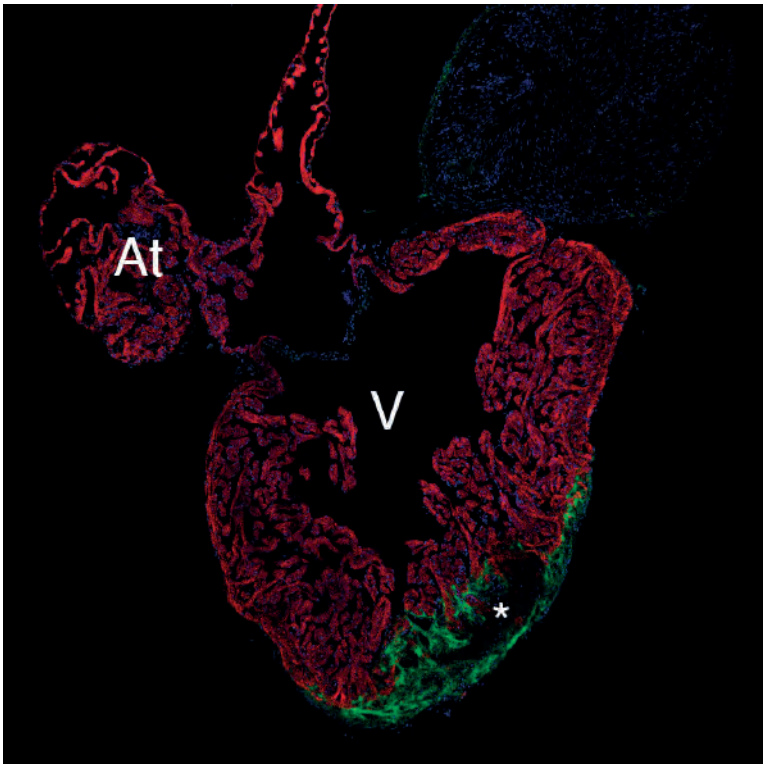
Predoctoral Researchers:
Carolina García Poyatos
Héctor Sánchez Iranzo
Marcos Sande Melón

Masters Students:
Andrés Sanz Morejon
María Claudia Quiñones

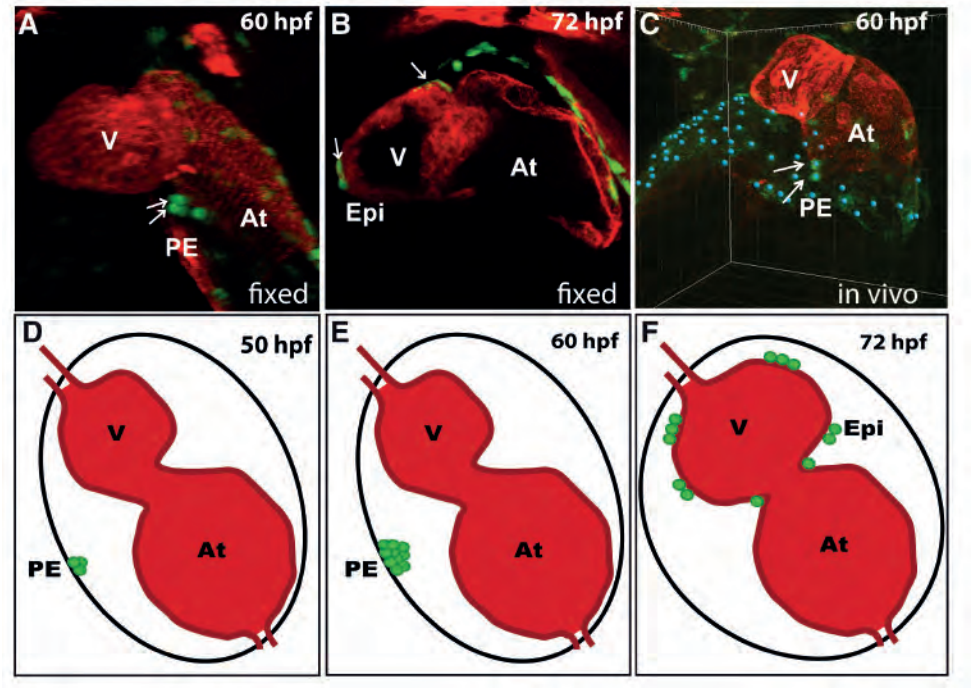
Graduated Technicians:
Ricardo Costa
María Galardi Castilla

Visiting Scientists:
Ana Belén García Redondo
Davide Seruggia
Javier Langa

Visiting Students
Laura Martínez López
David Bazaga



Heart regeneration upon cryoinjury in the zebrafish is preceded by fibrotic tissue deposition. Immunofluorescence with anti-collagen 1 (green) and anti-myosin heavy chain (red) antibodies. The asterisk marks the cryoinjured region. At, atrium; V, ventricle.



In vivo imaging of the developing epicardium in the zebrafish. A and B show whole-mount immunostaining of a transgenic reporter line labeling proepicardial and epicardial cells in green and the myocardium in red. At 60 hours postfertilization (hpf), proepicardial (PE) cells are visible at the inflow tract of the embryonic heart tube. At 72 hpf, some of these cells have attached to the myocardial wall, contributing to epicardium formation. C is a snapshot of a heart in a live embryo of the same transgenic line. This model allows tracking of PE cells and pericardial cells to determine the morphgenetic events leading to epicardium formation. D-F are schematic illustrations of epicardium formation. At, atrium; Epi, epicardium; PE, proepicardium; V, ventricle.

MAJOR GRANTS

- *Ministerio de Economia y Competividad (BFU2011-25297)*
- *Comunidad de Madrid (P2010/BMD-2321)*
- *Tercel (Red de Terapia Celular) (PI: M. Torres)*
- *European Commission. European Research Council Starting Independent Researcher Grant (ERC-337703 2013)*

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Bednarek D, González-Rosa JM, Guzmán-Martínez G, Gutiérrez-Gutiérrez Ó, Aguado T, Sánchez-Ferrer C, Marques IJ, Galardi-Castilla M, de Diego I, Gómez MJ, Cortés A, Zapata A, Jiménez-Borreguero LJ, Mercader N*, Flores I*. **Telomerase is essential for Zebrafish Heart Regeneration.** *Cell Rep.* (2015) 12: 1691-703
*Co-corresponding author

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*Equal contribution

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Rodius S, Nazarov PV, Nepomuceno-Chamorro IA, Jeanty C, González-Rosa JM, Ibberson M, da Costa RM, Xenarios I, Mercader N, Azuaje F. **Transcriptional response to cardiac injury in the zebrafish: systematic identification of genes with highly concordant activity across in vivo models.** *BMC Genomics* (2014) 15: 852

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Genetic control of organ development and regeneration

Head of Laboratory
Miguel Torres

RESEARCH INTEREST

We are interested in understanding the cellular basis of developmental processes and how this is contolled by transcription factor networks (TFN). We have developed genetic methods in the mouse that allow us to trace cell lineages in clonal analysis or functional mosaics. Furthermore, we have established culture methods for the live analysis of developmental processes in embryonic stem cells and in the early mouse embryo. Using these new approaches we have described the relevance of cell competition in the early mouse embryo and in the cardiomyocyte lineage of the developing and adult heart. We are currently exploring the molecular and cellular mechanisms underlying cell-cell comparison and loser-cell elimination.

In recent years we have identified the role of *Meis* transcription factors in organogenesis, including limb, eye, cardiovascular and hematopoietic system development. We have formulated new molecular models underlying the activity of the Meis TFN in pattern formation and organ regeneration. Furthermore, we have identified Myc-driven cell competition as a strategy for stimulating the proliferation and replacement of adult cardiomyocyte populations without compromising cardiac function. A current focus of the lab is the transcriptional control of cardiomyocyte proliferation in the adult heart and its impact on cardiac function and repair. Based on evidence from animal models, we hypothesize that the Myc and Meis TFNs play essential roles in controlling adult cardiomyocyte proliferation and cardiac repair. We are currently developing animal models to test these ideas.

Research Scientists:
Laura Carramolino
Cristina Clavería

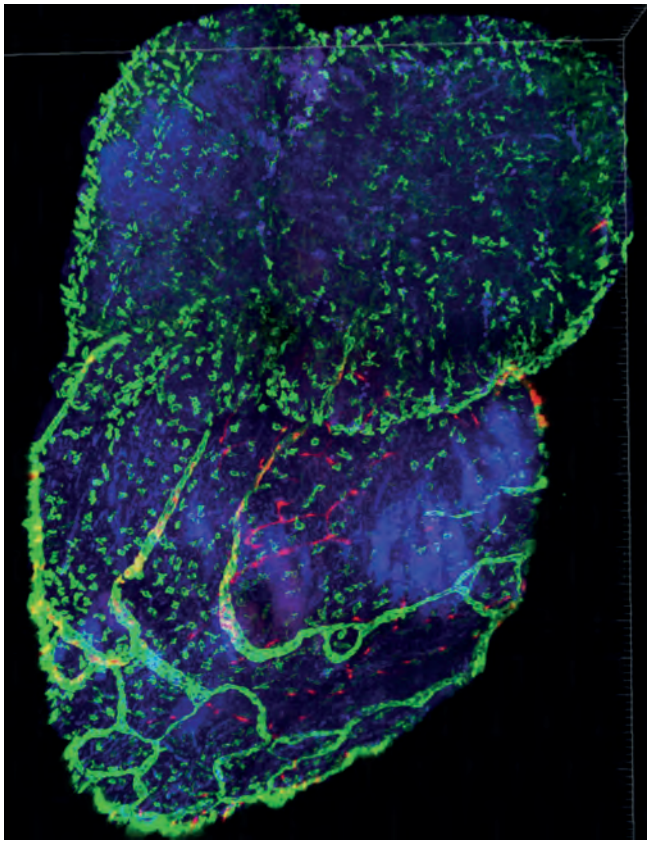
Postdoctoral Researchers:
Irene Delgado
Kenzo Ivanovitch

Predoctoral Researchers:
Covadonga Díaz Díaz
Ghislaine Lioux
Alejandra Cristina López Delgado
Noelia Muñoz Martín
José Antonio Valverde López
Cristina Villa del Campo

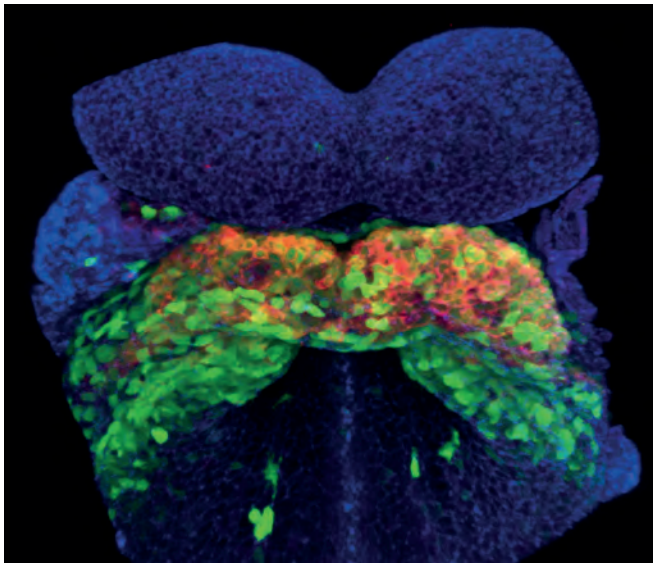
Masters Students:
Isaac Esteban Varela
Ester de la Cruz Crespillo
Lin Li

Technicians:
Vanessa Carolina Cadenas Rodríguez
Rocío Sierra Muñoz
Susana Temiño Valbuena

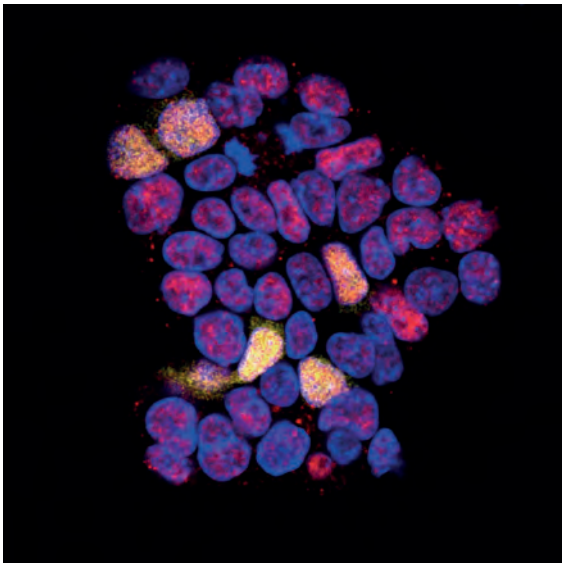
Visiting Scientist:
Juan José Sanz-Ezquerro



Clonal analysis of heart lineages. Image: Ghislaine Lioux
3D confocal reconstruction of a neonatal heart, showing lymphatics (green) and random clones (red) affecting the lymphatic and glial cell lineages.



Early mouse heart development. Image: Kenzo Ivanovitch
3D confocal reconstruction of a neonatal heart, showing all cardiac mesoderm (green) and differentiated primitive cardiomyocytes (green+red).



Myc expression dynamics in ES cells. Image: Covandonga Díaz
An ES cell colony showing nuclei (blue), Myc expression levels (pink) and clonally related cells (yellow).

MAJOR GRANTS

- *Comunidad de Madrid (S2010/BMD-2315)*
- *Ministerio de Economía y Competividad. FIS RETICS (TERCEL: RD12/0019/0005)*
- *Ministerio de Economía y Competividad (BFU2012-310862013-15)*
- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600) (Coordinador E. Lara)*
- *Ministerio de Economía y Competividad. Red de Excelencia Temática. (BFU2015-70193-REDT)*
- *ERANET-NEURO European Commission and Ministerio de Economía y Competividad. (PCIN-2015-020)*
- *Ministerio de Economía y Competividad. (EUIN2015-62897)*
- *Ministerio de Economía y Competividad. Juan de la Cierva Incorporación. (IJCI-2014-19108). PI: I. Delgado*

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*Co-corresponding authors

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Meis1 coordinates a network of genes implicated in eye development and microphthalmia. *Development* (2015) 142: 3009-20
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CLINICAL STUDIES

PESA CNIC-SANTANDER

Fuster-CNIC-Ferrer Cardiovascular Polypill and SECURE Trial

STEMI trials: The Metroprolol program

TAN SNIP

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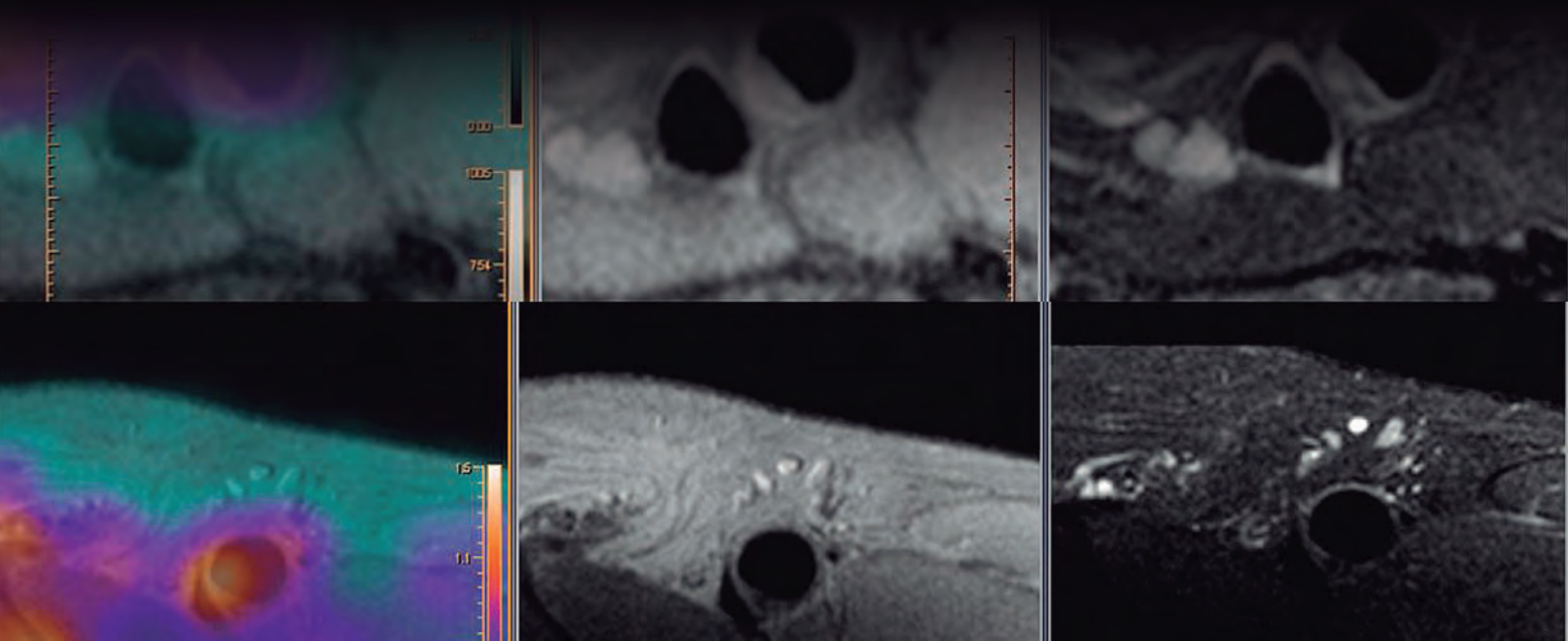


PESA CNIC-SANTANDER

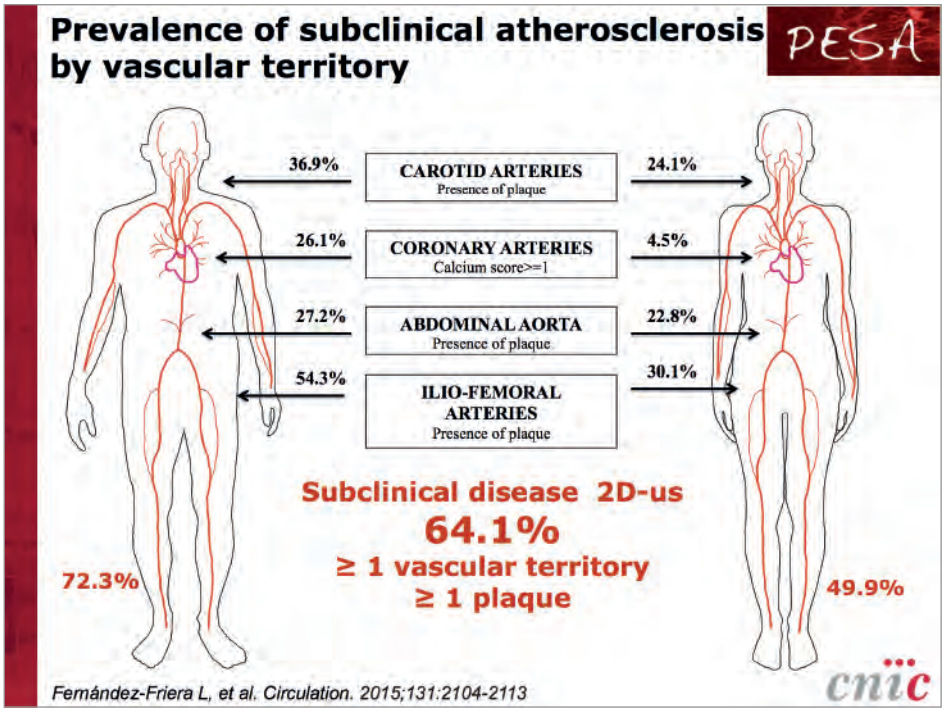
(Progression of Early Subclinical Atherosclerosis)

Noninvasive imaging techniques provide invaluable tools for identifying individuals with subclinical alterations indicating increased risk of cardiovascular events. This field has been boosted by the development of basic techniques (2D/3D vascular ultrasound and coronary calcium score by computed tomography) and advanced imaging techniques (magnetic resonance imaging and positron emission tomography) that can be applied to large populations. Several studies currently underway, such as the High-Risk Population (HRP) study led by Valentín Fuster in the USA, are pioneering the application of these techniques to population studies. Most studies to date have examined populations composed of individuals above the age of 60 years. However, atherosclerotic disease in this group has already several decades of evolution and may be too advanced for prevention of future events.

The PESA CNIC-Santander trial is an ambitious study designed to identify new imaging and biological factors associated with the presence and progression of early phases of atherosclerosis. PESA has recently completed the prospective enrolment of 4184 healthy subjects aged 40 to 54 years (2635 men and 1549 women) who have undergone a multi-territory screening for subclinical atherosclerosis by noninvasive 2D/3D ultrasound in the carotid, abdominal aorta and ilio-femoral arteries together with coronary artery calcium score by computed tomography. Participants have additionally been assessed for a complete set of cardiovascular risk factors (including lifestyle and psychosocial factors) and have provided blood samples for advanced "omics" and future biobanking analyses. In addition, 940 individuals in whom a significant plaque burden was detected by ultrasound and CT underwent advanced imaging by ^{18}FDG PET/MRI at the CNIC Advanced Imaging Unit during 2013 and 2014. The study has also received approval for research into the association between atherosclerosis initiation/progression and telomere dysfunction in circulating leukocytes, and leukocyte samples have been collected from a subgroup of 1456 PESA participants.

