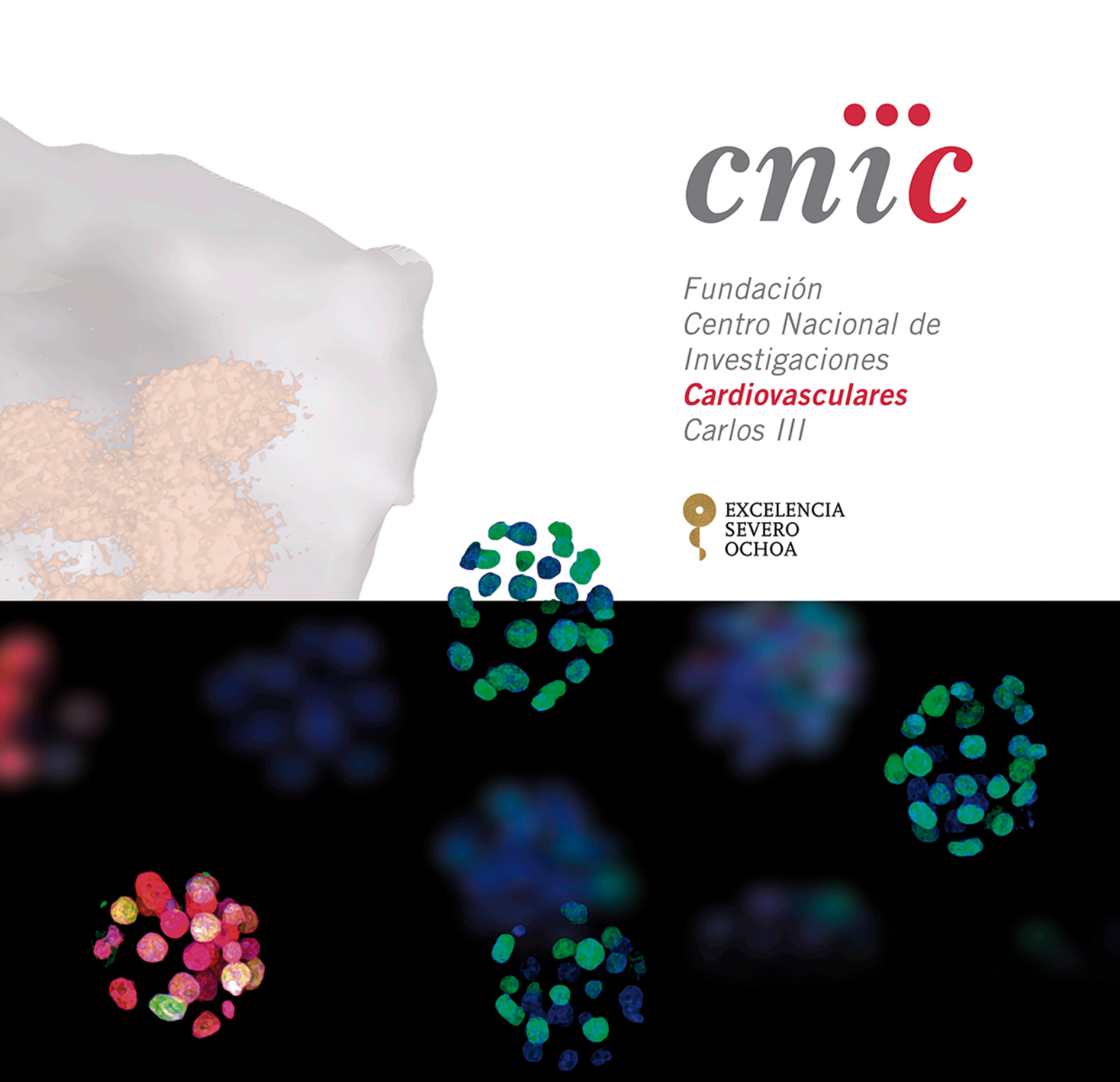


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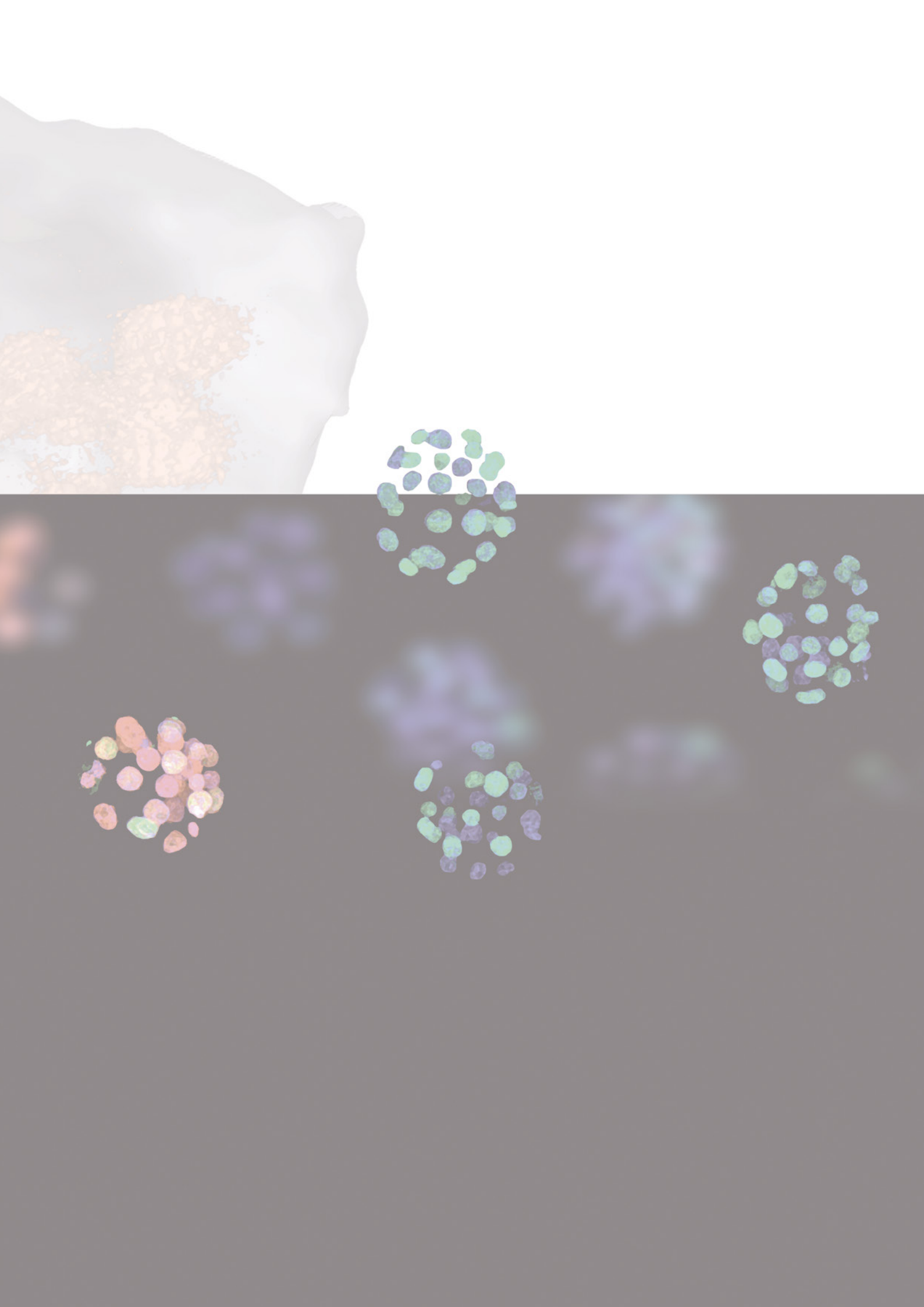
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Centro Nacional de
Investigaciones
Cardiovasculares
Carlos III

EXCELENCIA
SEVERO
OCHOA

SCIENTIFIC REPORT

2014

SCIENTIFIC REPORT 2014





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Valentín Fuster

Over the past few years the CNIC has established itself as a center of research excellence in the international scientific arena through its scientific productivity and its innovative organizational and funding pattern.