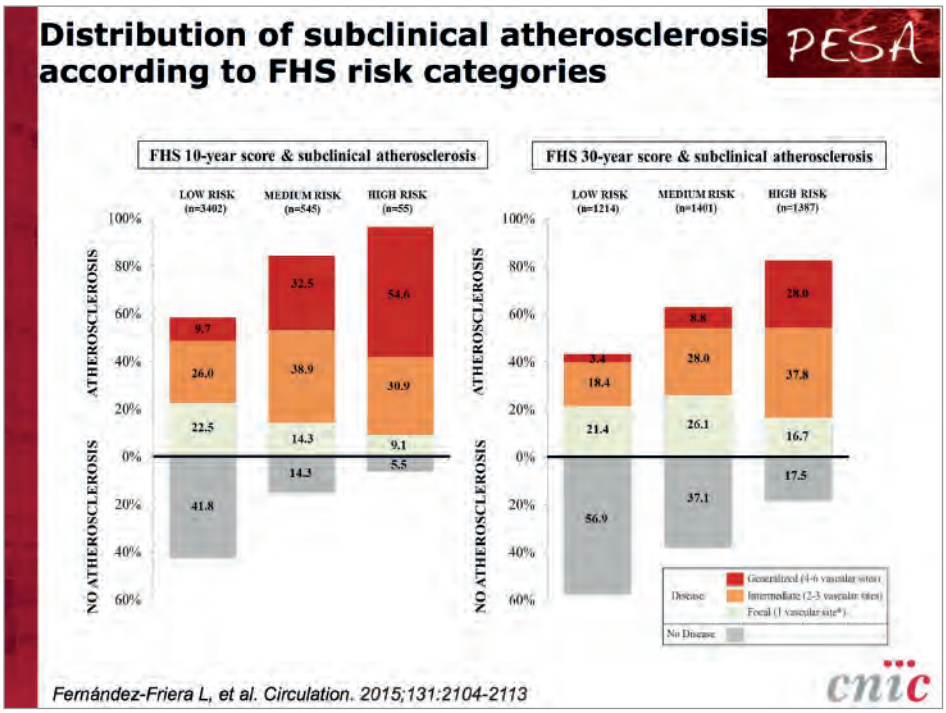
All PESA participants are followed-up at 3 and 6 years to assess the progression of atherosclerotic plaques and to determine how the detection of subclinical disease impacts the risk of future cardiovascular events. By the end of 2015, more than 2600 participants had already had their 3-year follow-up visit (visit 2). Similarly, in July 2015 we began a 3-year follow-up MRI analysis of the 940 individuals assessed with advanced PET/MRI technology at baseline. This intermediate vascular MRI study includes cardiac MR sequences that will allow comprehensive characterization of subclinical myocardial disease.

In Jun 2015, the PESA trial baseline findings were published in *Circulation* (2015). The results of this analysis show that subclinical atherosclerosis is highly prevalent in this middle aged asymptomatic population. Interestingly, the most frequently affected vascular site in the early stages of atherosclerosis is the iliofemoral territory. Subclinical atherosclerosis was found in most individuals classified at high risk on traditional scales (FHS 10- and 30-year scores), but was also present in nearly 60% of participants classified at low risk, with intermediate or generalized disease in one third of participants. Ongoing PESA follow-up over at least 6 years will enable the study of associations between subclinical disease evaluated at baseline and subsequent cardiovascular events.



PESA CNIC-SANTANDER

(Progression of Early Subclinical Atherosclerosis)



Distribution of subclinical atherosclerosis detected by noninvasive imaging according to Framingham Heart Study risk (FHS) categories. Vascular sites examined were the right and left carotids, the abdominal aorta, and the right and left iliofemoral arteries (presence of plaque by 2D ultrasound), as well as the coronary vessels (coronary artery calcification score). FHS risk scores were classified as low (<10%), moderate (≥10%–20%), or high (>20%).

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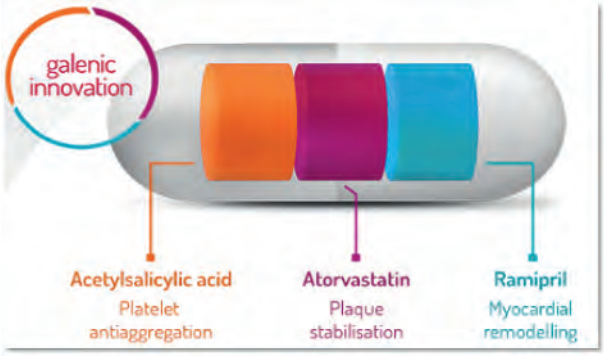
Fernandez-Ortiz A, Jimenez-Borreguero LJ, Penalvo JL, Ordovas JM, Mocoroa A, Fernandez-Friera L, Laclaustra M, Garcia L, Molina J, Mendiguren JM, Lopez-Melgar B, de Vega VM, Alonso-Farto JC, Guallar E, Sillesen H, Rudd JH, Fayad ZA, Ibanez B, Sanz G and Fuster V. **The progression and early detection of subclinical atherosclerosis (PESA) study: rationale and design.** *Am Heart J* (2013) 166: 990-8

Fuster-CNIC-Ferrer Cardiovascular Polypill

It's been nearly a decade since the CNIC and Ferrer teamed up to realize Dr. Fuster's vision of a cardiovascular polypill to make cardiovascular treatment accessible worldwide, improve treatment adherence, and provide a cost-effective public health strategy for the prevention of myocardial infarction.



The Fuster-CNIC-Ferrer CV Polypill includes aspirin (100 mg), ramipril (in doses of 2.5, 5, or 10 mg to allow for titration), and atorvastatin (20 mg). This first-in-class medication has so far been approved by the medicines agencies of 15 European countries (Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Ireland, Italy, Poland, Portugal, Rumania, Spain, and Sweden) for use in secondary prevention of CV events. These regulatory approvals add to the existing marketing in Mexico, Guatemala, Dominican Republic, Nicaragua, Honduras, Argentina and, more recently, Chile.

The Fuster-CNIC-Ferrer CV Polypill is indicated for secondary prevention of CV events as substitution therapy in adult patients adequately controlled with the monocomponents given concomitantly at equivalent therapeutic dosages. Currently the polypill has been marketed in Spain, Portugal, Romania and Germany under two different brand names: Trinomia® and Sincronium ®.

In the coming years, the Fuster-CNIC-Ferrer CV polypill will be launched in more European countries and worldwide.

SECURE Trial

secure

SECONDARY PREVENTION OF CARDIOVASCULAR DISEASE IN THE ELDERLY

HORIZON 2020

SECURE (Secondary Prevention of Cardiovascular Disease in the Elderly Population): the first clinical trial to investigate the efficacy of a Polypill in reducing cardiovascular mortality in secondary prevention.

PI: Dr. Valentin Fuster, MD, PhD
Co-PI: Jose M Castellano, MD, PhD
Scientific Coordinator: Hector Bueno, MD, PhD
Project Director: Ester Cunha Pavon

Cardiovascular disease (CVD) has become the number one cause of death among men and women aged over 65 in Europe, and the magnitude of the burden of CVD is expected to grow in parallel with the projected population aging. Moreover, the number of EU citizens over 65 is projected to almost double by 2060 – rising from 85 million in 2008 to 151 million in 2060. Improving the survival of CHD patients has created a large population of older adults eligible for secondary prevention.

SECURE Trial

Despite ground-breaking advances in therapy, CVD mortality rates remain high, mainly because patients do not follow ideal medical management (either through nonadherence or lack of access to medications). One of the barriers to adherence that has been consistently highlighted in registries, studies and trials is pill number and treatment complexity. The last decade has seen a surge of technical innovation to develop a polypill strategy that would improve adherence and at the same time improve medication access in low and middle income countries.

The FOCUS (Fixed-dose Combination Drug for Secondary Cardiovascular Prevention) study, funded under the EU Seventh Framework Programme and coordinated by the CNIC under the direction of Dr. Valentín Fuster, was the first to demonstrate that a polypill strategy significantly improves adherence in a secondary prevention population.

The CNIC was recently granted H2020 funding to carry out the first ever clinical trial to test the impact of a polypill strategy on hard cardiovascular outcomes. The SECURE (Secondary Prevention of Cardiovascular Disease in the Elderly Population) trial, led by Drs Fuster and Castellano, will enroll 3600 patients over 65 years of age in Spain, Italy, Germany, France, Czech Republic, Hungary and Poland. Patients will be randomized to the Fuster-CNIC-Ferrer Cardiovascular Polypill and followed for 2-4 years. The kick-off meeting of the SECURE project was held in Madrid in May 2015. Patient recruitment will begin in early 2016. The results of the SECURE study will help shape clinical recommendations for better use of medication in patients with ischemic heart disease across the world.

The Fuster-CNIC-Ferrer Cardiovascular Polypill is now a reality worldwide. The polypill has been approved for commercialization in more than 25 countries and has been approved by the major regulatory agencies. After the success of FOCUS, SECURE will provide the final evidence to enable millions of patients worldwide to benefit from simpler, more effective and cost-effective chronic treatments to decrease cardiovascular mortality and morbidity.

SELECTED PUBLICATIONS

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STEMI trials: The Metoprolol program

Acute myocardial infarction (AMI) is the main cause of death in western countries. The best strategy to limit myocardial damage is to perform an early coronary reperfusion. However, reperfusion itself comes at a price of additional myocardial damage, known as ischemia/reperfusion (I/R) injury.

The duration of ischemia can only be shortened through coordinated healthcare policies aimed at early detection and transfer of patients to hospitals with angioplasty capabilities. I/R injury, on the other hand, could potentially be reduced by pharmacological approaches; but despite great efforts, no therapy has been shown to consistently limit this phenomenon.

ß-blockers are a class of drugs that have been used to treat cardiovascular conditions for several decades. ß-blockers reduce mortality when administered after an AMI, and are a class IA indication in this context. There is a lack of information on the infarct-limiting effect of ß-blockers in patients undergoing reperfusion (current state-of-the-art treatment for infarction). Based on strong preclinical data, the CNIC initiated a program of clinical research with the long-term aim of demonstrating a reduction of events by the prereperfusion metoprolol administration in STEMI patients. The first trial was METOCARD-CNIC, recruiting patients with anterior STEMI presenting early (<6 hours from symptom onset). The EARLY BAMI trial is the validation study, recruiting a less restricted population with STEMI in any location presenting within 12 hours of symptom onset. In both trials, metoprolol or comparator (control/placebo) was administered before mechanical reperfusion.

The METOCARD-CNIC multicenter randomized clinical trial has already been completed. A total of 270 patients were recruited mainly by the emergency medical services. Metoprolol administration was associated with significantly smaller infarctions as evaluated by cardiac magnetic resonance (CMR) one week after infarction (Circulation 2013;128:1495-503), and with better long-term LVEF on 6-month CMR (J Am Coll Cardiol. 2014;63:2356-62). Metoprolol also significantly reduced the incidence of severe cardiac dysfunction and the incidence of heart failure readmissions.

The EARLY BAMI trial is a multinational randomized clinical trial conducted in Holland and Spain. More than 600 STEMI patients have been recruited to date. The primary endpoint is infarct size evaluated by CMR one month after reperfusion. All CMR studies are being analyzed in the central core lab at the CNIC. It is anticipated that more than 300 patients will undergo CMR to meet the power calculation. The CNIC is coordinating the Spanish branch of the trial. EARLY BAMI is the result of a multidiciplinary effort bringing together several partners. Patients are recruited by the Emergency Medical Service SUMMA112 during transit to one of the following participating hospitals within the codigo infarto Madrid: Hospital Fundación Jiménez Díaz, Hospital 12 de Octubre, Hospital Clínico San Carlos, Hospital Puerta de Hierro, Hospital Gregorio Marañón, Hospital de la Princesa, Hospital Ramón y Cajal, Hospital Fundación Alcorcón, and Hospital Principe de Asturias. All CMR studies in Spain are being performed at the CNIC using a unique magnet system. Reporting of the primary outcome is expected during 2016.

After these two trials testing the effect of early intravenous metoprolol on infarct size, the next step will be a larger multinational events-powered clinical trial led by the CNIC. More than 1200 STEMI patients will be recruited in more than 3 European countries.

Members of the METOCARD-CNIC and EARLY BAMI research group.

TAN SNIP: *Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis & Prevention*

TANSNIP unites 4 projects with the shared goal of building a model of cardiovascular risk based on detection, quantification and characterization of subclinical atherosclerosis and using this model to improve risk stratification and enable novel targeted therapies and risk reduction strategies. This transatlantic network brings together leading international experts from complementary fields, pools data from existing patient cohorts, and combines resources and knowhow in state-of-the-art imaging modalities, sophisticated biomarker platforms, and population sciences.

Existing tools for characterizing atherosclerosis and determining the risk of its complications are inadequate, and these deficiencies limit effective management across the spectrum of this common disease. Consequently, opportunities for early, cost-effective interventions in subclinical disease are missed, while high-risk populations with manifest disease are administered treatment almost indiscriminately. This leads to a high numbers-needed-to- treat (NNT), unnecessary patient risk, wasted resources, and unsustainable costs for health care providers.

The CNIC's international partners in TANSNIP are the US-based High-Risk Plaque Initiative (HRP), the Icahn School of Medicine at Mount Sinai (ISMMS) in New York, and the VU University Medical Center in Amsterdam. Within Spain, partners are the *Consejería de Sanidad de la Comunidad autónoma de Madrid*, *Banco Santander*, the *Sociedad Española de Cardiología*, and the *Fundación Interhospitalaria para Investigación Cardiovascular*.

The different research partners work on complementary aims under the TANSNIP umbrella, and the CNIC's current focus is on Aim 1, based on the PESA-CNIC cohort. This aim examines whether a personalized worksite based lifestyle intervention, driven by imaging data (3D-ultrasound of carotid and ilio-femoral arteries and coronary calcification) results in changes in behavior, improved control of risk factors and reduced progression of subclinical atherosclerosis plaque burden (SAPB).

AIM 1 (PESA-CNIC cohort)

Design

The study population for this part of the TANSNIP study consists of participants in the PESA study: employees aged 40 to 60 years of the Banco de Santander Headquarters in Madrid (Spain). Two parallel randomized controlled trials (RCT) are being conducted within the PESA cohort population. One RCT focuses on a sample of employees with high imaging-defined CV risk, whereas the second RCT is being conducted on a sample with low imaging-defined CV risk. In both RCTs, the participants are randomized to receive a comprehensive 3-year worksite lifestyle intervention or standard occupational health care. The worksite-based lifestyle intervention program consists of three elements: (A) twelve 1-hour sessions of personalized lifestyle counseling; (B) provision of a pedometer (Fitbit) for self-monitoring of physical activity; and (C) use of a sit-to-stand workstation (optional). Data will be collected at baseline and at follow-up at 1 year (T1), 2 years (T2), and 3 years (T3).

Endpoints

The primary outcome measure is BEWAT (a compilation score of moderate-vigorous physical activity, sedentary behavior, dietary fruit and vegetable intake, smoking, body weight and blood pressure), which will be assessed at baseline and years 1, 2 and 3. **Secondary outcomes** are changes in lifestyle (physical activity, standing behavior, diet, smoking, vitality, and quality of life), risk-factor profile, anthropometric measures, blood biomarkers, work-related outcomes (including work productivity and sickness absenteeism), health care comsumption, and intervention process evaluation measures.

Hypothesis

We predict that individual awareness of CVD risk stratification based on subclinical atherosclerosis imaging, accompanied by a comprehensive 3-year worksite-based lifestyle intervention, will lead to a reduction in the prevalence of CV risk factors related to lifestyle and an increase in physical activity, compared to standard practice. We further predict that the level of compliance with the 3-year worksite-based lifestyle intervention will be higher in the high imaging-defined CV risk group than in the low imaging-defined CV risk group.

TAN SNIP: *Trans-Atlantic Network to Study Stepwise Noninvasive Imaging as a Tool for Cardiovascular Prognosis & Prevention*

AIM 1 (PESA-CNIC cohort)

Inclusion data and intervention program

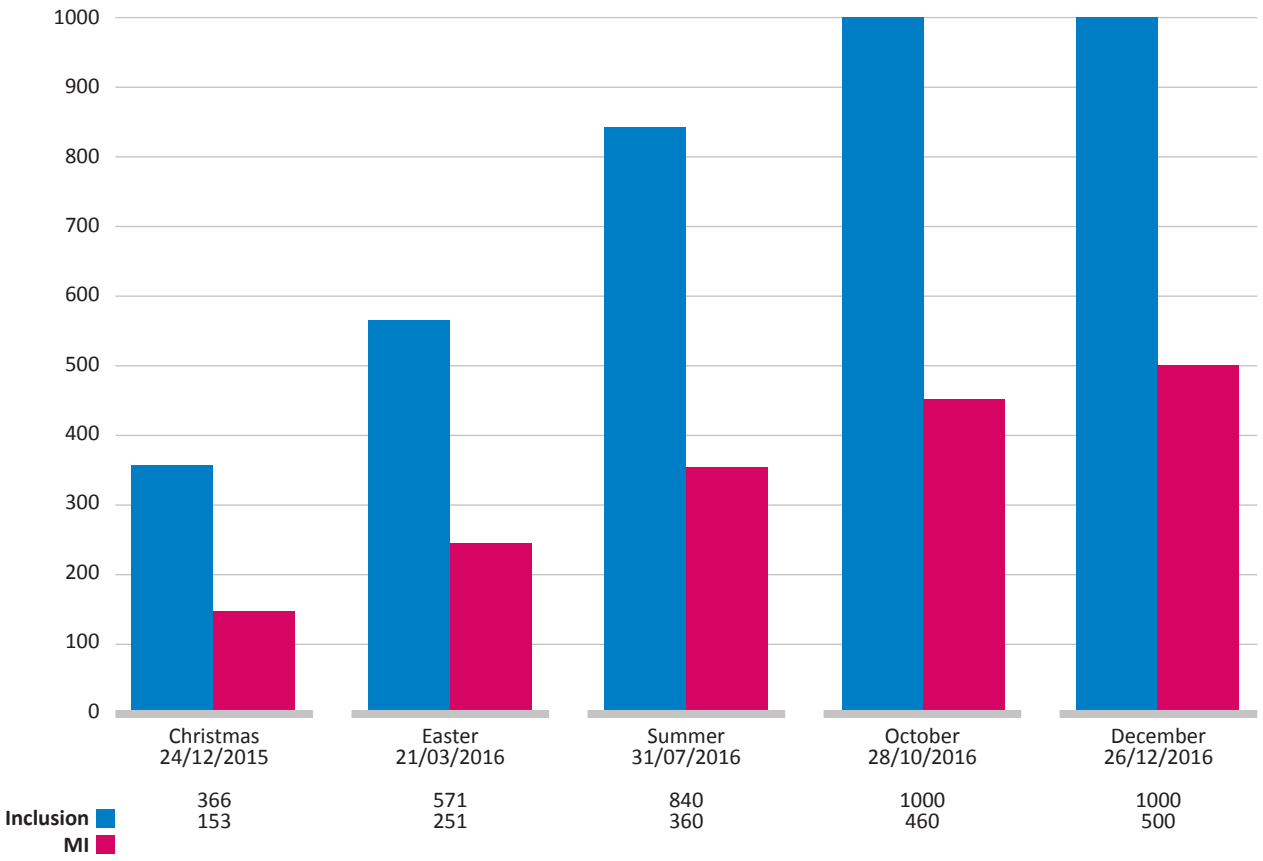
TANSNIP started including participants in May 2015 and the first MI session took place in June 2015. So far, a total of 339 participants have been included in the trial (170 in the control group and 169 in the intervention group). Of these, 95 participants belong to the high-risk imaging-defined RCT and 244 to the low-risk RCT. In the intervention group 154 participants have already received at least one motivational interview, 125 participants are using the Fitbit activity monitor, and 128 participants are willing to use the sit-stand workstation (54 workplace workstations have been installed so far). The expected inclusion and motivational interview schedule is shown in Figure 2. Inclusion is expected to be complete in October 2016, at which stage 500 motivational interviews will have been performed. The study is scheduled for completion in September 2019.

On December 2, 2015 the first scheduled 6-month focus group was held, with 5 participants from the intervention group who had completed the first 7 motivational interviews. Overall, the participants expressed themselves very satisfied with the intervention program.

As a quality-control measure, every 6 months randomly-selected motivational interviews are recorded and the study technicians are asked to complete a survey.

Scientific output

An abstract of the protocol paper has been submitted to the annual meeting of the International Society of Behavioural Nutrition and Physical Activity (ISNBPA), to be held in Cape Town, June 8-11, 2016. The full protocol paper is being finalized, and next year attention will be given to raising the study's media profile

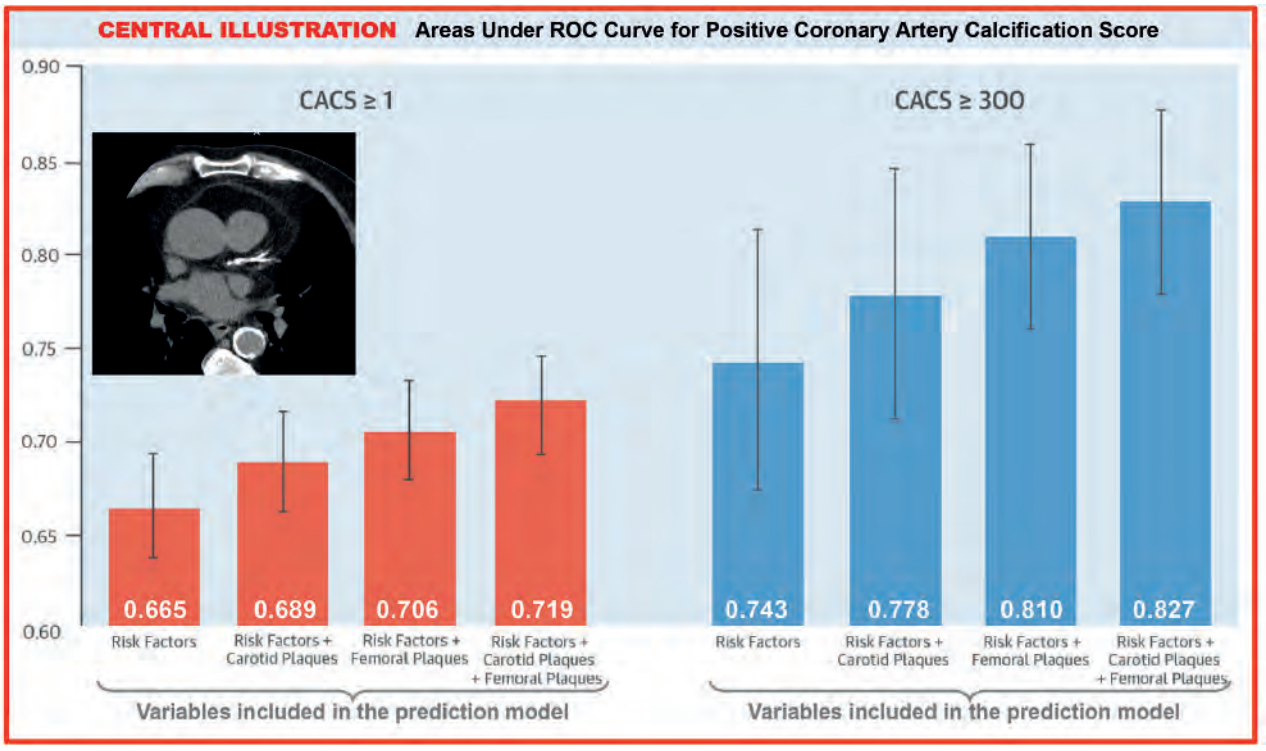


AWHS

The Aragon Workers Health Study (AWHS) is is a project conducted in collaboration with the *Instituto Aragonés de Ciencias de la Salud* (IACS) and the General Motors factory in Zaragoza. The AWHS was designed to evaluate the trajectories of traditional and emergent CVD risk factors and their association with the prevalence and progression of subclinical atherosclerosis in a population of middle-aged men and women in Spain. The study examines the development of cardiovascular disease and its risk factors by monitoring factory workers at their annual medical checkups.

The AWHS is an observational, prospective cohort study including more than 5000 participants. Recruitment began in 2009 and all workers at the factory fulfilling the inclusion criteria and willing to participate have now made their initial visit. In 2011, a screen was begun to detect subclinical atherosclerosis among 40-54-year-old participants, based on vascular 2D and 3D ultrasound in carotid, aorta and ilio-femoral arteries and on measurement of coronary artery calcification by computed tomography (CT). At the end of 2014, more than 2500 participants had been studied and the screen was concluded.

In 2012, the study's general methods were published (1) in an open access journal to support a more focused future publication of the ongoing research subprojects and to provide a clear description of the study to support fund-attracting strategies. The main results of vascular imaging studies at baseline will be published early in 2016 (2). Subclinical atherosclerosis was highly prevalent in this middle aged male cohort and, interestingly, association with risk factors and coronary calcium was found to be stronger for femoral plaques than for plaques in the carotid arteries. These data strongly support the screening of femoral plaques as a strategy for improving cardiovascular risk scales and predicting coronary disease.



Modified from Laclaustra M et al. *J Am Coll Cardiol* 2016 (in press)

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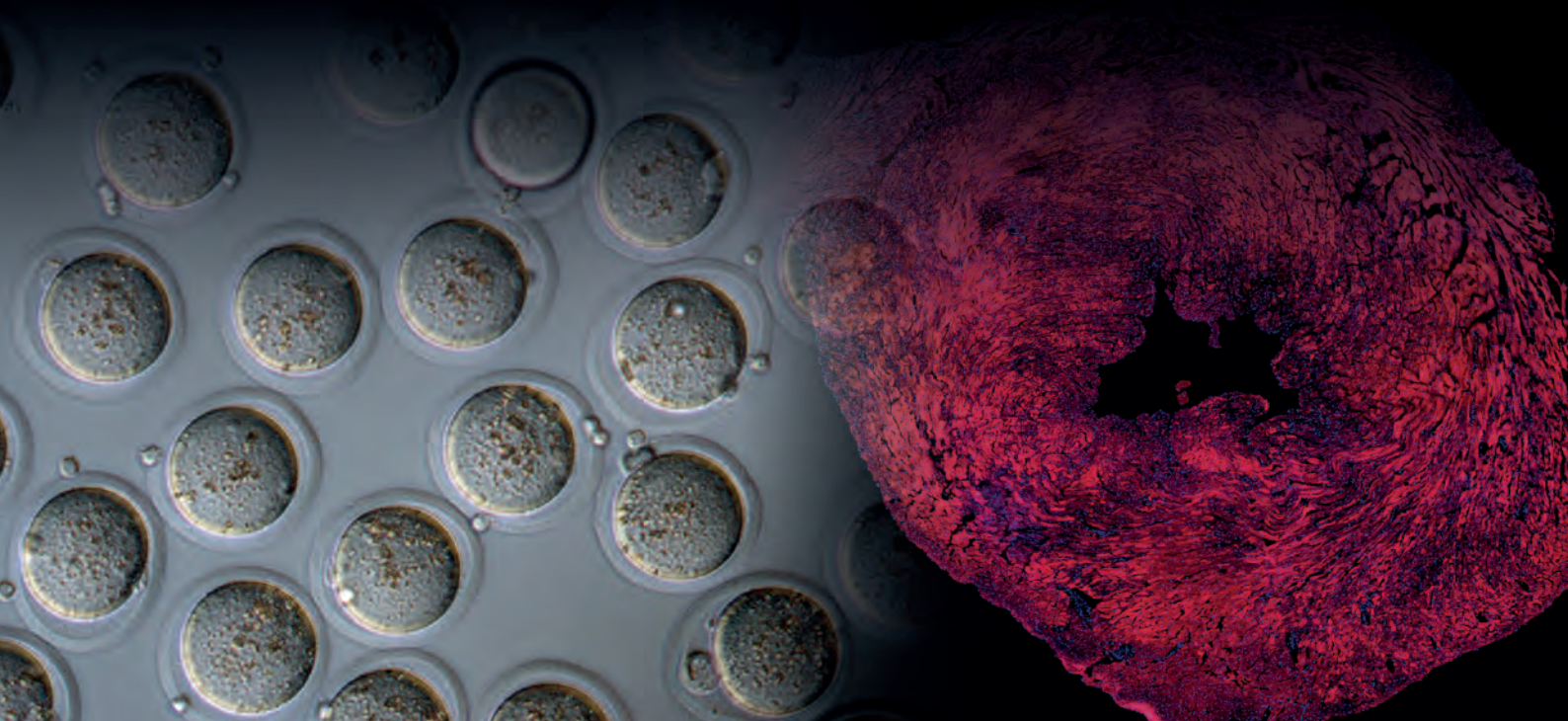
Laclaustra M, Casasnovas JA, Fernández-Ortiz A, Fuster V, León-Latre M, Jimenez-Borreguero LJ, Pocovi M, Hurtado-Roca Y, Ordovas JM, Jarauta E, Guallar E, Ibañez B, Civeira F. **Femoral and carotid subclinical aterosclerosis: risk factors and coronary calcium. The Aragon Workers' Health Study.** *J Am Coll Cardiol* (accepted)

Casasnovas JA, Alcaide V, Civeira F, Guallar E, Ibañez B, Borreguero JJ, Laclaustra M, León M, Peñalvo JL, Ordovás JM, Pocovi M, Sanz G, Fuster V. **Aragon workers' health study--design and cohort description.** *BMC Cardiovasc Disord* (2012) 12: 45

TECHNICAL UNITS

- Advanced Imaging
- Bioinformatics
- Cellomics
- Comparative Medicine
- Genomics
- Microscopy and Dynamic Imaging
- Pluripotent Cell Technology
- Proteomics/Metabolomics
- Transgenesis
- Viral Vectors



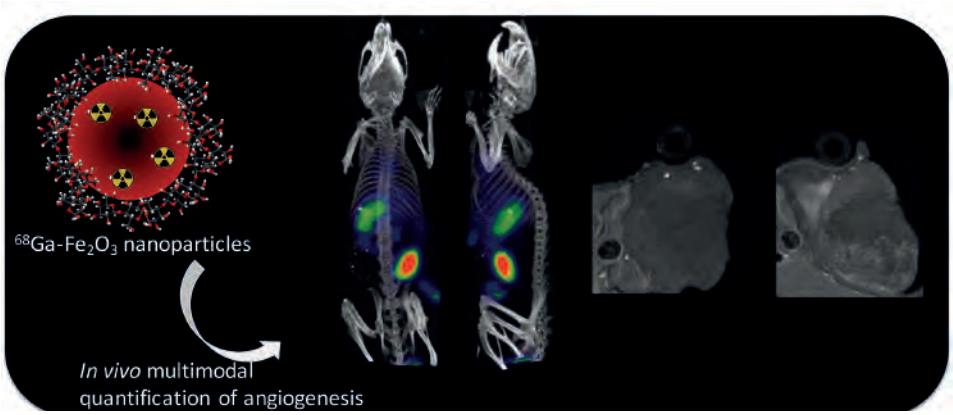
Advanced Imaging

Head of Unit

Jesús Ruiz-Cabello Osuna

RESEARCH INTEREST

The Advanced Imaging Unit (AIU) is a multidisciplinary group working on the development of new imaging applications and molecular imaging tools that will expand knowledge of the molecular and cellular events underlying cardiovascular disease. The three core areas of the AIU's research and service are 1) cardiovascular imaging, 2) nanomedicine and radiochemistry, and 3) metabolomics (research only). The AIU offers the CNIC and the wider scientific community support and expertise in cardiovascular imaging using five state-of-the-art modalities: MRI, X-ray CT, nuclear imaging (PET), ultrasound (echocardiography) and optical (2- and 3-dimensional luminescence and fluorescence). For its nanomedicine and radiochemistry program, the AIU has a dedicated nanotechnology and bioorganic chemistry laboratory dedicated to developing new nanoparticles, molecular probes, techniques for site-directed biofunctionalization of biomacromolecules (peptides, proteins and antibodies), and tools for the oriented immbolization of these molecules for the diagnosis and treatment of cardiovascular diseases. Currently the unit produces multifunctional nanoparticles for all imaging techniques available at the CNIC. The range of nanoparticles includes iron oxide, liposomes, carbon dots and gold nanoparticles, and all of them are functionalized with specifc cardiovascular biomarkers. The Unit's radiochemistry laboratory is now fully operative for ^{68}Ga and ^{89}Zr, providing the Center with specific PET radiotracers for cardiovascular nuclear imaging. On a daily basis, the imaging unit works with conventional (cyclotron obtained) radiotracers (^{18}F-FDG, ^{18}F-FMISO, ^{18}F-NaF, etc.) for the noninvasive assessment of different cardiovascular diseases. The Unit also has long experience in metabolic data analysis using ^{18}F-FDG PET, magnetic resonance spectroscopy (^{13}C, ^{31}P, ^{1}H) and mass spectrometry, as well as statistical and image and spectroscopic processing tools developed in-house. The Unit is also engaged in developing new techniques for cardiovascular imaging (PET, CT and MRI), which are tested and validated on small and large animal models and finally transferred to human applications. Our research in these areas ranges from technical developments and chemistry advances to *in vitro* studies and tracking of biological processes *in vivo*.



Nano-radiochemistry applications. *Left*: ^{68}Ga core-doped iron oxide nanoparticles for angiogenesis quantification. *Right*: PET/CT imaging of tumor-bearing mice 1 hour after injection of ^{68}Ga-C-IONP-RGD, showing strong activity in the tumor. *Right*: Axial T1-weighted spin echo MRI of the tumor area in a mouse before injection of ^{68}Ga-C-IONP-RGD and 24 hours post-injection.

Postdoctoral Researchers:

Fernando Herranz
Jesús Mateo de Castro
Samuel España
Teresa Arias
Marco Filice
José Luis Izquierdo
Arnoldo de Jesús Santos Oviedo

Predoctoral Researchers:

Ana Victoria Lechuga
Hugo Groult
Juan Pellico
Riju Bhavesh
Ehsan Yazdanparast
Carlos Velasco

Technicians:

Izaskun Bilbao
Marina Benito
Coral Velasco
Yeny Rojas
Natalia Moñivas

Res@CNIC Fellow:

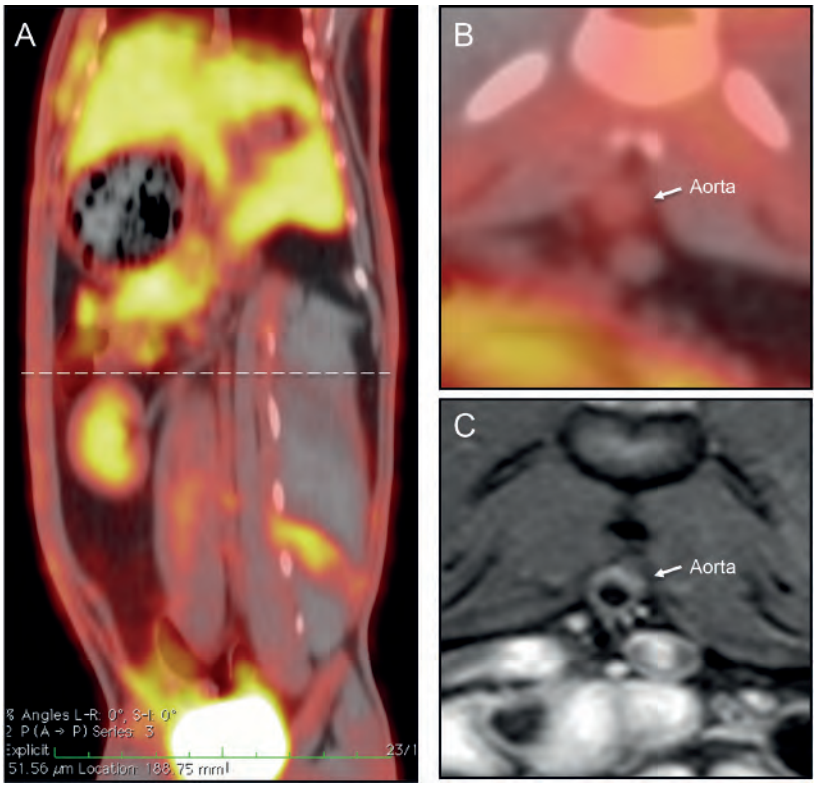
Ana Vega

Masters Students:

Adriana Mota
Almudena González
Irene Fernández-Barahona

Visiting Scientists:

Ignacio Rodríguez
Palmira Villa
Sandra Pérez Rial
José Gabriel Venegas
Clara Uriel



In vivo **^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) of metabolic rate/ inflammation, and magnetic resonance imaging (MRI) monitoring of atherosclerotic plaque progression**. A, Combined PET/computed tomography (CT) coronal view of the abdominal aorta, illustrating the ^{18}F-FDG uptake of an atherosclerotic rabbit after 8 months on an atherogenic diet. B, Axial view of the slice indicated with a dotted line in the coronal view, showing increased FDG uptake in the aorta. C, Corresponding T1-weighted MRI showing development of an aortic atherosclerosis lesion.

MAJOR GRANTS

- *Ministerio de Sanidad y Consumo (CIBERES CB06/06/1090)*
- *European Commission FP7-PEOPLE-2010-ITN (П-NET 264864) (NO CNIC).*
- *European Commission FP7-PEOPLE-2013-ITN (CardioNext PITN-GA-2013-608027)*
- *Ministerio de Economía y Competitividad. FIS RETICS (Terapia Celular: RD12/0019/0005) PI: Miguel Torres, Colaborador Jesus Ruiz Cabello*
- *Ministerio de Economía y Competitividad. Modalidad Generación Conocimiento (MAT2013-47303-P) PI: Fernando Herranz*
- *Instituto de la Salud Carlos III. FIS-FEDER (PI14/01427) PI: Jesús Mateo*
- *Ministerio de Economía y Competitividad. SAF2014-58920-R PI: Samuel España*
- *Ministerio de Economía y Competitividad. SAF2014-59118-JIN. PI: Marco Filice*
- *Madrid-MIT M+Visión (PRMIT2013) PI: Samuel España*
- *Madrid-MIT M+Visión (MIT14 - AAE867002) PI: Teresa Arias*
- *Madrid-MIT M+Visión (MIT14 - X7118248R) PI: Arnoldo Santos*

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Mateo J, Bilbao I, Vaquero JJ, Ruiz-Cabello J, España S. **In vivo ^{18}F-FDG-PET imaging in mouse atherosclerosis.** *Methods Mol Biol* (2015) 1339: 377-86

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Pellico J, Lechuga-Vieco AV, Benito M, García-Segura JM, Fuster V, Ruiz-Cabello J, Herranz F. **Microwave-driven synthesis of bisphosphonate nanoparticles allows in vivo visualisation of atherosclerotic plaque**. *RSC Advances* (2015) 5: 1661-5

Groult H, Ruiz-Cabello J, Pellico J, Lechuga-Vieco AV, Bhavesh R, Zamai M, Almarza E, Martín-Padura I, Cantelar E, Martínez-Alcázar MP, Herranz F. **Parallel multifunctionalization of nanoparticles: a one-step modular approach for in vivo imaging.** *Bioconjug Chem* (2015) 26: 153-60

Bioinformatics

Head of Unit

Fernando Martínez

Support Scientists:

Fátima Sánchez Cabo
Carlos Torroja
Manuel José Gómez Rodríguez

Predoctoral Researchers:

Wencke Walter
(from the Nuclear Receptor Signaling Laboratory, led by Mercedes Ricote)
Alberto Gatto
(from the Molecular Regulation of Heart Development and Disease Laboratory, led by Enrique Lara-Pezzi)
Girolamo Giudice
(from the Molecular Regulation of Heart Development and Disease Laboratory, led by Enrique Lara-Pezzi)

RESEARCH INTEREST

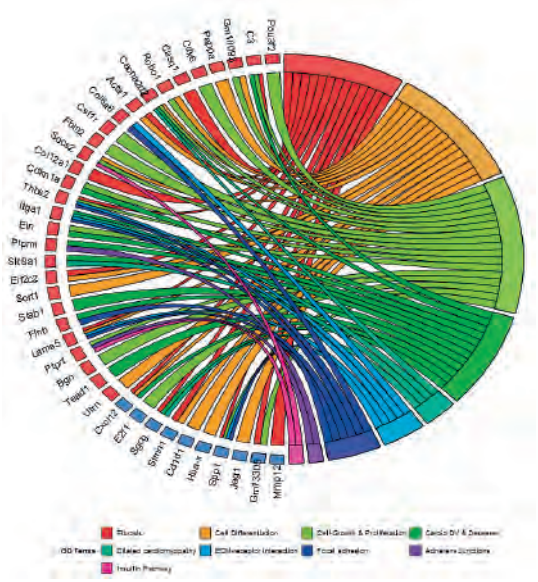
The Bioinformatics Unit provides *ad-hoc*, state-of-the-art bioinformatic, data analysis and computational biology solutions to support and enhance CNIC research projects in a collaborative environment.

During 2015the Unit worked with several research groups to has developed two new bioinformatics tools to visualize and gain insight into the biology of complex systems explored with omics technologies. (i) GOplot (Walter W et al, *Bioinformatics* 2015) is a Bioconductor package to ease the integration of omics data with functional analysis results (Figure 1). (ii) ATtRACT (Giudice G et al, *Database* 2016) is a database of RNA binding protein motifs that integrates resources disseminated in various repositories to facilitate the identification of enriched motifs in the sequence of sets of alternatively spliced genes (Figure 2).

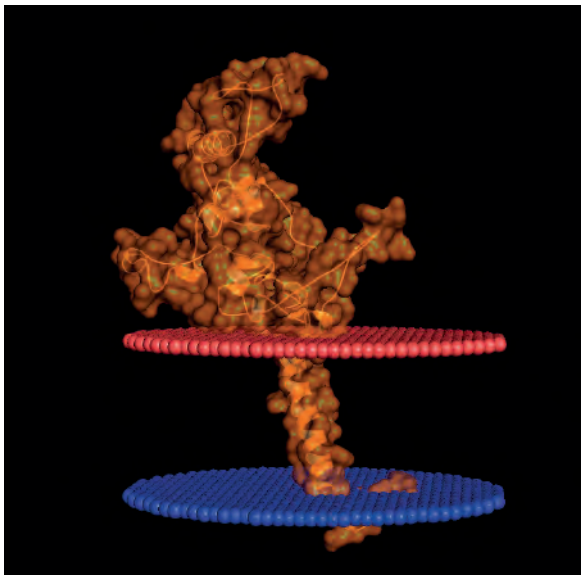
Another main focus during 2015 was the implementation of state-of-the-art software suites, tools and pipelines for structural bioinformatic analysis, modeling and protein docking (Figure 3). These solutions give CNIC researchers a new view of the 3D and protein interaction environments to help them in their research.

In addition, the Unit is moving towards translational bioinformatics by automating the already implemented pipelines to allow the analysis of hundreds of samples of large human cohorts. We are also involved in the implementation of tools and methodology to integrate heterogeneous *omics* data, as well as in the development of pipelines for the analysis of single-cell data, in collaboration with the Genomics Unit and members of the EU consortia funded by the H2020 APERIM program (http://aperim.eu/).