The CNIC's impressive record is built on our diverse activities, which in addition to a dynamic research base include training programs, technological innovation and partnerships with the healthcare sector and industry. Our success is also the fruit of an open institutional environment that brings together investigators from diverse disciplines to work together to achieve the Center's goals through innovation, efficiency, cooperation and enthusiasm. The CNIC's management structure was praised as a model for other Spanish enterprises last June when Prime Minister Mariano Rajoy attended the annual governing council meeting of the Pro-CNIC Foundation, the panel of private companies whose continued support is so vital.

Another key event that attracted much media attention last year was the approval of the CNIC Polypill for commercialization in 22 countries, including Spain. Production of the CNIC Polypill was inaugurated in September in a ceremony presided over by Spanish State Secretary of Social Services and Equality, Susana Camarero. The results of the Focus Polypill trial were the focus of two key studies published last year in the *Journal of the American College of Cardiology*, and were a centerpiece of the European Society of Cardiology meeting held in Barcelona.

Also coming to fruition last year was the METOCARD-CNIC study, which examined the benefits of early intervention after a heart attack with the beta-blocker metoprolol. The positive follow-up findings were published in the *Journal of the American College of Cardiology*, and the groundbreaking advance signaled by this trial won strong praise at the ACC meeting. The AHA considered the METOCARD-CNIC trial one of the 10 most relevant studies in cardiovascular field worldwide, and invited CNIC researchers to present a dedicated session at the AHA meeting in Washington DC.

The Center's commitment to pioneering translational research was reaffirmed with a new project called TAN SNIP. This transnational study pools the expertise of the CNIC, the Icahn School of Medicine at Mount Sinai, the Framingham Heart Study, and the VU University Medical Center in Amsterdam, and enjoys financial backing from AstraZeneca. The goal of this transatlantic network is to develop tools for stepwise non-invasive imaging for cardiovascular prognosis and prevention.

Delivering on our commitment to improving public cardiovascular health also involves us in a range of public educational programs, which form an essential part of the Center's mission. The CNIC's public profile was reinforced in February through a partnership with Spanish broadcaster *Radio Televisión Española* (RTVE) and *Fundación para el Conocimiento MADRI+D* (an initiative of the Madrid regional government). This health awareness campaign consists of a series of televised conversations with RTVE staff about the importance of taking care of cardiovascular health.



Miguel Torres

The CNIC's outstanding research productivity continued last year, as detailed in the pages of this report. And this strength was rewarded by the Center's continued success in securing competitive funding. National funds secured last year exceeded €6 million, while international funding exceeded €4 million. It is especially pleasing to see the CNIC's success in obtaining European Research Council funding. Rui Benedito was awarded an ERC Starting Grant to study the formation of new blood vessels, and Susana González and Simón Méndez were awarded ERC Consolidator Grants for the study of heart muscle aging and stem cell niche biology, respectively.

CNIC researchers also won recognition in the form of prestigious prizes and honors. Rui Benedito was awarded the *Princesa de Girona* prize for his work on angiogenesis, and on International Women's Day Guadalupe Sabio was garlanded by the Madrid regional government as one of the outstanding Spanish women scientists for her work on stress signaling in cardiovascular diseases. Another young investigator, Ana García Álvarez, received a Young Researcher Award from The European Society of Cardiology for her work on pulmonary circulation and right ventricular function. And one of us (VF) was deeply honored to receive the title of Marquis from Don Juan Carlos I of Spain, in one of his last acts as King, for "outstanding and unceasing research efforts and … educational outreach work".