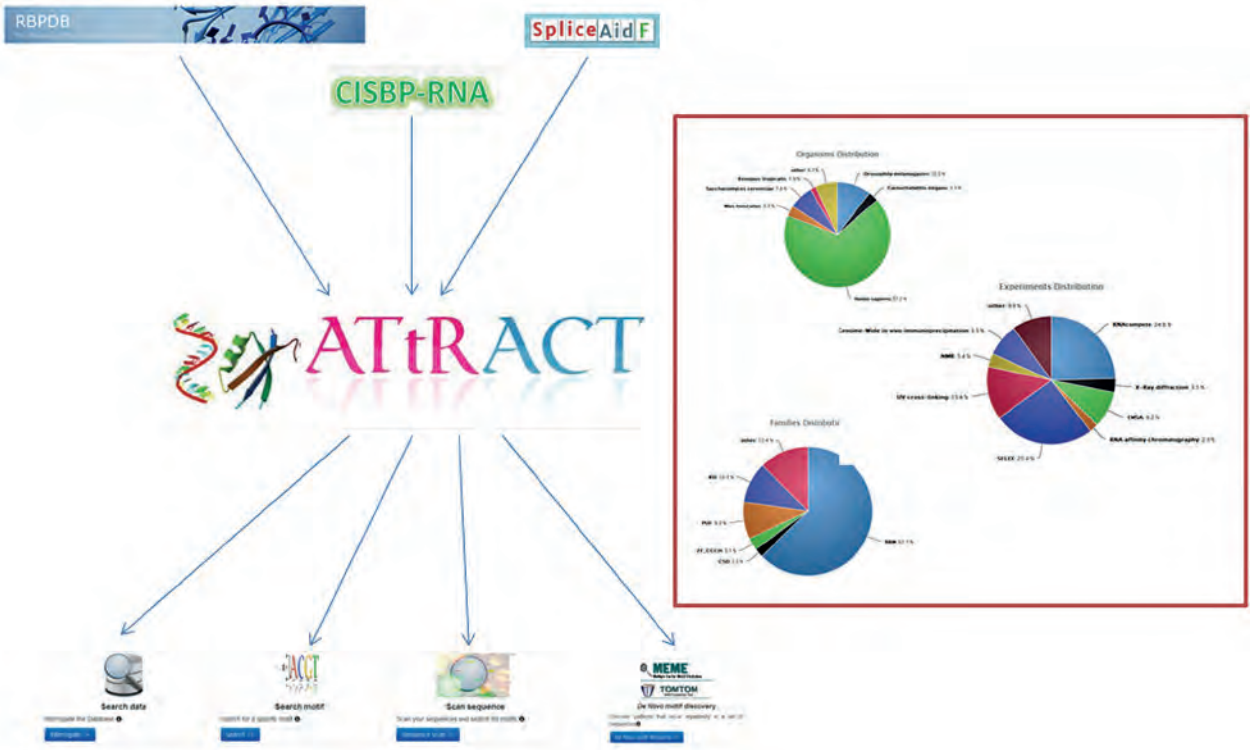
The Unit has also continued to provide customized advice and training to CNIC researchers on the analysis and interpretation of their experimental data. In this regard, we have completed the implementation of the Bioinformatics Unit Galaxy Platform, which will be soon opened to CNIC researchers, allowing them to analyze their own *omics* data.



Example of a circos plot produced with the GOplot package (Walter et al, 2015) to display the association between differentially expressed genes and the corresponding enriched pathways (D'Amato et al, 2015)



3D view of the emerin protein embedded in the nuclear membrane



Summary of the Attract database (Giudice G, Sánchez-Cabo F, Torroja C*, Lara-Pezzi E*, ATtRACT - A database of RNA binding proteins and associated motifs. Accepted for publication in *Database*)

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Walter W, Sanchez-Cabo F*, Ricote M*. **GOplot: an R package for visually combining expression data with functional analysis**. *Bioinformatics* (2015) 31: 2912-4
*Co-corresponding authors

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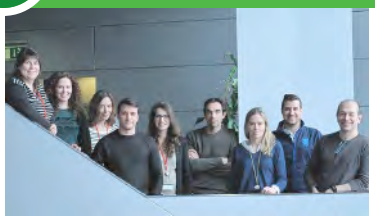
Bednarek D, Gonzalez-Rosa JM, Guzman-Martinez G, Gutierrez-Gutierrez O, Aguado T, Sanchez-Ferrer C, Marques IJ, Galardi-Castilla M, de Diego I, Gomez MJ, Cortes A, Zapata A, Jimenez-Borreguero LJ, Mercader N, Flores I. **Telomerase is essential for zebrafish heart regeneration**. *Cell Rep* (2015) 12: 1691-703

Cellomics

Head of Unit

María Montoya



RESEARCH INTEREST

The Cellomics Unit provides the CNIC with the two principal cell analytical techniques, flow cytometry and high content screening (HCS), and supports quantitative image-based research.

In 2015, we implemented spectral flow cytometry technology with a newly acquired Sony SP6800 Spectral analyzer (Fig. 1). We also organized the High Content Screening Workshop at the CNIC, which brought together researchers from academia and industry to discuss state-of-the-art methodology and the latest computing solutions for high content screening technology. In partnership with the Integrin Signaling group, we successfully developed an HCS assay for analyzing extracellular matrix remodeling based on multiparametric image analysis of a combination of texture and intensity parameters obtained from fibronectin signals. In control experiments Y27 induced a chaotic phenotype (Z' = 0.5) whereas TGFβ induced an organized phenotype (Z' = 0.28) (Fig. 2). The Unit has also programmed an automatic segmentation and tracking computational pipeline for confocal 4D time-lapse videos of cell membrane signals recorded in cultured cells. This work has been done in partnership with the Genetic control of organ development and regeneration laboratory, and allows the automated extraction of phenotypic parameters. An additional data analysis and cell lineage tree tracing pipeline was developed in MATLAB (Fig.3B). The Unit also developed customized image analysis tools for a variety of purposes, for example, quantification of collagen deposition or blood vessels in heart and tumor immunohistological tissue sections.

Research Scientists:

José Manuel Ligos
Laura Fernández
Daniel Jiménez

Predoctoral Researcher:

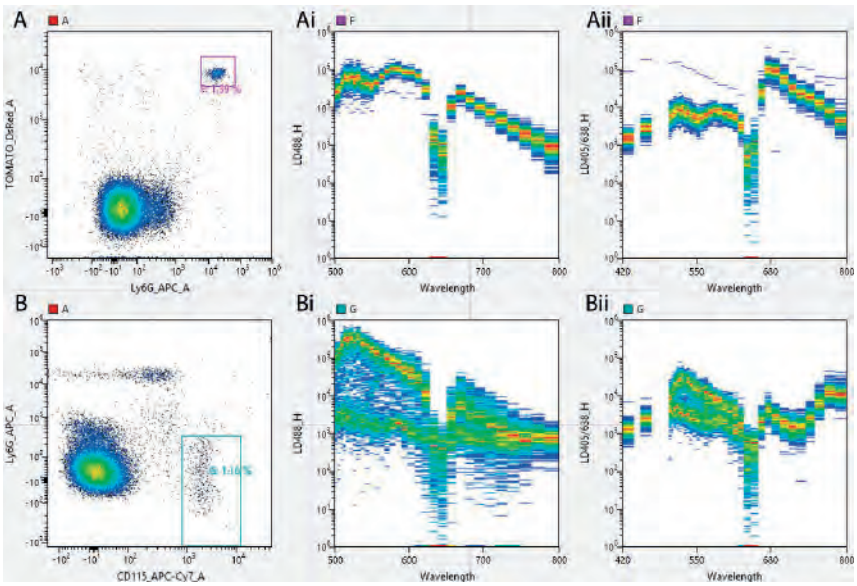
Antonio Quilez
(since 1 December)

Technicians:

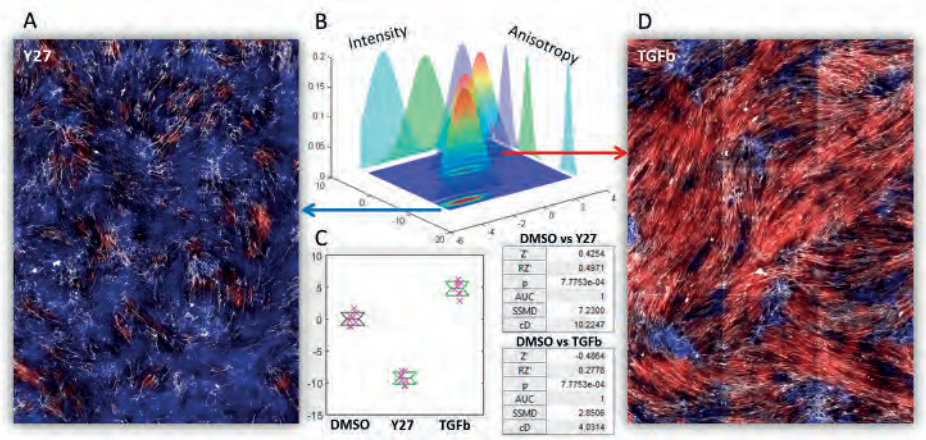
Raquel Nieto
Mariano Vitón
Irene Palacios Doiztua
Elena Prieto
(since 1 December)

Visiting Scientist:

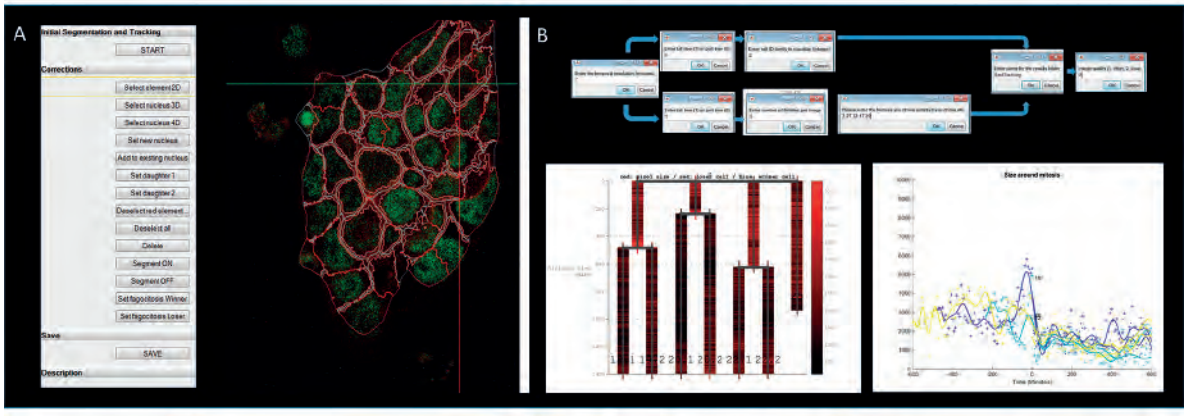
Marco Cordani



Detection of myeloid subpopulations by spectral flow cytometry. Mouse peripheral blood cells labeled with Ly6C FITC, Ly6G APC, and CD115 APC-CY7. **A,B)** Dot plot representations of subpopulations of interest TdTomato+/Ly6G+ (F) and CD115+/Ly6G- (G). Spectra are shown for analysis of the F **(Ai, Aii)** and G **(Bi, Bii)** subpopulations, and were obtained by exciting with lasers at 488 nm **(Ai, Bi)** and 638 nm **(Aii, Bii)**.



Quantification of the organization of the extracellular matrix of using multiparametric image analysis. **A, D)** Fn staining of Hela cells treated with Y27 (left) or TGFβ (right) displaying chaotic (blue) and organized (red) phenotypes. **B)** Gaussian mixture model of texture and intensity parameters. **C)** The resulting value of texture and intensity combination for eight independent wells of the indicated treatments.



Cell segmentation, tracking and data analysis tool. **A)** Segmentation and tracking computational pipeline with edition interface developed in Definiens. **B)** Data analysis and cell lineage tree tracing developed in MATLAB, showing visualization options and an example of the resulting graphics.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (BIO2014-62200-EXP)*
- *European Union (641639) (H2020 ITN-BIOPOL)*

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Pellico J, Ruiz-Cabello J, Saiz-Alía M, del Rosario G, Caja S, Montoya MC, Laura Fernández de Manuel L, Puerto Morales M, Gutiérrez L, Galiana B, Enríquez JA, Herranz F. **Fast synthesis and bioconjugation of 68Ga core-doped extremely small iron oxide nanoparticles for PET/MR imaging**. *Contrast Media Mol Imaging* (accepted)

Leiva M, Quintana JA, Ligos JM, Hidalgo A. **Haematopoietic ESL-1 enables stem cell proliferation in the bone marrow by limiting TGFβ availability**. *Nat Commun* (accepted)

Rallon NI, Mothe B, Lopez Bernaldo DE Quiros JC, Plana M, Ligos JM, Montoya MC, Muñoz MA, Esteban M, Garcia F, Brander C, Benito JM; RISVAC03 Study Group. **Balance between activation and regulation of HIV-specific CD8 T cells response after MVA-B therapeutic vaccination**. *AIDS* (doi: 10.1097/QAD.0000000000000966. Epub 2015 Nov 19)

Jimenez-Carretero D, González G., Rodríguez-López S., Kumamaru KK, George E, San José ER, Ledesma-Carbayo MJ (2015). **Automated axial right ventricle to left ventricle diameter ratio computation in computed tomography pulmonary angiography**. *PloS One* (2015) 10: e0127797

Petitjean C, Zuluaga MA, Bai W, Dacher JN, Grosgeorge D, Caudron J, Ruan S, Ayed IB, Cardoso MJ, Chen HC, Jimenez-Carretero D, Ledesma-Carbayo MJ, Davatzikos C, Doshi J, Erus G, Maier OM, Nambakhsh CM, Ou Y, Ourselin S, Peng CW, Peters NS, Peters TM, Rajchl M, Rueckert D, Santos A, Shi W, Wang CW, Wang H, Yuan J. **Right ventricle segmentation from cardiac MRI: A collation study**. *Med Image Anal* (2015) 19:187-202

Comparative Medicine

The Unit develops and manages laboratory animal models to reproduce the principal human cardiovascular diseases, working closely with the CNIC research teams and apllying the 3 Rs. The Unit tries to refine these animal models by identifying factors that could interfere with research project aims, be a source of non-representative data, or have a major impact on animal welfare.

The Comparative Medicine Unit's support for in vivo work at the CNIC is organized into five core work areas.

- *Animal Husbandry*. The Unit's technicians, managers and veterinarians are trained to work under the facility's SPF conditions and take charge of the daily husbandry of the animal colonies. The Unit enacts an environmental enrichment program to support species-specific behaviors to maximize animal welfare and wellbeing.

- *Pathology Core* (PC). The Histopathology Laboratory provides specialized hispathological services including animal necropsy, paraffin and OCT processing and sectioning, histochemical and immunohistochemical staining of tissue sections, digital scanning and image analysis, optical projection tomography with an OPT 3001 scanner and general support to CNIC researchers with phenotyping and histopathological evaluation of their animal models.

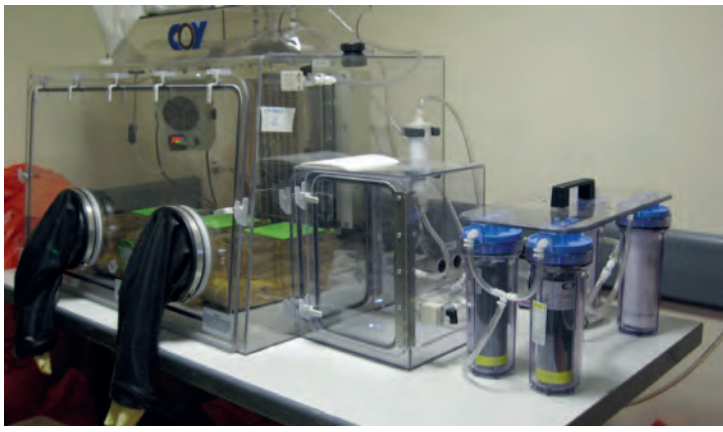
- *Phenotyping Core* (PhC). In this area, we have added new equipment to meet the needs of the CNIC research groups, including a coagulation analyzer and a metabolic cages system.

- *Veterinary Medicine and Experimental Surgery Core* (VMESC). The VMESC provides highly specialized expertise in the surveillance and monitoring of animal health status, disease follow-up, development of surgical animal models with enphasis on minimally invasive procedures, life support, setting up new experimental strategies that reproduce human cardiovascular diseases, and acquisition of pathophysiological data. The VMESC team is run by two clinical veterinarians with extensive expertise in laboratory animal science and four specialist veterinary technicians.

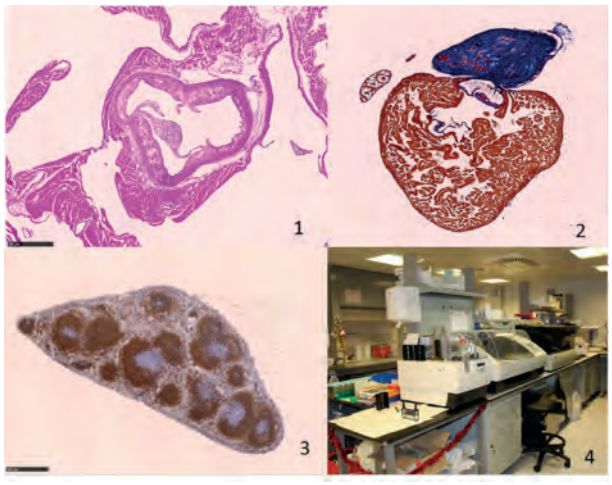
- *Quality Control Core* (QCC). The QCC follows the recommendations of the latest FELASA report (*Laboratory Animals* 2014, 48(3):178-192).

The Comparative Medicine Unit maintains ISO 9001 accreditation for all five core work areas.

PRIMUS anesthesia equipment



Hypoxic chamber



1. Atheroma plaques in a mouse aortic valve stained with H&E. **2**. Zebrafish heart stained with Acid Fuchsin Orange G to visualize collagen (blue). **3** Immunohistochemistry staining with anti-PAX5 antibody in a mouse spleen highlighting the B lymphocytes of the white pulp. **4**. Histopathology laboratory.

Genomics

Head of Unit

Ana Dopazo

Support Scientists:

Sergio Callejas
Alberto Benguría

Technician:

Rebeca Álvarez

RESEARCH INTEREST

The Genomics Unit currently focuses on second generation sequencing (NGS) technologies for genome analysis using the Illumina HiSeq 2500 and MiSeq sequencers.

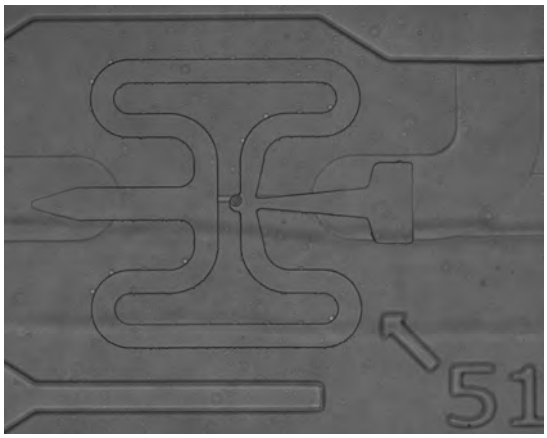
The Unit provides these cutting-edge genomic technologies to the scientific community at the CNIC and beyond, offering a wide variety of NGS applications (RNA Seq, Low input RNA Seq, small RNA-Seq, ChIP Seq, PCR Seq, Exome Sequencing, targeted resequencing, etc). On each sequencing project the Unit's tasks include project consultation, sample quality check, sample library preparation, and data generation. Several of the top CNIC scientific publications in 2014 and 2015 include NGS experiments performed in the Genomics Unit.

One of the team´s scientific and technological research interests focuses on low-input OMICS, including genomics, transcriptomics and miRNomics, with special emphasis on the study of the transcriptome at the single-cell level. The Unit has started to perform single-cell RNA seq using the Fluidigm C1 Single-Cell Auto Prep System. This microfluidic device captures individual cells, and facilitates the generation of single-cell cDNA libraries for Illumina mRNA sequencing.

The Unit continues to automate the newly incorporated NGS library preparation protocols by using an open liquid handling platform. This is especially essential when working at the single-cell level since automation allows handling the processing of the needed higher number of samples per experiment. Additionally this step avoids the bottleneck created by the high sample number typically sequenced in the Unit, and also reduces the risk of human error.

Other services include DNA fragmentation using a Covaris E220 ultrasonicator, the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT machines) and a TaqMan array processing service.

In addition to providing these high-quality genomics services, the Unit performs its own research.



Individual human liver tumor cell isolated using the C1 Single-Cell Auto Prep System.

MAJOR GRANTS

-Ministerio de Economía y Competitividad. FIS (PI14/02120)
-Ministerio de Economía y Competitividad. EXPLORA Tecnología (BFU2014-62250-EXP)

SELECTED PUBLICATIONS

Blanco FJ, Ojeda-Fernandez L, Aristorena M, Gallardo-Vara E, Benguria A, Dopazo A, Langa C, Botella LM, Bernabeu C. **Genome-wide transcriptional and functional analysis of endoglin isoforms in the human promonocytic cell line U937**. *J Cell Physiol* (2015) 230: 947-58

Stateva SR, Salas V, Benguría A, Cossío I, Anguita E, Martín-Nieto J, Benaim G, Villalobo A. **The activating role of phospho-(Tyr)-calmodulin on the epidermal growth factor receptor.** *Biochem J* (2015) 472: 195-204

Hill R, Kalathur R, Callejas S, Colaco L, Brandao R, Serelde B, Cebria A, Blanco-Aparicio C, Pastor J, Futschik M, Dopazo A, Link W. **A novel Phosphatidylinositol 3-Kinase (PI3K) inhibitor directs a potent FOXO-dependent, p53-independent cell cycle arrest phenotype characterized by the differential induction of a subset of FOXO-regulated genes.** *Breast Cancer Res* (2014) 16: 482

Microscopy and Dynamic Imaging

Head of Unit

Valeria R Caiolfa

Staff Scientists:

Moreno Zamai
Antonio Manuel Santos Beneit
Elvira Arza
Verónica Labrador Cantarero

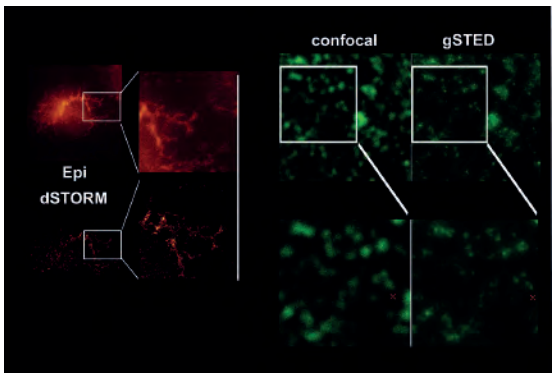
Visiting Scientists:

AntonioTrullo
Mª Eugenia Pérez-Ojeda Rodríguez

RESEARCH INTEREST

In 2015 we have continued the development of the super-resolution platform and Single Plane Illumination Imaging (SPIM). We have introduced 2-color 3D STED imaging for three-dimensional imaging of cells at resolution higher than 80 nm in the x,y and z planes, providing critical capacity for colocalization studies of complex cellular organelles and subcellular structures. Super-resolution image analysis was improved by applying newly developed deconvolution approaches.