The Center's research profile was broadened and deepened with three key additions. Jorge Alegre-Cebollada leads the Single-Molecule Mechanobiochemistry laboratory, investigating the links between protein changes and the mechanical properties of the heart. José Jalife reinforces our work in the field of arrhythmias, and his Cardiac Arrhythmia laboratory maintains a formal collaboration with the University of Michigan. José María Castellano takes on a crucial role as the new Coordinator of the Clinical Research Unit. We are very pleased to welcome these three experts to the CNIC team.

2014 also saw a new training initiative, made possible through a strategic alliance with the *Fundación Interhospitalaria para la Investigación Cardiovascular* (FIC). The program, called FICNIC, was launched in March and is tailored for young cardiologists. Our training partnership with the *Fundación La Caixa* continues, with an injection of funding last year for four new grants under the *La Caixa-Severo Ochoa* International PhD Program.

As the consolidation of our project continues, it will be important to maintain an agile organizational infrastructure that fosters fluid cooperation in multidisciplinary projects. This openness and flexibility is an essential foundation for ensuring the best use of knowledge and resources, and thus lead to improved results and their scientific and social translation.

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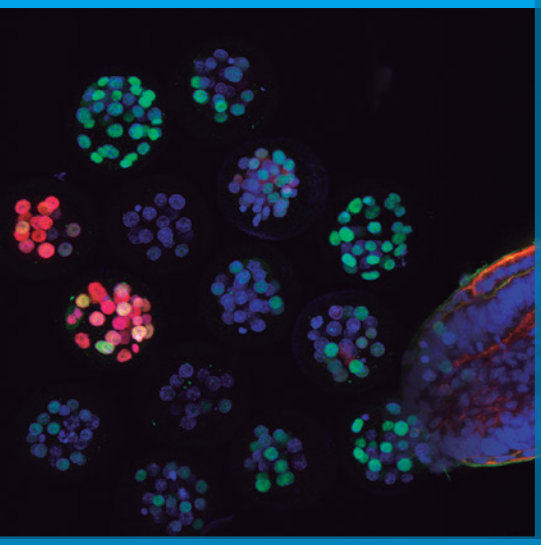
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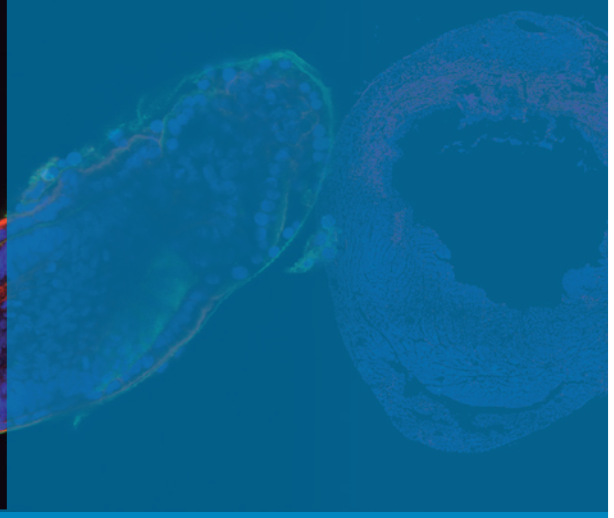
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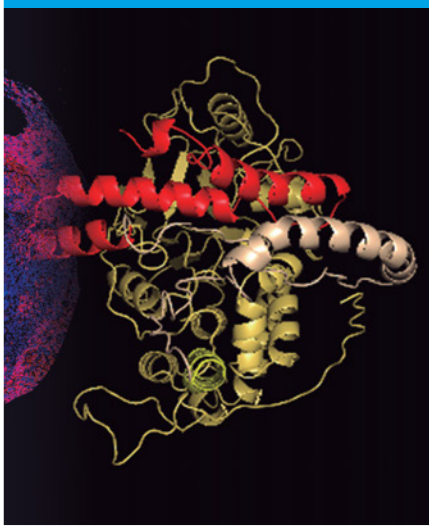
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RESEARCH DEPARTMENTS

Cardiovascular Development and Repair







RESEARCH DEPARTMENTS



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1. Cardiovascular Development and Repair

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The Department of Cardiovascular Development and Repair seeks to understand how the cardiovascular system is built, maintained and in some instances repaired. Our research programs examine the molecular and cellular basis of cardiovascular development, cardiovascular homeostasis and repair, and the role of stem-cell biology in these processes.

A. Cardiovascular Developmental Biology

We study how cardiac lineage specification occurs and how proliferation and patterning of the different cardiac regions that will form the mature heart are regulated. We want to unravel how alterations to these mechanisms lead to cardiovascular disease and how they can be manipulated to repair the diseased heart.

Program Coordinator: *José Luis de la Pompa*

B. Stem Cell Biology

Our aim is to understand the role of stem and progenitor cells in the development and maintenance of the cardiovascular system, as well as their contribution to the repair of the diseased state. We study different stem-cell populations—including embryonic, mesenchymal, cardiac and hematopoietic populations—in order to understand common and type-specific aspects of stem-cell biology that can be translated to the cardiovascular setting.

Program Coordinator: *Miguel Manzanares*

C. Tissue Homeostasis and Repair

We aim to understand the molecular and cellular processes that control the response of the cardiovascular system to acute and chronic damage resulting from large and small scale injury. We are interested in how cells and tissues adapt to and regulate oxygen availability, how the cardiovascular system communicates with other body systems, and how innate cardiovascular repair mechanisms function and could be enhanced to treat disease.

Program Coordinator: *José Antonio Enríquez*



Genetic control of organ development and regeneration

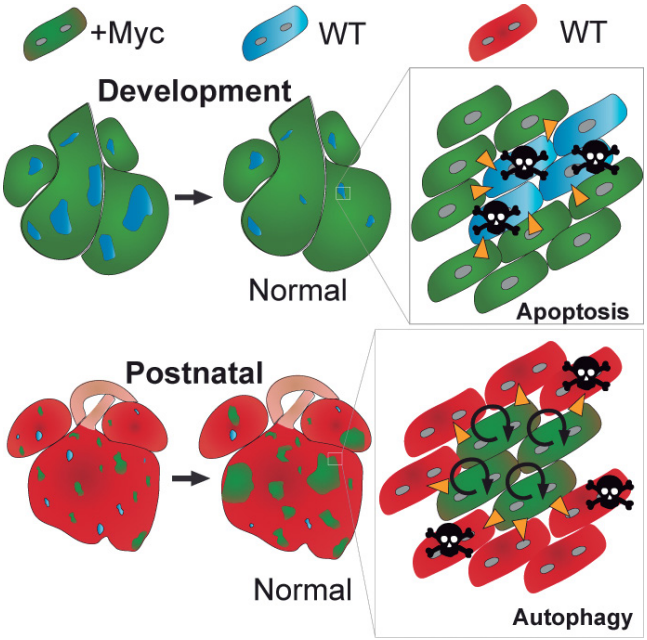
RESEARCH INTEREST

We are interested in understanding the cellular basis of developmental processes and how this is contolled by transcription factor networks (TFN). To study the cellular basis of developmental processes we have developed genetic methods in the mouse that allow us to trace cell lineages in clonal analysis or functional mosaics.

Using mosaic methods, we have described the involvement of cell competition in the selection of the fittest pluripotent cells during embryonic development. We recently found that cardiomyocytes and other cardiac lineages remain susceptible to induced cell competition during fetal and adult life, without compromising heart function. This finding opens the door to exploration of the roles of cell competition in cardiac repair and plasticity. We have also developed an *in vitro* embryonic stem cell competition model in which we can track the endogenous levels of Myc, a major determinant of cell competitive ability. This model is allowing us to study in great detail the live dynamics of cell competition and its cellular/molecular mechanisms.

Using clonal analysis methods, we have continued our efforts to trace the cellular history of the cardiovascular system. Starting with the initial specification steps, we have addressed the origin and relationships between the earliest blood and vascular cells. Unexpectedly, we found that the hemangioblast model of a common origin of vascular and blood cells does not hold for the *in vivo* situation in the early mouse embryo. Instead, we found initial independent specification of the two lineages followed by activation of an endothelial hemogenic program in the pre-circulation mouse yolk sac. In this area, we are now developing new methods for live analysis of early mouse development, which are allowing us to move toward live analysis of cellular contribution to early cardiovascular development.