We have exploited the flexible configuration of our multiphoton microscopes to investigate novel meso-scale bone-mimicking models that bridge the gap between microfluidic, macro-scale studies and high-throughput screening of the effects of multiple variables on the vascularization of bone-mimicking tissues. We have studied the influence of endothelial cell (EC) density and the relative proportions of ECs, mesenchymal stem cells and osteo-differentiated MSCs cultured in hydrogel-type matrices. In close collaboration with the Galeazzi Hospital in Milan, these studies allowed the identification of synergistic correlations among critical parameters driving microvascular network development within a bone-mimicking environment, and could be translated to any vascularized tissue.



Comparison of the detection of calreticulin in the endoplasmic reticulum by (left) epifluorescence and single molecule localization microscopy in direct STORM (dSTORM) and (right) confocal imaging and gated stimulated emission depletion (gSTED).

The Unit continued its training activities through one-to-one programs and workshops, and and contributed to the CNIC-JOVEN training plan (ACERCATE, CICERONE and the Master Program) through theoretical and practical sessions.

MAJOR GRANTS

- INFRA-MINECO-2013 - Plataforma Biomédica Avanzada CNIC en Nanoscopía multimodal

SELECTED PUBLICATIONS

Bersini S, Gilardi M, Arrigoni C, Talo G, Zamai M, Zagra L, Caiolfa V, Moretti M. Human in vitro 3D co-culture model to engineer vascularized bone-mimicking tissues combining computational tools and statistical experimental approach. *Biomaterials* (2015) 76: 157-72

Groult H, Ruiz-Cabello J, Pellico J, Lechuga-Vieco AV, Bhavesh R, Zamai M, Almarza E, Martin-Padura I, Cantelar E, Martinez-Alcazar MP, Herranz F, **Parallel multifunctionalization of nanoparticles: a one-step modular approach for in vivo imaging.** *Bioconj Chem* (2015) 26: 153-60

Sanchez SA, Mendez-Barbero N, Santos-Beneit AM, Esteban V, Jimenez-Borreguero LJ, Campanero MR, Redondo JM. **Nonlinear optical 3-dimensional method for quantifying atherosclerosis burden.** *Circ Cardiovasc Imaging* (2014) 7: 566-9

Pluripotent Cell Technology

Head of Unit

Giovanna Giovinazzo

Support Scientists:

Francisco Gutiérrez
Elisa Santos

Technicians:

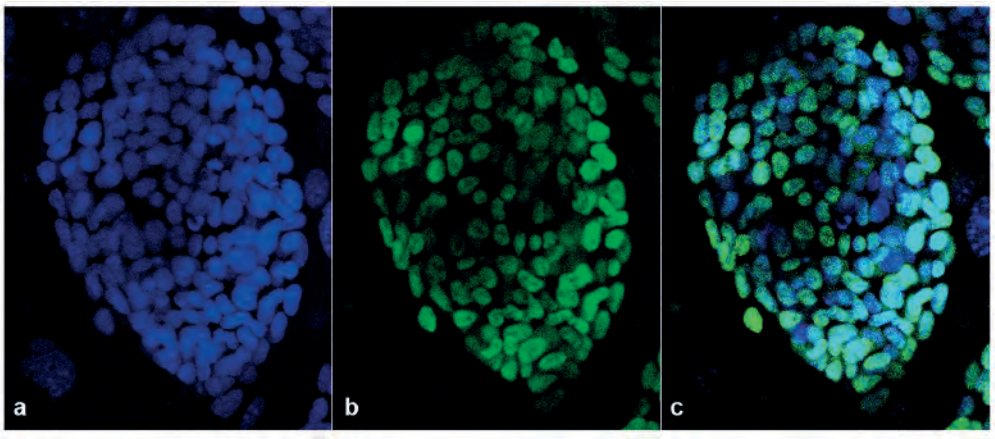
María Ángeles Sanguino
Carles Moreno Soriano

RESEARCH INTEREST

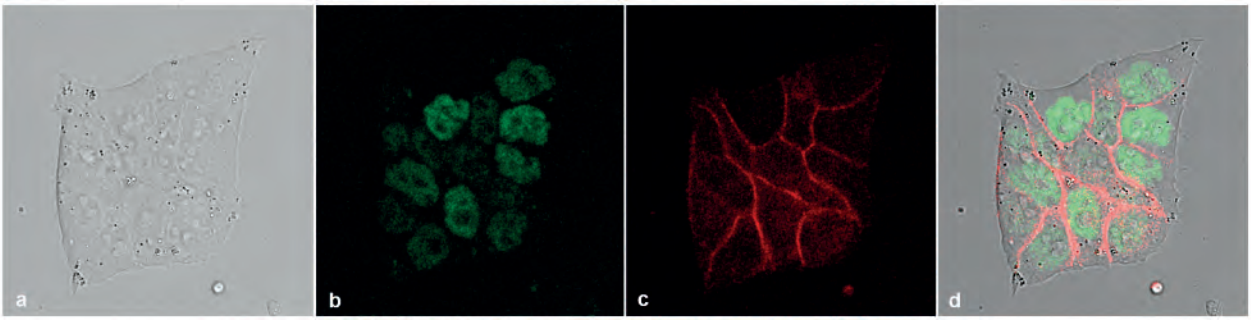
The Pluripotent Stem Cell Technology Unit supports CNIC researchers whose research involves the culture and manipulation of mouse and human pluripotent stem cells. Our staff offer expertise and comprehensive training in successful stem cell culture, supply protocols and validated reagents, and give expert advice on the maintenance and differentiation of stem cells.

One of the Unit's core functions is to facilitate gene-targeting experiments to produce quality-controlled genetically-modified mESCs, an essential requirement for germline transmission and the generation of knockout, knockin and conditional mutant mice. The Unit undertakes all key steps in the gene-targeting protocol: electroporation of the targeting vector, selection, karyotyping, culture, and the preparation of cells for blastocyst injection. The Unit's service portfolio also includes Neo removal, random insertion and screening by Southern blot. On request, we can also assist CNIC researchers with the design of targeting vectors and screening strategies. The Unit also applies its wide expertise in genetic modification to create *in vitro* models of pluripotent cells using the CRISPR/cas system, and we supply knockout stem-cell lines for diverse research projects. On request, we can also assist CNIC researchers in fine-tuning differentiation protocols for a specific lineage.

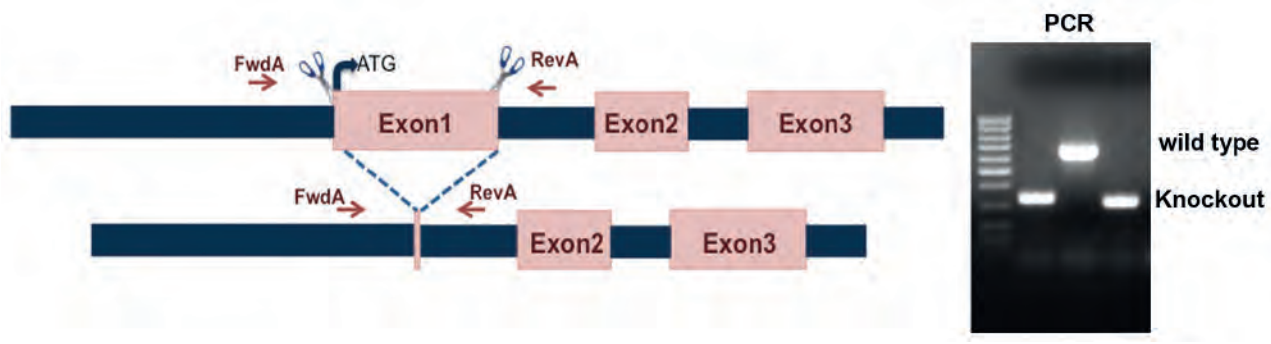
Human induced pluripotent stem cells (hiPSC) are an extraordinarily valuable source of cells for basic and translational research, including drug development and disease modeling. The Unit is also able to provide expert advice on the design of experiments involving hPSCs and provide the latest cutting-edge technology for genome editing for generating in vitro models of cardiovascular disease.



Immunocytochemistry detection of pluripotent cell markers in mESCs. a) DAPI. b) Nanog. c) Merged view.



Confocal image of mycEGFP mESCs a) Brightfield image of an undifferentiated colony. **b)** Nuclear localization of mycEGFP protein (green). c) Membrane marker expression linked to dtTomato fluorescent protein. d) Merged view



Generation of knockout mESC lines using the CRISPr/Cas9 strategy. Strategy for creating a knockout stem cell line and PCR screening to detect genetically modified clones.

SELECTED PUBLICATIONS

Rosello-Diez A, Arques CG, Delgado I, Giovinazzo G, Torres M. **Diffusible signals and epigenetic timing cooperate in late proximo-distal limb patterning.** *Development* (2014) 141: 1534-43

Gonzalez-Lazaro M, Rosello-Diez A, Delgado I, Carramolino L, Sanguino MA, Giovinazzo G*, Torres M*. **Two new Targeted alleles for the comprehensive analysis of Meis1 functions in the mouse** *Genesis* (2014) 52: 967-75
*Co-corresponding authors

Proteomics/Metabolomics

Head of Unit

Juan Antonio López

Support Scientists:

Enrique Calvo
Emilio Camafeita
Iakes Ezkurdia

Technicians:

Raquel Mesa
Rocío Campo (since 1 December)
Ricardo Magni (since 1 December)

Visiting Scientist:

María Gómez Serrano (IIB-CSIC)

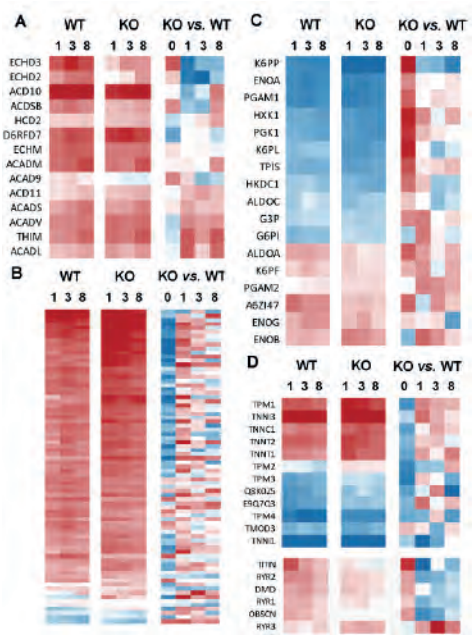
RESEARCH INTEREST

The CNIC Proteomics Unit devotes considerable effort to technological innovation, through the continuous development of new methods of interest to the research community. Throughout 2015, the Unit worked on improvements to the quantitative analysis of protein abundance by shotgun and targeted proteomics using high-throughput technologies based on nano-liquid chromatography coupled to mass spectrometry.

Progress was also made on quantitative proteomics using multiplexed stable isobaric labeling (iTRAQ and TMT). Particular improvements were made in the development of chromatographic conditions for HPLC-based peptide fractionation, and optimization of the recently incorporated Orbitrap Trybrid Fusion mass spectrometer. We also made progress in the statistical analysis of TMT-derived quantitative data and systems biology interpretation of the results using algorithms developed in house.

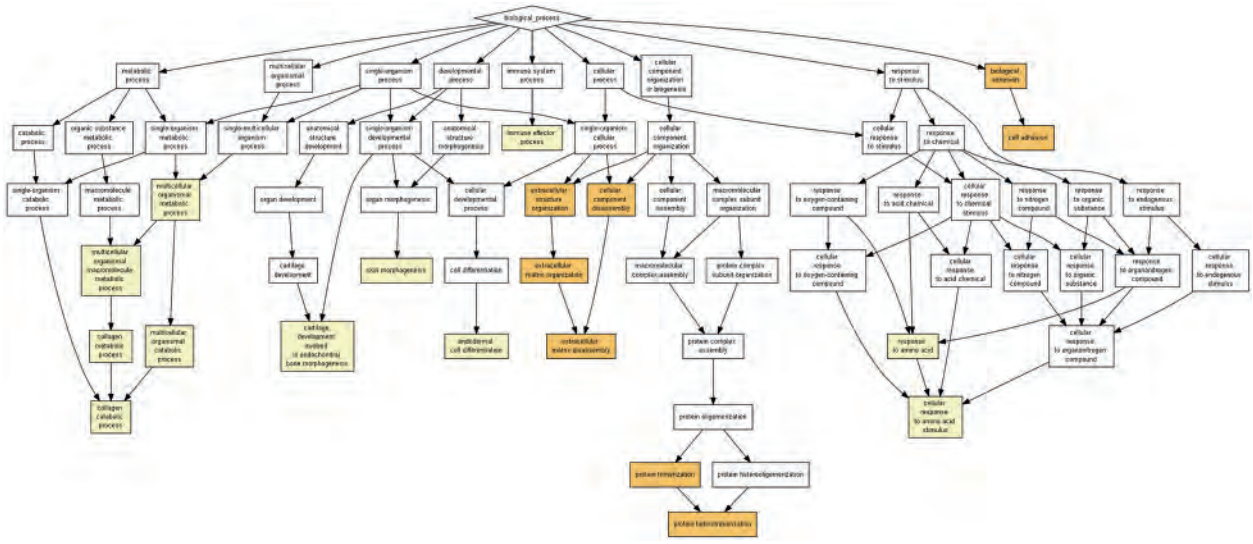
These approaches are also being applied to the quantitative analysis of posttranslationally modified peptides, directly identified by database searches or using enrichment protocols. For biomarker discovery in the clinical setting we are analyzing dozens of plasma samples using depletion protocols of the most-abundant proteins and multiplexed quantitation.

We are developing technological and statistical methodologies for data-independent scanning approaches, which mix targeted and shotgun approaches and produce complete fragment profiles of all peptide species. This robust analytical platform is allowing us to manage large research projects that require qualitative and quantitative proteomic approaches to measure differential protein expression, characterize posttranslational modifications, and map protein-protein interactions in different biological systems.



Quantitative proteomics analysis showing metabolic changes due to deficiency in executioner caspases 3 and 7. (A-D) Relative abundance profiles of selected proteins during mouse development (0, 1, 3 and 8-month-old). The changes are expressed separately for KO and WT animals in relation to the abundances at t = 0, while in the rightmost column the abundance of proteins in the KO are compared with that of the WT animals at each time point. Selected proteins include those related to beta-oxidation (A), oxidative phosphorylation complexes (B), glycolysis (C), and structural and contractile proteins (D).

Modified from Cardona *et al*. 2015. doi:10.1371/journal.pone.0131411.g003.



Proteomics analysis of the adipose-derived mesenchymal stem cell (ADMSC) secretome. White matter injury restoration after ADMSC cell administration in an experimental model of subcortical ischemic stroke. Gene onthology enrichment (GO) analysis of the proteins identified reveals important biological functions.
Modified from Otero-Ortega *et al*. Stem Cell Research & Therapy 2015. doi:10.1186/s13287-015-0111-4

SELECTED PUBLICATIONS

Martín-Alonso M, García-Redondo AB, Guo D, Camafeita E, Martínez F, Alfranca A, Méndez-Barbero N, Pollán Á, Sánchez-Camacho C, Denhardt DT, Seiki M, Vázquez J, Salaices M, Redondo JM, Milewicz D, Arroyo AG. **Deficiency of MMP17/MT4-MMP proteolytic activity predisposes to aortic aneurysm in mice**. *Circ Res* (2015) 117: e13-26.

Navarro-Lérida I, Teijo Pellinen, Sanchez SA, Guadamillas MC, Wang Y, Mirtti T, Calvo E, Del Pozo MA. **Rac1 nucleocytoplasmic shuttling drives nuclear shape changes and tumor invasion.** *Develop Cell* (2015) 32: 1–17

Burillo E, Lindholt JS, Molina-Sánchez P, Jorge I, Martinez-Pinna R, Blanco-Colio LM, Tarin C, Torres-Fonseca MM, Esteban M, Laustsen J, Ramos-Mozo P, Calvo E, Lopez JA, Vega de Ceniga M, Michel JB, Egido J, Andrés V, Vazquéz J, Meilhac O, Martin-Ventura JL. **ApoA-I/HDL-C levels are inversely associated with abdominal aortic aneurysm progression.** *Thromb Haemost.* (2015) 113: 1335-46

Sánchez-Gómez FJ; Calvo E; Bretón-Romero R; Fierro-Fernández M, Anilkumar N, Ajay M Shah; Katrin Schröder; Ralf P Brandes; Vázquez J; Lamas, S. **NOX4-dependent hydrogen peroxide promotes shear stress-induced SHP2 sulfenylation and eNOS activation**. *Free Rad Biol Med* (2015) 89: 419-30

Martin-Rojas T, Mourino-Alvarez L, Alonso-Orgaz S, Rosello-Lleti E, Calvo E, Lopez-Almodovar LF, Rivera M, Padial LR, López JA, de la Cuesta F & Barderas MG. **iTRAQ proteomic analysis of extracellular matrix remodeling in aortic valve disease**. *Sci Rep* (2015) 5:17290.

Transgenesis

Head of Unit

Luis-Miguel Criado Rodríguez

Support Scientists:

José Mª Fernández Toro

Juan de Dios Hourcade Bueno

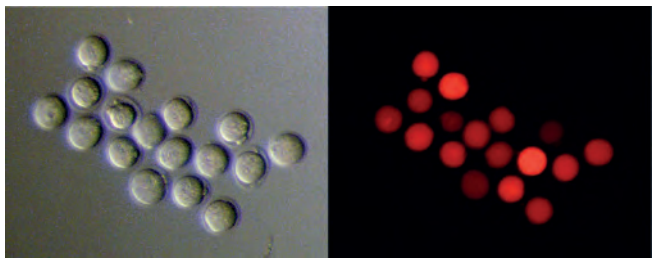
RESEARCH INTEREST

The main goal of the Unit is to provide genetically modified mouse strains, including knockout and knockin strains, to the CNIC research groups. This is achieved using well-established techniques, mainly the direct injection of DNA molecules into zygote pronuclei or the injection of genetically modified mouse embryonic stem cells (mESC) into 8-cell and blastocyst mouse embryos to obtain chimeric mice. Following the global trend toward the production of transgenic mice by gene edition with engineered nucleases, the Unit has successfully produced genetically modified mice using zinc finger nucleases (ZFN) and the CRISPR/Cas9 system, and in 2015 this methodology has been definitively established.

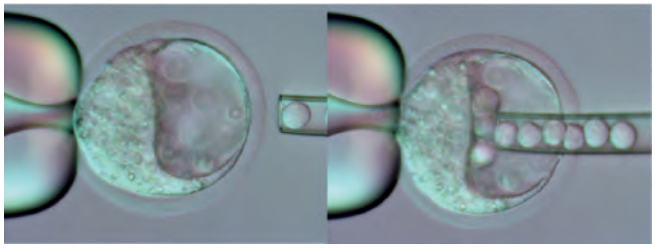
Other impontant activities include the rederivation of mouse strains by embryo transfer, cryopreservation of mouse strains by freezing embryos and sperm, mouse in vitro fertilization (mIVF), and intracytoplasmic sperm injection (ICSI). Moreover, the Unit also cryopreserves sperm from zebrafish (*Danio rerio*) and carries out in vitro fertilization with fresh and frozen sperm from this important vertebrate model organism.

In addition to its routine work, the Unit collaborates with several CNIC groups on specific aspects of their research programs, and Unit members participate in the CNIC's training programs by providing theoretical and practical sessions.

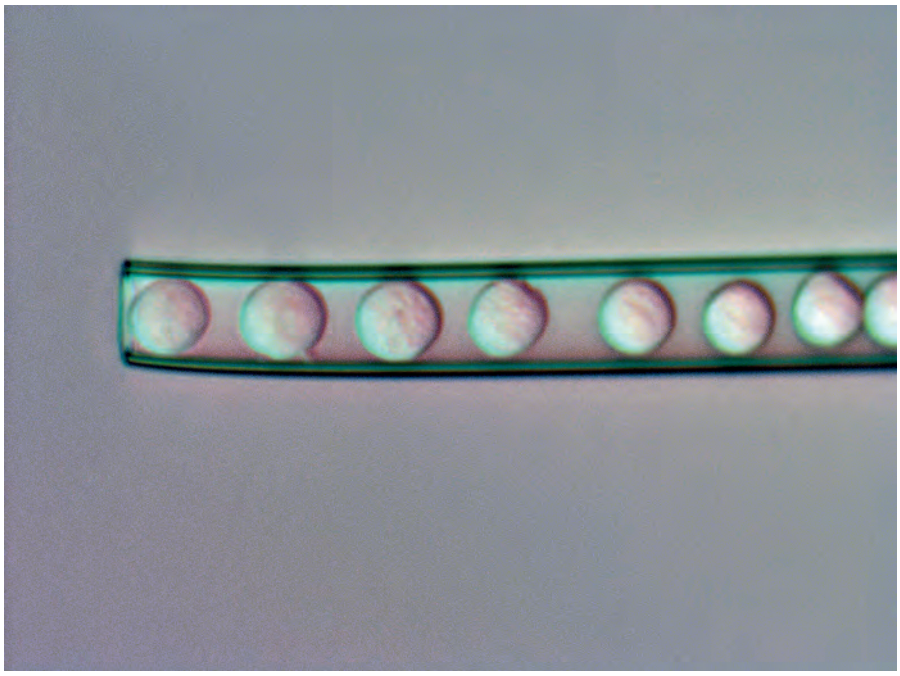
B6CBAF1/J mouse zygote showing the *zona pellucida* (glycoprotein structure surrounding the plasma membrane) and the two pronuclei containing nucleoli.