In our work on TFNs, we are investigating the role of the TALE-Hox network in cardiac development and cardiomyocyte pool turnover in the adult heart. We study the molecular interaction and regulation of this TFN in two classical models of patterning; the limb and the main embryo axis. During this last year we proposed a new model for limb P-D specification involving an epigenetic timing mechanism.



Cell competition promotes phenotypically silent cardiomyocyte replacement in the mammalian heart (from Villa del Campo et al., 2014)



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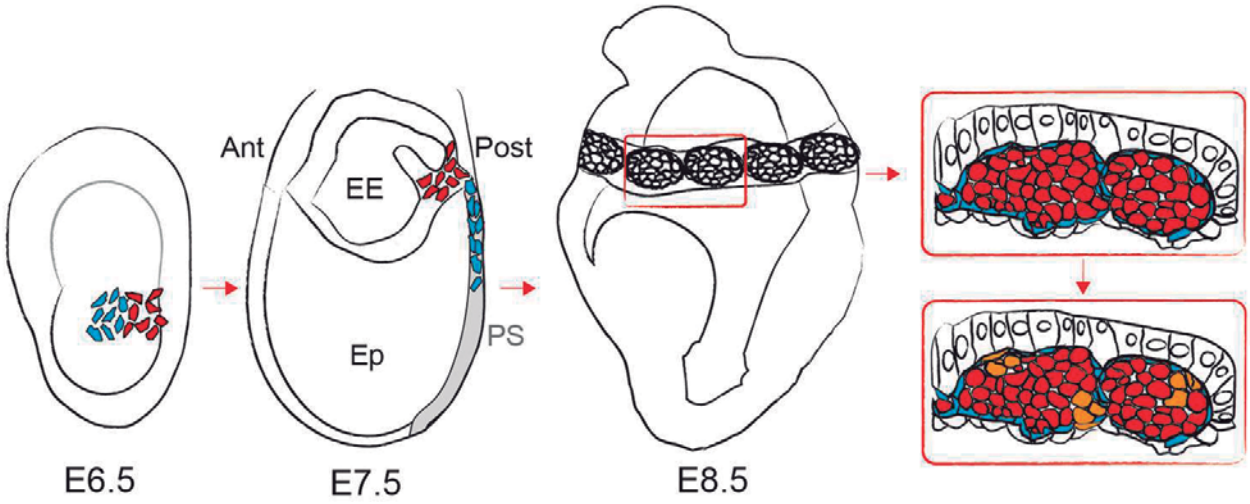
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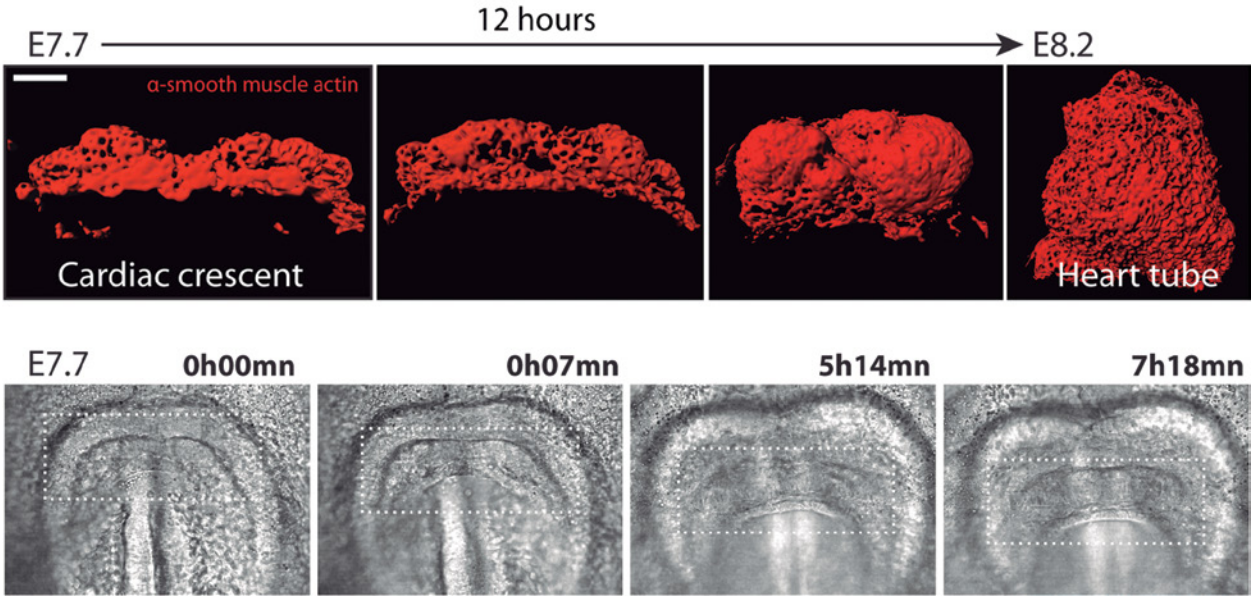
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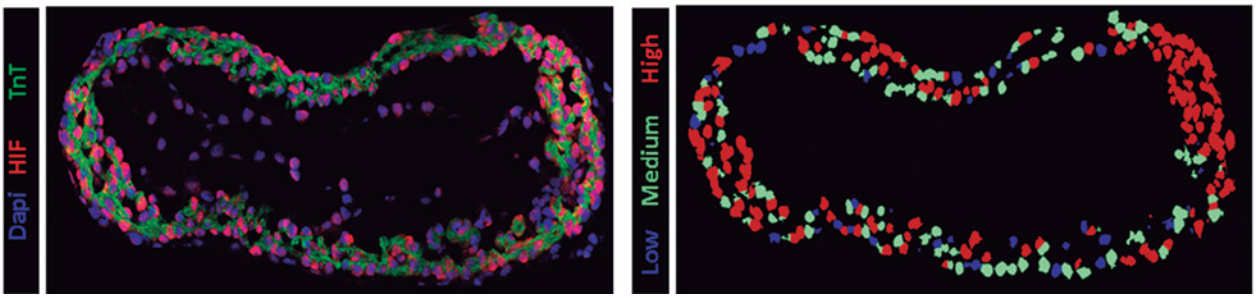


Model of the emergence of hemogenic endothelium in the YS blood islands from mesodermal precursors in the mouse embryo (from Padrón-Barthe et al., 2014)



The morphogenetic transformation of the cardiac crescent into the heart tube. The upper panels show 3D reconstructions revealing the morphology of the developing cardiac crescent and heart tube. The lower panels show a time-lapse images of a cultured embryo, revealing the early transition from the cardiac crescent to the early heart tube stage.

Another research line of our lab, led by Silvia Martín Puig, is the study of the role of hypoxia in the homeostasis of the cardiovascular system, with a particular focus on heart development and disease. We have generated several gain- and loss-of-function mouse models of the hypoxia pathway paying special attention to early cardiovascular populations that will contribute to the different functional cell types and structures composing the mature heart. Using these genetic tools we are investigating whether changes in oxygen tension influence the migration, proliferation or differentiation of cardiac progenitors and regulate mammalian cardiogenesis. Our data indicate that hypoxia, through the VHL/HIF axis, is essential for the proper formation of the ventricular chambers, maturation of the myocardium, and the correct development of the coronary vasculature. We are currently unraveling the mechanisms underlying the phenotypic alterations found in the mouse models generated. The molecular characterization of these phenotypes will help to elucidate the participation of the VHL/HIF axis in congenital heart disease and may have therapeutic applications.