B6CBAF1/JxC57BL/6J mouse zygotes after electroporation in the presence of tetramethylrhodamine dextran (TMR-D): visible light (left) and fluorescent light (right).



Microinjection of a C57BL/6J mouse blastocyst with genetically modified mESCs for the production of chimeric mice: before (left) and after (right) injection.



Blunt injection needle containing genetically modified mESCs ready for microinjection into mouse blastocysts.

C57BL/6J mouse oocytes produced by ultra-superovulation of sexually immature females.

Viral Vectors

Head of Unit

Juan Antonio Bernal

Support Scientists:

Cristina Sánchez-Ramos
Raúl Torres

Technicians:

Joan García
Cristina Márquez
Aida García

Visiting Scientist:

Catarina Reis
(CNIO)

RESEARCH INTEREST

The main purpose of the Viral Vectors Unit (ViVU) is to provide investigators with the scientific resources necessary to produce state-of-the-art recombinant viral vectors for in vivo and in vitro use in gene transfer experiments. The ViVU currently produces lentivirus, adenovirus and adeno-associated virus (AAV) serotypes 8 and 9, and maintains a P2 facility with the appropriate expertise, equipment and permissions.

Viral vectors are widely used for gene transfer and gene expression in vitro, and our aim is to develop these tools for new applications. Viral vectors are an attractive choice because of their high transduction efficiency and their ease and flexibility of application; these vectors can be used to genetically express or inhibit one gene or a combination of genes in specific areas and periods of time, while avoiding compensation phenomena or other drawbacks associated with traditional animal models.

We have developed an alternative to transgenic animals, in which AAV vectors, widely used for gene-therapy approaches, express disease-causing mutated genes to generate disease models in wild-type mice. We have also used AAV vectors to compensate genetic defects by knocking down specific genes in vivo.

Our work demonstrates that single systemic injection of AAV is more versatile, cost-effective, simpler, and time-efficient than transgenic approaches for generating this types of mutant model. These studies set the basis for our future vector development.

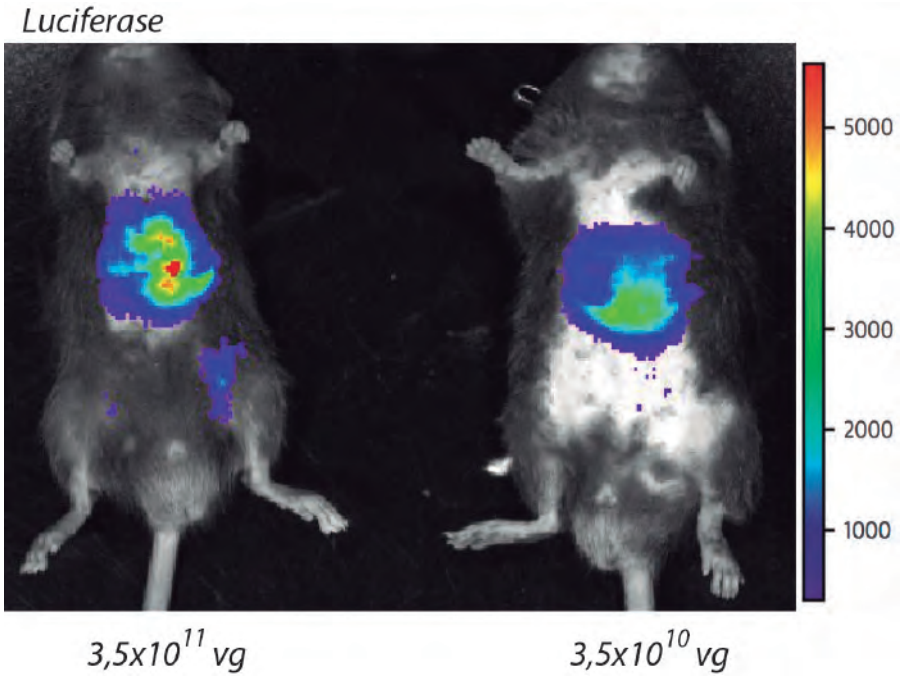


Figure 1. Infection with adeno-asociated virus serotype 9 *in vivo*. (A) Representative live-animal bioluminescence imaging of luciferase (Luc) transgene expression in C57BL6J mice injected intravenously (femoral vein) with AAV2–based vector in packaging serotype 9 at doses of $3.5x10^{10}$ and $3.5x10^{11}$ viral genomes (vg). Images were acquired 4 weeks after inoculation.

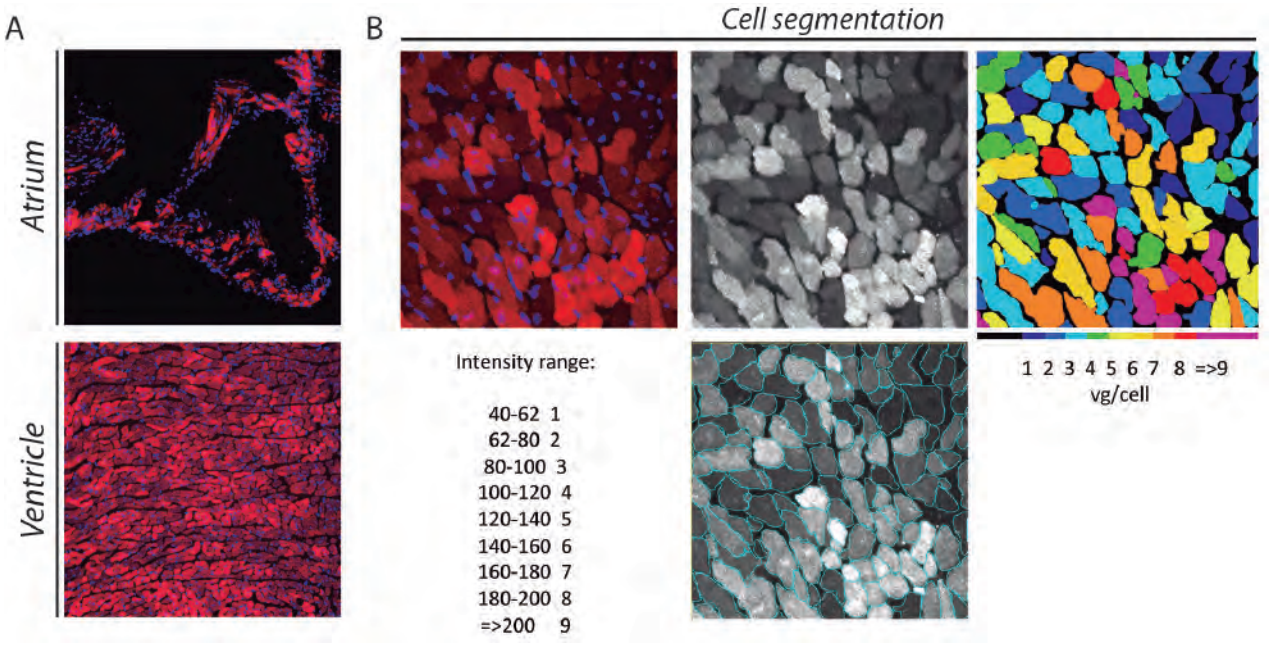


Figure 2. Cardiac expression driven by the specific cardiac promoter TnT. **(A)** Representative fluorescence microscopy images of cross sections of AAV-transduced hearts, illustrate showing expression of EGFP throughout the left atrium and ventricle. **(B)** Magnified images show the mosaic cellular distribution of wild-type cardiac PKP2 expression in the heart. **(B)** Fluorescence intensity segmentation and quantification of transduced protein expression, used to assign the number of integrated viral genomes per cardiomyocyte cardiomyocyte.

SELECTED PUBLICATIONS

Enrique Gallego-Colon, Maria Villalba, Joanne Tonkin, Francisco Cruz, Juan Antonio Bernal, Luis Jesús Jiménez-Borreguero, Michael Schneider, Enrique Lara-Pezzi, and Nadia Rosenthal. Intravenous delivery of adeno-associated virus 9-encoded IGF-1Ea propeptide improves post-infarct cardiac remodeling. *npj Regenerative Medicine* (accepted).

Torres R, Rodriguez-Perales S, Ramirez JC. **The use of innovative tools to reproduce human cancer translocations: lessons from the CRISPR/Cas system.** *Curr Biotechnol* (2014): 273-78

Torres R, Martin MC, Garcia A, Cigudosa JC, Ramirez JC, Rodriguez-Perales S. **Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system.** *Nat Commun* (2014) 5: 3964

Torres R, Garcia A, Jimenez M, Rodriguez S, Ramirez JC. **An integration-defective lentivirus-based resource for site-specific targeting of an edited safe-harbour locus in the human genome.** *Gene Ther* (2014) 21: 343-52

Ramirez JC, Torres R.
The arrival of next generation lentiviral vectors. *Nucleic Acids Res* (2014) 42: e53; reply

RESEARCH OFFICE

RESEARCH OFFICE

The Research Office provides wide-ranging support to CNIC Research Groups and Technical Units, including grant and fellowship applications, budget monitoring, personnel recruitment, laboratory organization, basic laboratory techniques, evaluation of laboratory equipment, administrative tasks, and the organization of scientific events.

Managers:

Ferreiro Villanueva, Beatriz *(Coordinator)*
Castillo Varón, Ana Isabel
Ciprés Blanco, Ángel
Grau Martínez, Laura
Quesada Navidad, Antonio Jesús
Soininen, Eeva Inari

Logisticians:

Asperilla Cabrera, Ana
Casaseca Ontoria, Teresa
Cuadrado Grande, Nuria
Mateos Villa, Javier
Merchán Martín, Álvaro
Oliva de la Morena, María Ángeles
Rico Jiménez, Yolanda

Assistants:

Bieger Vera, Eduardo
Fernández Fernández, Almudena
Gutiérrez Llaneza, Ana
Ramón Moreno, Marta
Román Hernando, Isabel

Technicians:

Daniel Palomares, Elisabet
El Maimoudi, Bahia
Fernández López, Iván
Martín Salamanca, Sandra
Ortega Rodríguez, Inés
Quintana de Blas, Andrea
Santos Martín, Eva

APPENDIX

APPENDIX

Publications

There were 215 CNIC publications in 2015, 195 of them in JCR-listed journals with an Impact Factor (IF). Of the total publications, 62% were produced through collaboration with foreign institutions and 29% with national institutions, and 9% were authored solely by CNIC researchers.

A CNIC scientist was a main author on 60% of the publications. The mean IF for all the articles was 6.984.

Articles with a CNIC Main Author

Pizarro G, Sánchez-Quintana D, Cabrera JA.
A Device to Narrow the Coronary Sinus for Angina.
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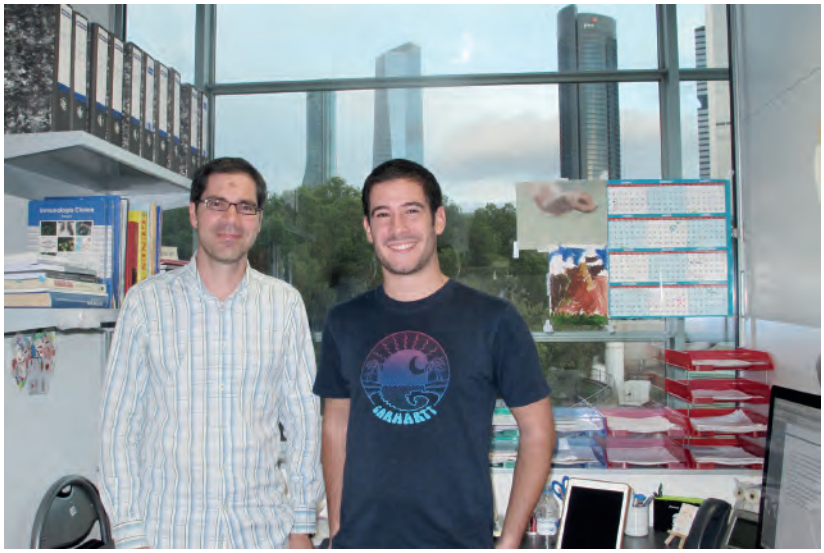

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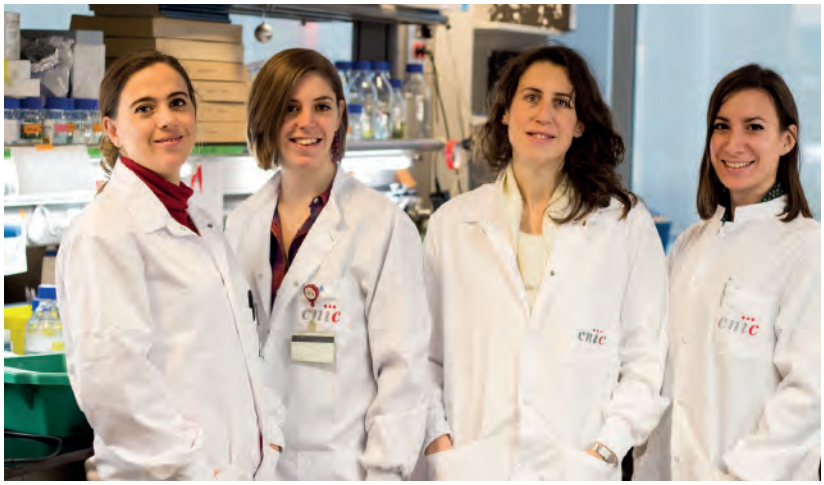
Publications



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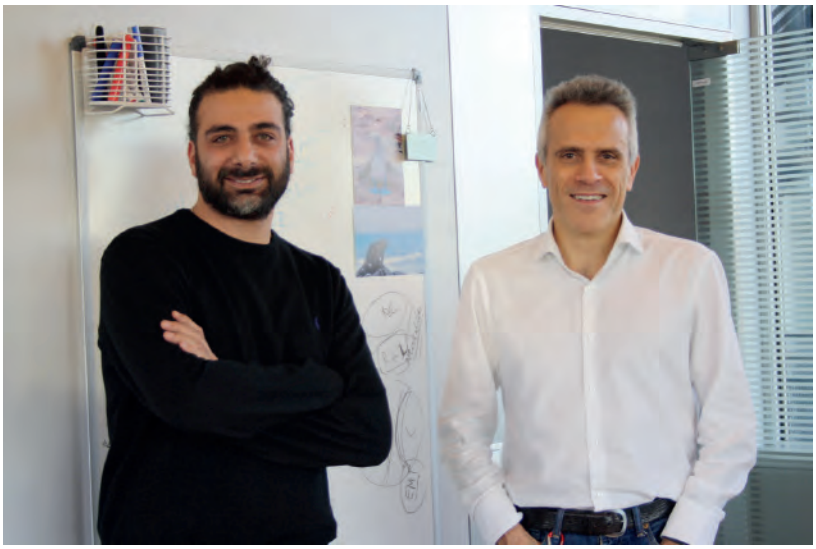

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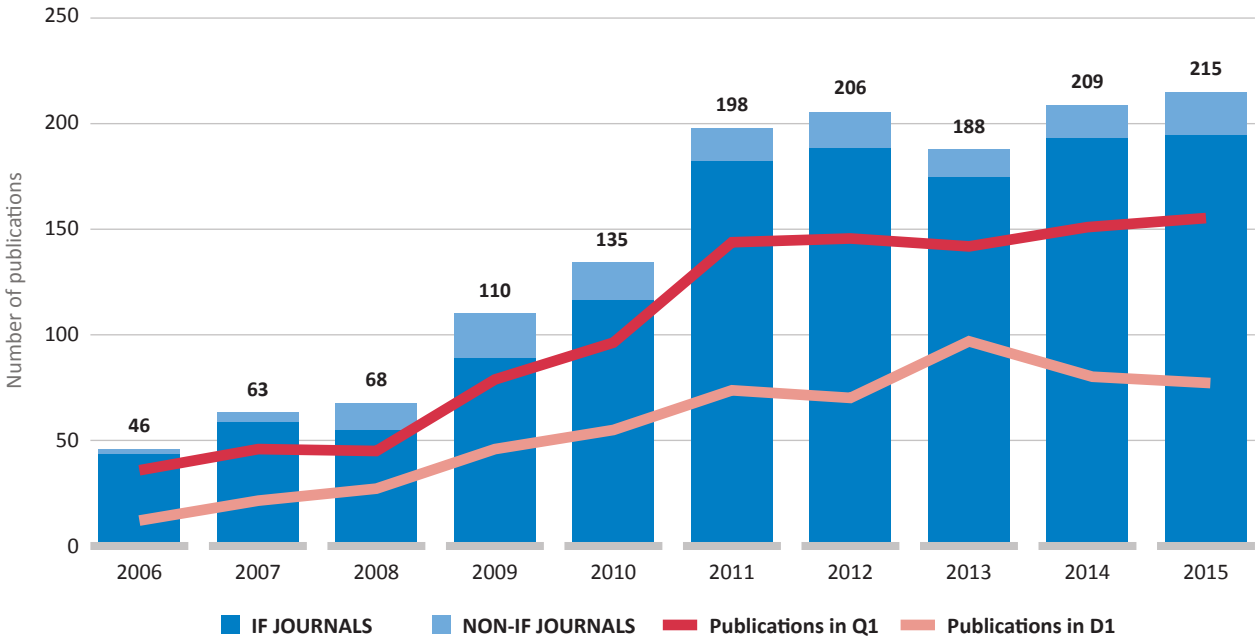
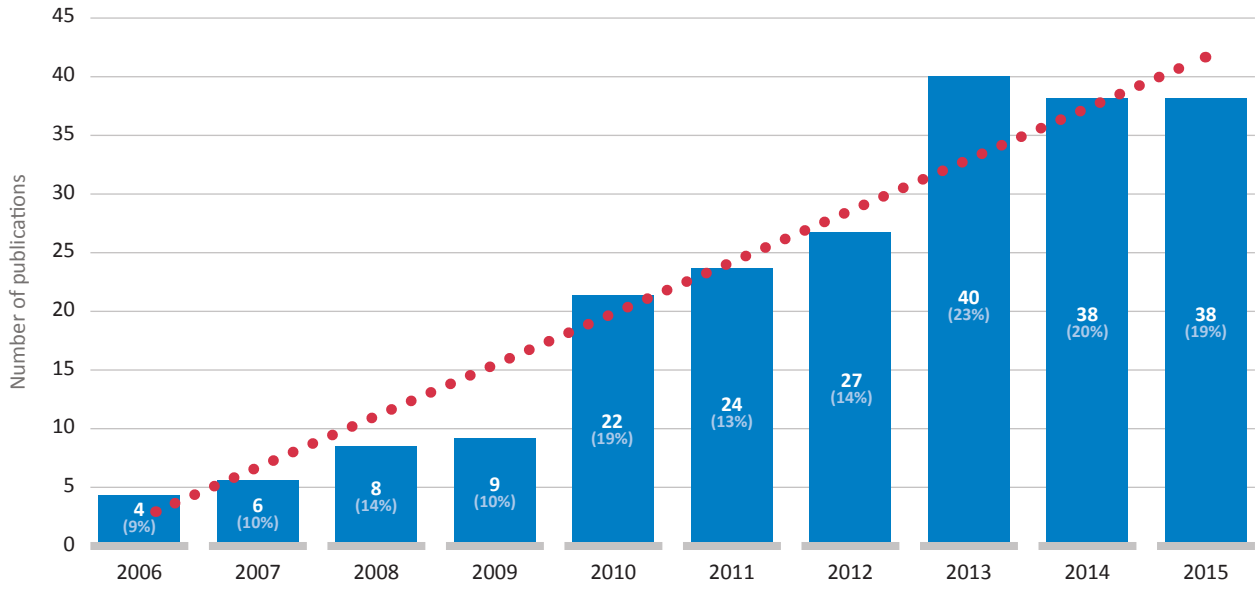
APPENDIX

Publications

SCIENTIFIC PRODUCTION



PUBLICATIONS IF>10



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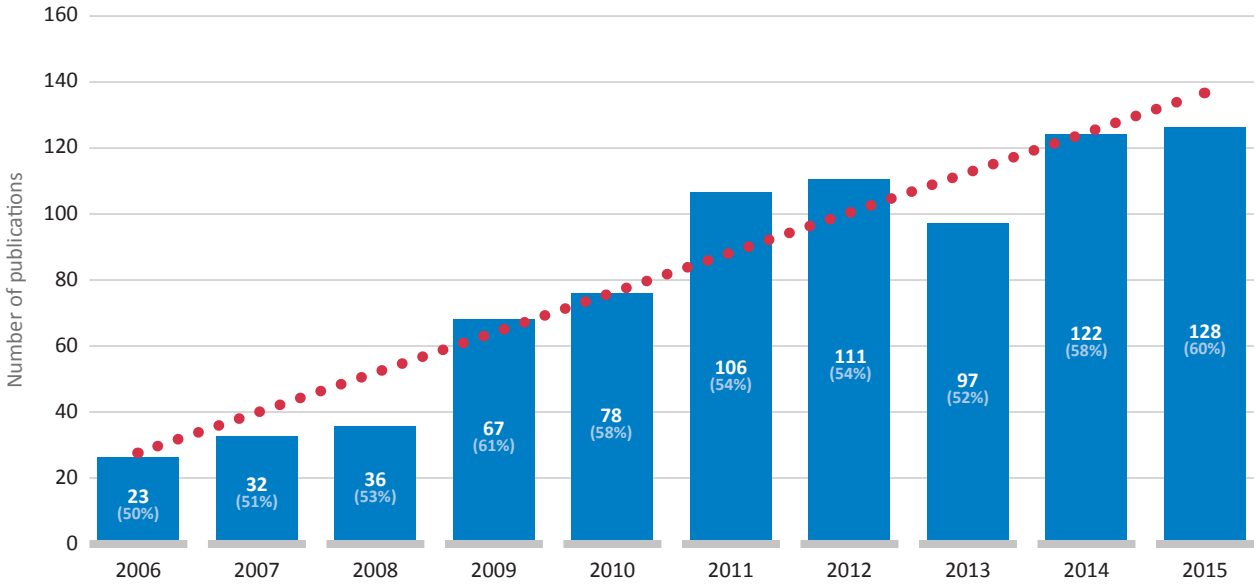
PUBLICATIONS CNIC MAIN AUTHOR



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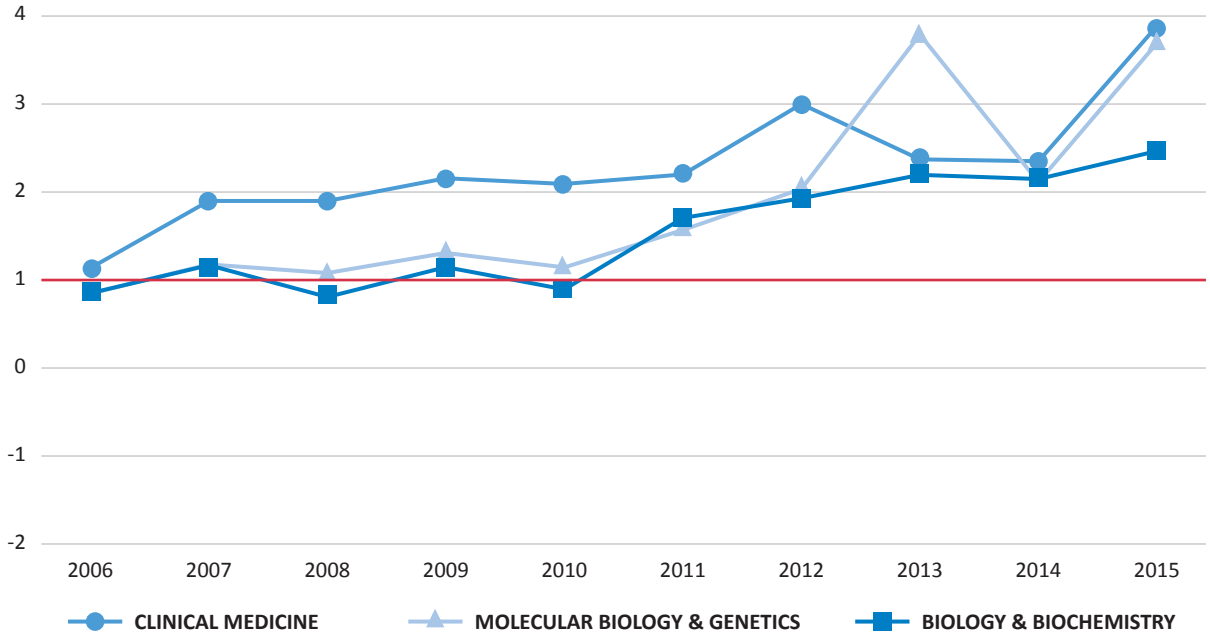
NORMALIZED CITATIONS BY CATEGORY



APPENDIX

Training Programs and Courses

Training is one of the CNIC's core activities, and the Center has devised a comprehensive training plan, **CNIC-JOVEN**, which includes programs for people at all levels, from senior high-school students to postdoctoral researchers and other professionals.

The CNIC-JOVEN Training Plan is designed to bring young people into biomedical research and create a strong base of talented researchers in the cardiovascular area.

Pre-university & Undergraduate Students

ACÉRCATE Program

The ACÉRCATE Program offers senior high school students studying natural and health sciences the chance to experience life as a biomedical researcher, with the aim of awakening interest in a career in research.

Participants spend two weeks at the CNIC, learning modern techniques used in biomedical research, conducting supervised experiments, operating sophisticated scientific equipment and presenting the results of their work, all under the supervision of our researchers.

Fellowships in 2015: *8*

CICERONE Program

The CICERONE Program is open to advanced undergraduate students studying toward a biomedicine-related university degree. Participants extend their scientific training through hands-on experience of laboratory-based biomedical research during the summer recess. In addition to carrying out a supervised research project, the students also attend CNIC seminars and workshops.

The aim of the program is to give university students first-hand knowledge of biomedical research so that they can make informed choices about the possibility of pursuing a scientific career.

Fellowships in 2015: *35*

Recent Graduates

CARDIOVASCULAR POSGRADUATE Program

The CNIC is developing a Cardiovascular Postgraduate Program, run through collaboration with Spanish universities. The first strand in this Program has been established through a formal agreement with the Universidad Autónoma de Madrid (UAM).

In the academic year 2014-2015, the CNIC partnered in the Masters in Molecular Biomedicine, offering a module in Cardiovascular Disease. This optional module provides a broad overview of cardiovascular biology, including perspectives from basic, clinical and translational research.

Dates: *13 January-18 February 2015*
Venue: *CNIC*
UAM MSc Students: *14*
CNIC PhD students: *17*

MASTER Program

This grants program provides individual funding for study towards a Masters degree at a Spanish university. The program is directed at students who are going to study for a PhD in one of the CNIC's laboratories: completion of an official Masters (Máster Oficial) has been introduced as an obligatory stage towards a PhD in Spain, in accordance with the Bologna process to standardize academic qualifications across Europe.

Fellowships in 2015: *14*

PREDOCTORAL (PhD) Program

The PREDOCTORAL Program provides a unified framework for all researchers at the CNIC who are working toward a doctoral degree. All predoctoral researchers are signed up to this program, independently of their funding source.

The aims of the program are as follows:

- To ensure uniform quality of predoctoral training at the CNIC
- To ensure fair and equal access of predoctoral researchers to training opportunities

Graduate students at the CNIC who obtained their PhD degrees in 2015: *10*
Graduate students studying for their PhD theses at the CNIC during 2015: *77*

LA CAIXA-SEVERO OCHOA INTERNATIONAL PhD Program

The *la Caixa* Foundation is a non-profit organisation funded by the third largest bank in Spain, the Caja de Ahorros y Pensiones de Barcelona (*la Caixa*). Since 1982, the *la Caixa* Foundation has run various fellowship programs to enable Spanish students to study postgraduate courses in Spain and abroad. Thanks to this support, thousands of students have been able to pursue their studies.

The *la Caixa* Foundation funds fellowships at the CNIC in recognition of the Center's status as one of the Spanish centers of excellence named in the first and second editions of the Severo Ochoa Award. In 2015 the *la Caixa* Foundation provided support for two highly qualified graduate students to carry out their experimental work towards obtaining a PhD degree at the CNIC within an International PhD Program.

la Caixa Fellowships in 2015: *2*

Graduates & Medical Professionals

RES@CNIC Program

The aim of the Res@CNIC Program is to offer medical professionals, during the first years of their specialization period as resident interns, the opportunity to learn about and become familiar with the latest techniques in cardiovascular research being used in the CNIC's laboratories, under the guidance of a CNIC scientist. Residents participating in RES@CNIC also receive training in theoretical aspects of cardiovascular research through a taught module run by experts. The Program also seeks to create links and collaborations so that on conclusion of their MIR specialization period, these professionals will have the chance to undertake research projects in their respective National Health System centers in partnership with the CNIC.

RES@CNIC was launched in 2012. Students selected for the fourth call will join the CNIC during January and February 2016.

Selected candidates for the fourth call: *6*

INVESMIR Program

The INVESMIR Program offers medical professionals during their specialization period as resident interns the opportunity to further their training through a research project in one of the CNIC's laboratories, under the supervision of a CNIC scientist.

An important aim of the program is that participants establish contacts and collaborations in the CNIC that will support them, after completion of their MIR specialization training, in pursuing their own research projects at their centers within the Spanish National Health System.

Fellowships in 2015: *2*

FICNIC Program

In 2014 the CNIC partnered with the Fundación Jesús Serra (FJS) and the *Fundación Interhospitalaria para Investigación Cardiovascular* (FIC) to create this program, aimed at promoting training in translational cardiovascular research. The program offers training fellowships to medical professionals specializing in cardiology or cardiovascular surgery.

The FICNIC Program is intended for medical professionals during the final year of their resident intern physician (MIR) specialization period or cardiologists or cardiovascular surgeons within three years of completing their specialization.

In 2015 the fellowship awarded in 2014 was renewed.

VALENTÍN FUSTER Program

The *Instituto de Investigación Hospital 12 de Octubre* (i+12) offers a position for a medical researcher/physician in the cardiovascular area at the *Hospital Universitario 12 de Octubre* (H12O), Madrid. The position is offered through the formal partnership between the H12O Research Foundation and the CNIC, established to promote research excellence in the cardiovascular field.

The aims of this program are to establish the profile of the translational researcher/physician, dedicated to the efficient translation of research results to the clinic and the generation of research hypotheses from a clinical standpoint and to contribute to the consolidation of the research component within the career of clinical researchers in the Spanish National Health System.

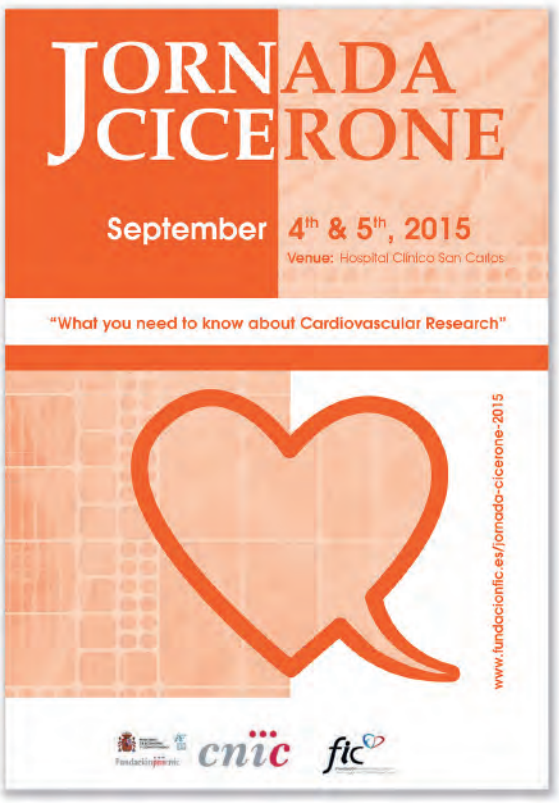
The position offered within this program was awarded to the candidate Héctor Bueno Zamora.

CICERONE Workshop: "What you need to know about cardiovascular research"

Fundación Interhospitalaria Investigación Cardiovascular

This lecture series provides a general introduction to cardiovascular research in Spain, and also gives participants the chance to question key researchers and opinion leaders in the field. Since 2012, the Jornada CICERONE has been run in collaboration with the *Fundación Interhospitalaria para la Investigación Cardiovascular* and takes place at the Hospital Clínico San Carlos, Madrid.

Dates: *September 4 and 5, 2015*
Attendees: *89*



CARDIOVASCULAR PATHOPHYSIOLOGY Course: From symptoms to genes

The CARDIOVASCULAR PATHOPHYSIOLOGY course is offered in collaboration with the Sociedad Española de Cardiología. This course offers a translational vision of cardiology to medical specialists by introducing them to the study of pathophysiology and basic research. Participants are given an overview of the molecular and genetic factors that underlie cardiac diseases and gain an up-to-date vision of cardiac physiology.

Dates: *December 11 and 12, 2015*
Venue: *CNIC Lecture Hall*
Attendees: *95*

VASCULAR BIOLOGY Course

Dr. Valentín Fuster delivers this lecture series, sponsored by FERRER, on "Vascular biology: basic and clinical research" as part of the summer program of the *Universidad Internacional Menéndez Pelayo* (UIMP).

Dates: *July 20 and 21, 2015*
Attendees: *323*

Research Professionals

CNIC International Postdoctoral Program

The CNIC International Postdoctoral Program (CNIC IPP) is aimed at supporting transnational mobility of postdoctoral researchers and broadening and deepening their individual competence, particularly in relation to the acquisition of complementary skills needed to become an independent group leader in the future. The program offers fellowships for researchers who hold a PhD Degree at the time of the application deadline.

Fellowships awarded in 2015: *5*

The CNIC-IPP is supported by the CNIC and the European Commission under the FP7 Marie Curie Actions- PEOPLE- COFUND Programme.

Seminars and Events

January

26 **Peter Tontonoz**
Howard Hughes Medical Institute and Laboratory Medicine University of California
Los Angeles, USA

February

6 **Shahin Rafii**
Weill Corner Medical College, Cornell University
New York, USA

9 **Michel Nussenzweig**
Howard Hughes Medical Institute, The Rockefeller University
New York, USA

March

6 **Alejo Efeyan**
Whitehead Institute for Biomedical Research
Cambridge, Massachusetts, USA

13 **Miguel López**
School of Medicine & the Research Centre of Molecular
Medicine and Chronic Diseases (CIMUS),
Universidad de Santiago de Compostela, Spain

23 **Edward E. Morrisey**
Perelman School of Medicine at the University of Pennsylvania
Philadelphia, USA

April

13 **Mitch Lazar**
Institute for Diabetes, Obesity, and Metabolism / Perelman School of Medicine at the University of Pennsylvania
Philadelphia, USA

17 **Leonard I Zon**
Boston Children's Hospital • HHMI
Boston, USA

27 **Matthias Nahrendorf**
MGH Center for Systems Biology, Harvard Medical School
Boston, USA

May

11 **Jean-Luc Balligand**
Institut de Recherche Experimentale et Clinique (IREC),
University of Louvain Medical School
Brussels, Belgium

19 **Sandeep V. Pandit**
Center for Arrhythmia Research, University of Michigan
Ann Arbor, USA

21 **Pedro Fernández Salguero**
Facultad de Ciencias, Universidad de Extremadura
Badajoz, Spain

25 **Fiona Watt**
Centre for Stem Cells and Regenerative Medicine, Kings College London
UK

29 **Yasuyuki Fujita**
Institute for Genetic Medicine, Hokkaido University Japan

June

8 **Stanley Nattel**
University of Montreal / Montreal Heart Institute Research Center / Canadian Journal of Cardiology
Montreal, Quebec, Canada

21-22 **V CNIC Conference**
Vulnerable Patient Meeting

13 **Ángel R. Nebreda**
Institute for Research in Biomedicine
Barcelona, Spain

17 **Mª Ángeles Moro**
Universidad Complutense de Madrid
Spain

18 **David del Álamo**
EMBO Journal
Heidelberg, Germany

26 **Pere Puigserver**
Harvard Medical School
Boston, USA

APPENDIX
Seminars, Events and Awards

July

6 **Bing Ren**
UCSD School of Medicine and Ludwig Institute for Cancer Research, University of California San Diego
La Jolla, USA

7 **Pura Muñoz**
ICREA and Pompeu Fabra University
Barcelona, Spain

13 **Jeff. W. Bulte**
Johns Hopkins Institute for Cell Engineering
Baltimore, USA

27 **Eva Nogales**
University of California
Berkeley, USA

September

4-5 **Jornada Cicerone 2013**
What You Need to Know About Cardiovascular Research

10 **Greg Lemke**
Salk Institute for Biological Studies
La Jolla, California, USA

15 **Michael Schnoor**
Leibniz-Institute of Arteriosclerosis Research.
University of Münster
Germany

21 **Duojia Pan**
School of Medicine & the Research Centre
Howard Hughes Medical Institute, Johns Hopkins University School of Medicine
Baltimore, USA

22 **Aleksander Popel**
Johns Hopkins University
Baltimore, USA

25 **La noche de los investigadores**
Bioingeniería: La investigación cardiovascular del futuro

October

5 **Mathias Gautel**
New Hunt's House , King's College London
UK

19 **Juhani Knuuti**
Turku PET Centre, University of Turku and Turku University Hospital
Finland

23 **CNIC-Perkin Elmer 2nd High Content Screening Workshop**

26 **James Eberwine**
PENN Genome Frontiers Institute, University of Pennsylvania,
Philadelphia, USA

November

3 **Sesión Informativa Horizonte 2020 sobre Convocatoria ITN 2016**
Acciones Marie Sklodowska – Curie ITN

11 ***Semana de la Ciencia*** **(Science Week)**
Ven a CNIC: Visita interactiva a sus departamentos para conocer la investigación cardiovascular

27 **CNIC PhDay**

30 **Jeroen Bax**
Leiden University Medical Center
The Netherlands

December

11-12 **IX Curso de Fisiopatología Cardiovascular**
Del síntoma a los genes

Awards

Fuster, Valentín

- Fundación Ferrer Investigación, XXI Premio Severo Ochoa de Investigación Biomédica.
- La Gran Cruz de la Orden Civil de Sanidad, España.
- American College of Cardiology Award for "Leadership in Global Population Health Education, Research, Science", Washigton DC, USA.
- American Association of Clinical Endocrinologists, "Frontiers in Science Award".
- Fellow of the European Respiratory Society, in Recognition of Excellence in Scientific and/or Educational Contributions to Respiratory Medicine, Amsterdem, The Netherlands.
- Honorary Professor, Instituto Universitario Escuela de Medicina del Hospital Italiano, Buenos Aires, Argentina.
- La Sociedad Ecuatoriana de Cardiologia. Recognition Award for Valuable Support in the Progress to Prevent Cardiac Diseases, Quito, Ecuador.

Jalife, José

- Douglas P. Zipes Lecture. Heart Rhythm Society.
- Doctor Honoris Causa. University of Valencia, Spain.

D'Amato, Gaetano

- Best poster Prize. 2015 Weinstein Conference on Cardiovascular Development, Boston MA, USA.

Filgueiras, David

- Premio Extraordinario de Doctorado. Universidad Autónoma de Madrid.

Izquierdo-García, José L.

- ISMRM MERIT AWARD Summa cum laude. International Society of Magnetic Resonance in Medicine.

Mittelbrunn, María

- Bolsas de investigación 2015 del programa L'Oréal–UNESCO For Women in Science.

Nicolás Ávila, José Ángel

- Premio Inmunotek Investigación Básica. Inmunotek.

Pellico, Juan

- EMIM 2015 Excellence Award. European Society for Molecular Imaging (EMIM).

Sanz Morejón, Andrés

- First Prize. Biological and Biomedical Sciences Area. Master work: Nuevas Estrategias para el Estudio de la Regresión de la Fibrosis Durante la Regeneración Cardíaca del Pez Cebra. XIV Certamen Arquímedes de introducción a la investigación.

APPENDIX
Strategic Alliances

The CNIC consolidates and expands its alliances to investigate, train, innovate and transfer.

In 2015, the CNIC signed 29 interinstitutional agreements to create or consolidate partnerships.

In the education sector, the CNIC expanded its already wide academic network (established agreements with 15 universities) by signing new collaboration agreements with universities in Spain (Universidad Politécnica de Valencia; Universidad de Granada; and Universidad Autónoma de Barcelona). Moreover, the CNIC also strengthened its links with foreign universities, mostly through the establishment of student exchange programs and short visits for practical work in the CNIC's laboratories. Five new international agreements were signed last year (Universidad Autónoma de Puebla, Mexico; Universities of Bern and Lausanne, Switzerland; Aarhus University, Denmark; and Universita degli Studi di Verona, Italy).

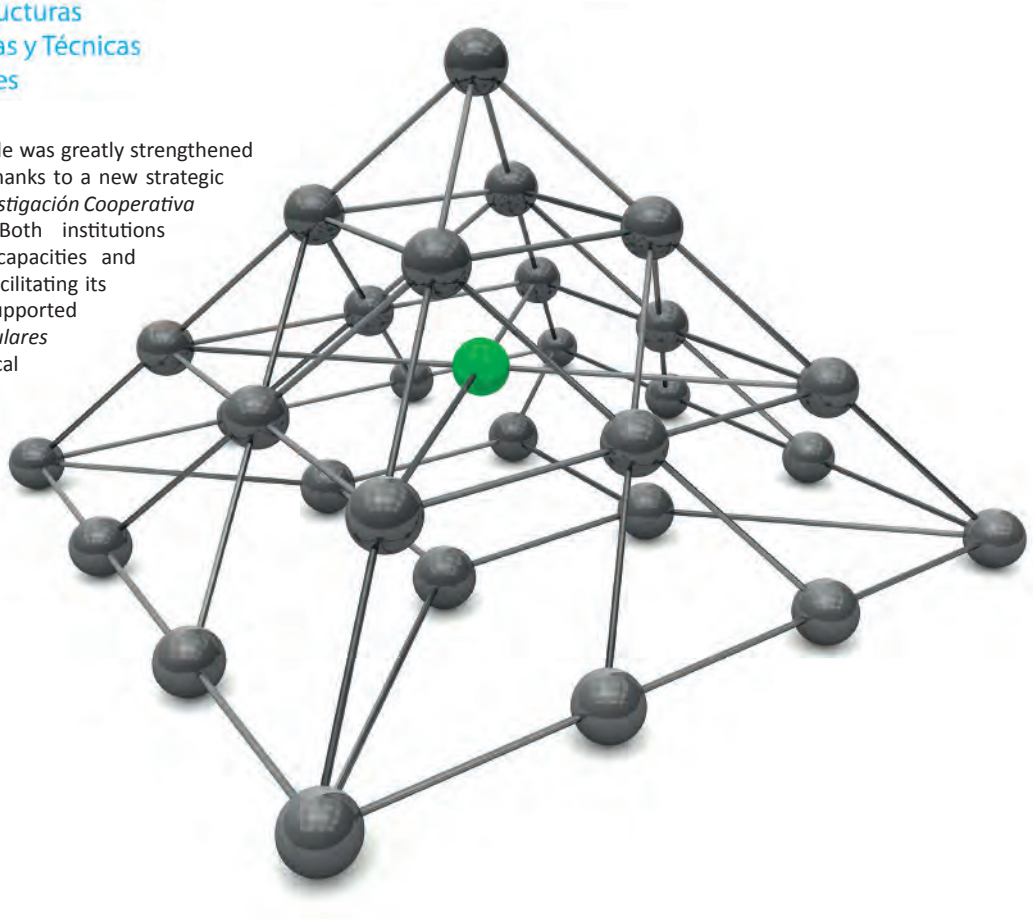
The Center also reinforced its postgraduate medical programs by establishing a new partnership with the Hospital Universitario 12 de Octubre (H12O) Research Foundation. Through this partnership, the Center has launched the VALENTIN FUSTER Program, which supports medical researchers/physicians to promote research excellence in the cardiovascular field. Thanks to this program, Dr. Hector Bueno has joined the CNIC.

Links with the clinical sector have been consolidated through the signing of new agreements with Spanish hospitals (Fundación Hospitales Madrid) and foreign clinical institutions (Memorial Sloan Kettering Cancer Center, New York; VU Medical Center, The Netherlands).

The CNIC also expanded its links with the commercial biotechnology sector with the signing of a research collaboration agreement with Pacesetter Inc. to study the role of atrial fibrillation in cardiac remodeling.

Infraestructuras Científicas y Técnicas Singulares

Finally, the CNIC's international profile was greatly strengthened in the area of biomedical imaging thanks to a new strategic alliance with the *Centro Vasco de Investigación Cooperativa en Biomateriales* (BiomaGUNE). Both institutions will integrate their technological capacities and equipment in biomedical imaging, facilitating its inclusion in the new government supported *Instalaciones Científico Técnicas Singulares* (ICTS: Specific Scientific-Technical Installations), which have been designed to place Spain in a more competitive position internationally.



Public-Private Partnership

In December 2005, the Spanish Government signed an agreement with a group of some of the most important Spanish businesses (Pro CNIC Foundation, **http://www.fundacionprocnic.es**) to sponsorship the CNIC.

Since the signing of this agreement, the CNIC's funding has been based on a public-private partnership (PPP) of a broad, socially-committed nature. The Pro CNIC Foundation does much more than provide the CNIC with money; it also contributes its accumulated managerial and business expertise. Representatives of the Pro CNIC Foundation sit on the CNIC's Board of Trustees and actively participate in the management, planning and decision taking related to the Center.

A major strength of this socially-committed PPP model is that it provides a more solid base than traditional forms of charitable financing, giving the CNIC a more stable financial base than it would have if it depended on sporadic donations from benefactors. This stability gives the CNIC greater freedom to commit itself to long-term, high-return research strategies in collaboration with public and private institutions, and allows for a more effective use of its own resources generated through competitive projects and the exploitation of intellectual property rights.

The current members of the Pro CNIC Foundation are **Acciona**, **BBVA**, **Endesa**, **Fundación Abertis**, **Fundación Mutua Madrileña**, **Fundación Mapfre**, **Banco Santander**, **Fundación Ramón Areces**, **Fundación Repsol**, **Gas Natural Fenosa**, **Grupo Prisa**, **Inditex**, **la Caixa**, and **Telefónica**.

Private Funding

Competitive Funding

From 2007 to 2015 the CNIC attracted **more than €51 million from national competitive sources**, mostly national public funding agencies.

The **CNIC has recently renewed its prestigious accreditation as a *Severo Ochoa* Center of Excellence** for a further 4 years (2016-2019).

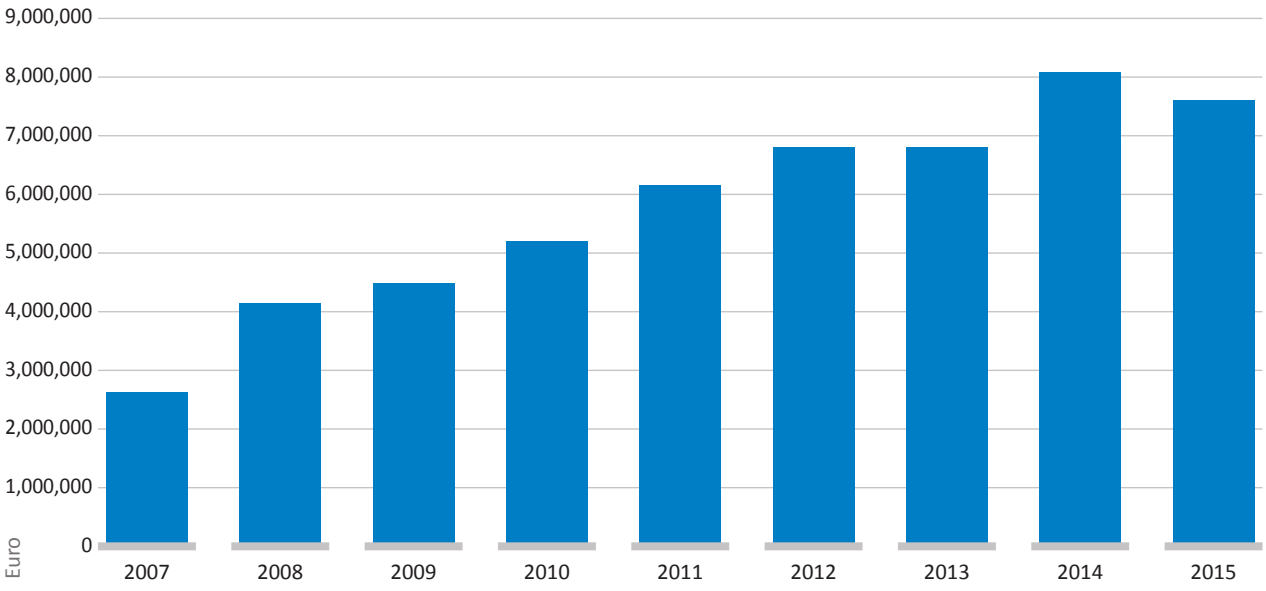
In 2015, CNIC researchers participated in more than 30 different national calls being successful in 48 applications.

In the same period (2007 to 2015), the CNIC attracted **more than €31 million from international competitive sources**.

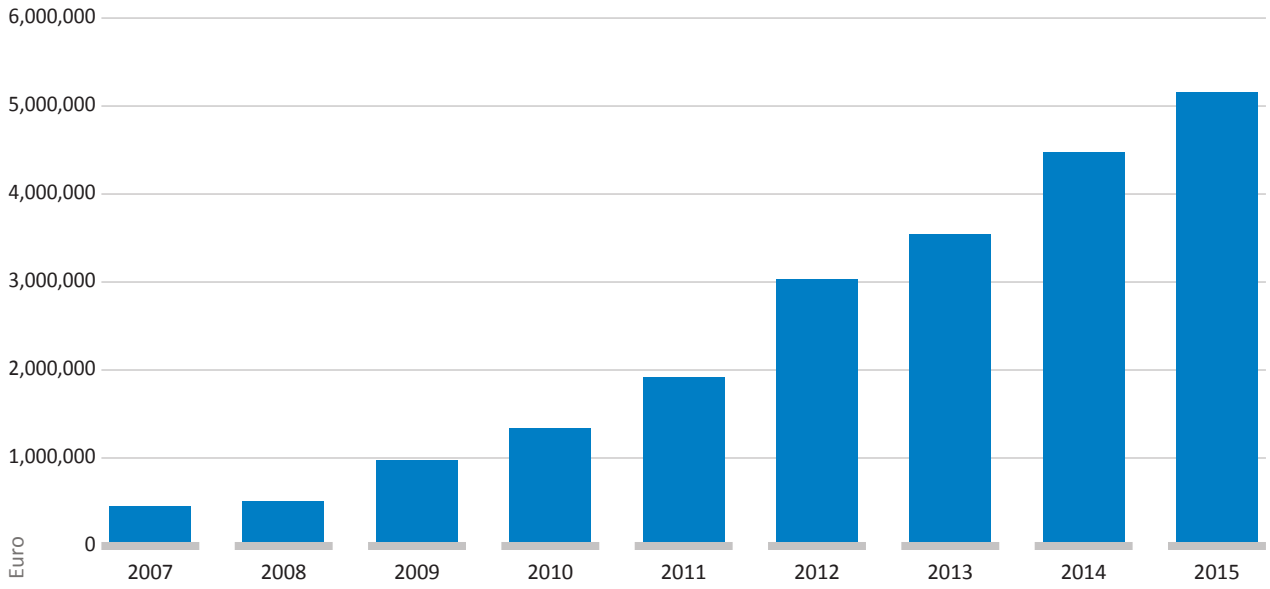
The CNIC participated in **34 projects funded under the European Commission's Seventh Framework Programme (FP7)** and is engaged in **11 projects** funded under the EU Research and Innovation **Horizon 2020 (H2020)** programme. Moreover, the Center is the top-ranking Spanish institution for funding awarded under the EC Societal Challenge *Health, Demographic Change and Wellbeing* (H2020-2014 call).

The international scientific competitiveness of the CNIC's research groups is highlighted by their high representation in projects funded by the **European Research Council (ERC)**, which funds Europe's brightest minds to tackle innovative research challenges. The CNIC contributes to the achievement of this goal through **5 ERC projects awarded under FP7 and 4 awarded under H2020**.

National Competitive Funding



International Competitive Funding



The distribution of funds per calendar year was obtained by dividing the total amount of each award by the foreseen duration of the project.

APPENDIX

Patent Portfolio

Twenty inventions are currently being filed, twelve of them in partnership with other institutions.

TECHNOLOGY OFFERS AVAILABLE FOR OUT-LICENSING

TITTLE	INVENTORS	APPLICANTS	PATENT APPLICATIONS
Methods of using the Calcineurin A variant CnAB1 for the treatment of cardiac hypertrophy	Enrique Lara Pezzi, Nadia Rosenthal, María López Olañeta, María Villalba Orero, Jesús Gómez Salinero	CNIC, EMBL	PCT, US, EP
Uso de agonistas selectivos de receptores beta-3 adrenérgicos para el tratamiento de hipertensión pulmonar	Borja Ibañez Cabeza, Valentín Fuster Carulla, Ana García-Álvarez	CNIC , CLINIC	PCT, JP, US, EP
Terapia neuroregeneradora/neurocompensatoria para el tratamiento de las neoplasias mieloproliferativas	Simón Méndez Ferrer, Lorena Arranz Salas, Joan Isern Marín	CNIC	PCT, JP, US, EP
AAV vectors for the treatment of ischemic and non-ischemic heart disease	Enrique Lara Pezzi, Borja Ibáñez Cabeza, Enda Joseph Clinton, Jesús María Gómez Salinero, María Villalba Orero, David Sanz Rosa, Juan Antonio Bernal Rodríguez	CNIC	EP, PCT
Micellar nanoparticles containing antitumoral glycosides	Hugo Groult, Fernando Herranz Rabanal, Jesús Ruiz-Cabello Osuna, Alfonso Fernández-Mayoralas Álvarez, Manuel Nieto Sampedro, Lorenzo Romero Ramírez, Isabel García Álvarez	CNIC, CSIC, CIBER	EP, PCT
Bimodal fluorophore-labeled liposomes and associated methods and systems	Carlos Pérez-Medina, Thomas Reiner, Jason S. Lewis, Wilem J.M. Mulder, Zahi A. Fayad	MSKCC, MSSMMOUNT SINAI, CIBERES, CNIC	US,PCT
Single core radionuclide-metal oxide nanoparticles: a new biocompatible nanosystem for dual hot spot imaging	Jesús Ruiz-Cabello Osuna, Fernando Herranz Rabanal, Riju Bhavesh, Juan Pellico Sáez	CNIC, UCM	EP, PCT
Method of predicting or prognosticating neurological performance in patients who have suffered a cardiac arrest and optionally comatose status due to ventricular fibrillation	David Filgueiras Rama, Esteban López de Sá y Areses, José Millet Roig, Conrado Javier Calvo Sainz	CNIC, UPV, Hospital Universitario La Paz	EP, PCT
Method and system for generating MR images of a moving object in its environment	Javier Sánchez González, Nils Dennis Nothnagel, Borja Ibáñez Cabeza, Rodrigo Fernández Jiménez, Valentín Fuster Carulla	Philips, CNIC	EP, PCT
Método de detección de predisposición a padecer cardiopatía dilatada	Pablo Garcia Pavía, Sofía Cuenca, Laura Padrón de Vaumas, Enrique Lara Pezzi	Fundación Investigación Hospital Puerta de Hierro, CNIC	ES
Optic device and method for detecting cardiovascular disease	Vicente Andrés García, Cristina Rius Leiva, Beatriz Julia Dorado de la Corte, Tobias Ackermann, Xavier Muñoz Berbel, Andreu Llobera Adan	CNIC, CSIC	ES
MiRNA compositions for the treatment of mature B-cell neoplasms	Almudena Rodríguez Ramiro, Nahikari Bartolomé Izquierdo, Virginia García de Yébenes Mena	CNIC	EP

APPENDIX

Patent Portfolio

TITTLE	INVENTORS	APPLICANTS	PATENT APPLICATIONS
p38 inhibitors for the treatment and prophylaxis of liver cancer	Ana Martinez Gil, Carmen Gil Ayuso-Gontán, Guadalupe Sabio Buzo, Antonia Tomás Loba, Bárbara González Terán, Elisa Manieri	CNIC, CSIC	EP
Biophotonic devices and methods of use	Vicente Andrés García, Vicente, Beatriz Julia Dorado de la Corte, Cristina Rius Leiva, Tobias N. Ackermann, Xavier Muñoz Berbel, Andreu Llobera Adan	CSIC, Aarhus University, CNIC	EP
Procedimiento de obtención de datos útiles para el diagnóstico de cardiomiopatías	María Pilar Martín Fernández, Raquel Sánchez Díaz, Adela Matesanz Marín, Luis Jesús Jiménez Borreguero, Francisco Sánchez Madrid	CNIC	EP

Patent Applications:
ES - Spain
PCT - International
EP - Europe
US – USA
JP- Japan

ACTIVE LICENSED AGREEMENTS

TITLE: "Capsule for the prevention of cardiovascular diseases"
APPLICANTS: CNIC, FERRER
LICENSEE: FERRER

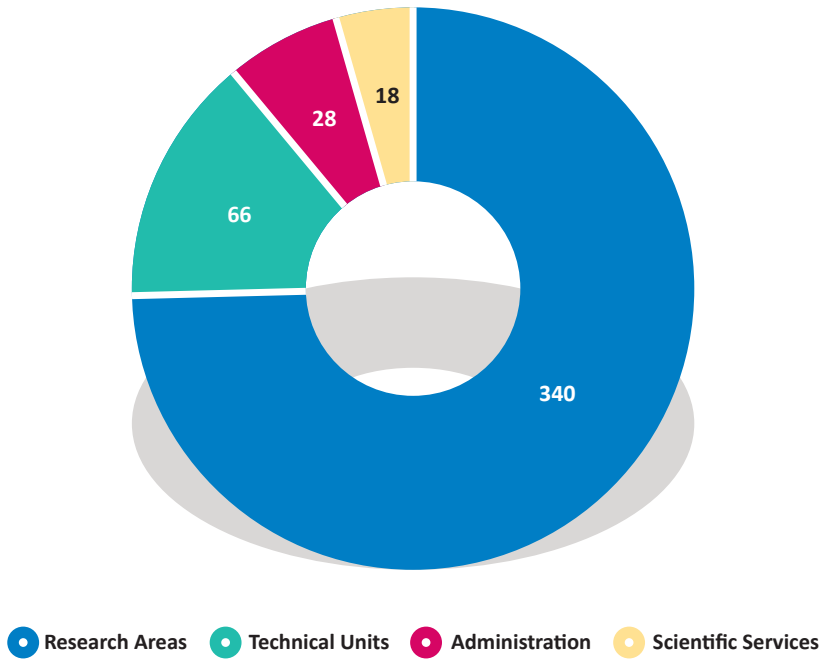
TITLE: "Method for identifying senescent mesenchymal stem cells"
APPLICANT: CNIC
LICENSEE: NIMGenetics

TITLE: "Vectores de expresión de proteínas: plásmidos pGEX-Calcineurina, pGEX-FKBP12 y pGEX-Ciclofilina A"
APPLICANT: CNIC
LICENSEE: PROTEIN ALTERNATIVES S.L.

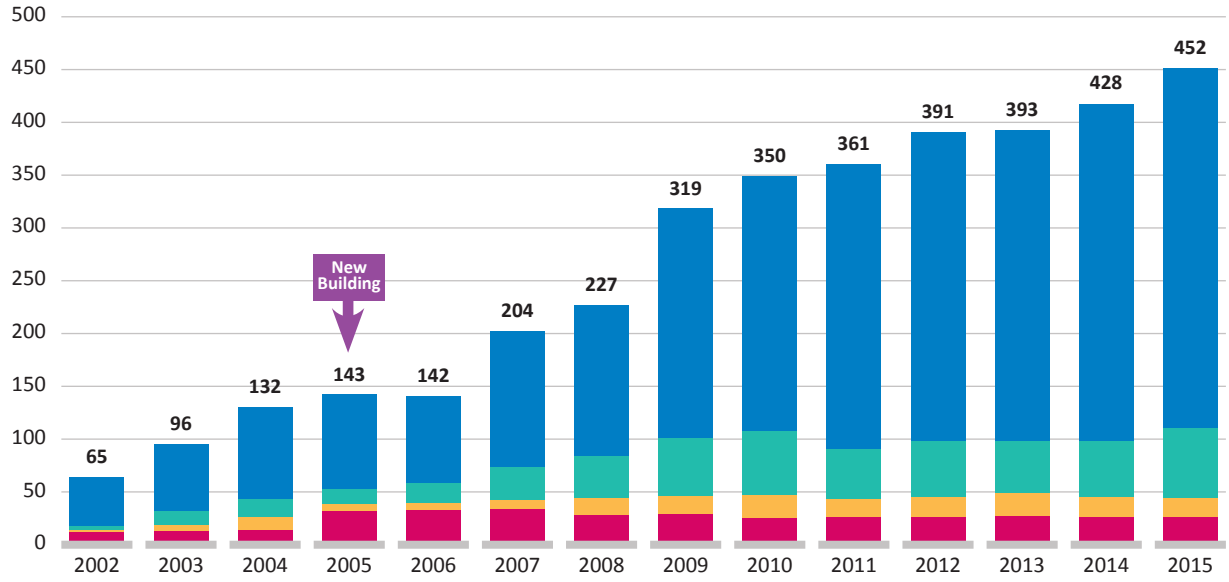
APPENDIX

Staff Figures

CNIC Staff 2015 (452)



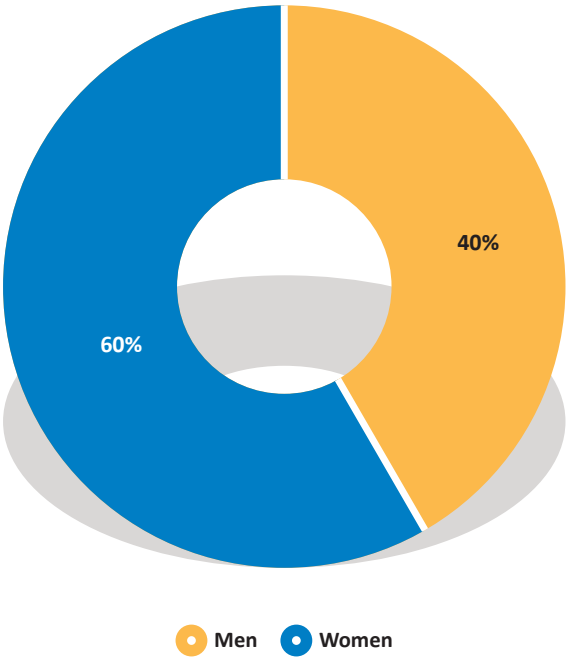
Gradual growth current status



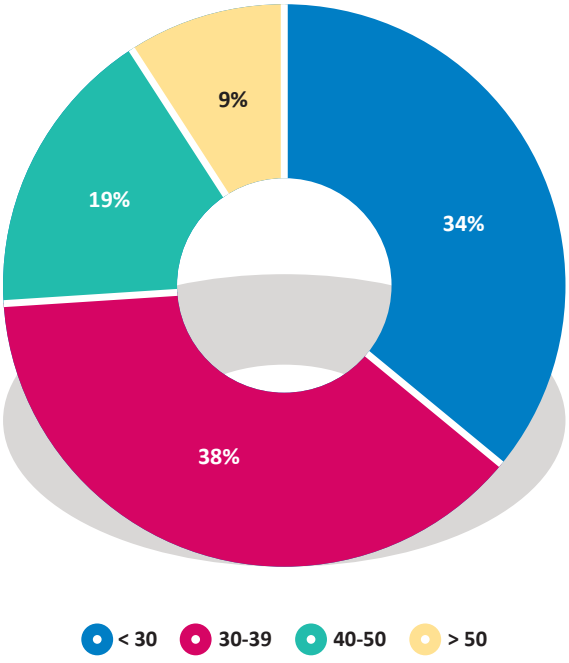
APPENDIX

Staff Figures

Gender Distribution 2015



CNIC Staff by age 2015 (452)



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Melchor Fernández Almagro, 3 - 28029 Madrid. Spain T. (34) 91 453 12 00 - F. (34) 91 453 12 45 - www.cnic.es