Distribution of HIF1alfa protein within the developing ventricular chambers of the early mouse heart. Left panel: HIF1alfa (red) is expressed in most of the primitive ventricular cardiomyocytes labeled by troponin T (green) in an E9.5 heart section. Right panel: Myocardial HIF1alfa intensity map generated with Cell Profiler Software and representing differential HIF1alfa protein expression levels (low, medium, high) in ventricular cardiomyocytes.

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 Ciencia e Innovación. FIS (CP09/00100). IP: S. Martín-Puig**
- **European Commission FP7. Marie Curie Career Integration Grant (276891). IP: S. Martín-Puig**
- **Ministerio de Ciencia e Innovación (SAF2011-29830). IP: S. Martín-Puig**
- **Comunidad de Madrid (S2010/BMD-2542). IP: S. Martín-Puig**

Selected Publications

Villa Del Campo C, Claveria C, Sierra R, Torres M **Cell competition promotes phenotypically silent cardiomyocyte replacement in the mammalian heart**. *Cell Rep* (2014) 8:1741-51

Padron-Barthe L, Temino S, Villa Del Campo C, Carramolino L, Isern J, Torres M **Clonal analysis identifies hemogenic endothelium and not hemangioblasts as the source of the blood-endothelial common lineage in the mouse embryo**. *Blood* (2014) 124:2523-32

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

Clavería C, Giovinazzo G, Sierra R, Torres M. **Myc-driven endogenous cell competition in the early mammalian embryo**. Nature (2013) 500:39-44

Penkov D*, Mateos San Martín D*, Fernandez-Díaz LC, Rosselló CA, Torroja C, Sánchez-Cabo F, Warnatz HJ, Sultan M, Yaspo ML, Gabrieli A, Tkachuk V, Brendolan A, Blasi F, Torres M. **Analysis of the DNA-binding profile and function of TALE homeoproteins reveals their specialization and specific interactions with Hox genes/proteins.** *Cell Rep*. (2013) 3:1321-33
*Equal contribution

Martin-Puig S, Tello D and Aragones J. **The PHD-HIF oxygen sensing pathway in cardioprotection**. Front. Physiol. (Cardiac Electrophysiol.) (accepted) Oxygen homeostasis in the cardiovascular system



Regeneration and aging



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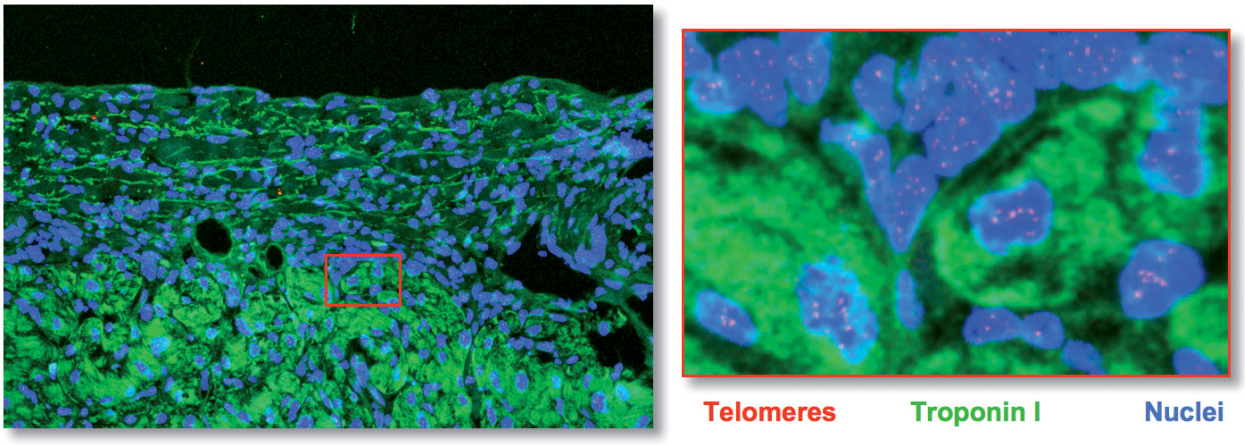
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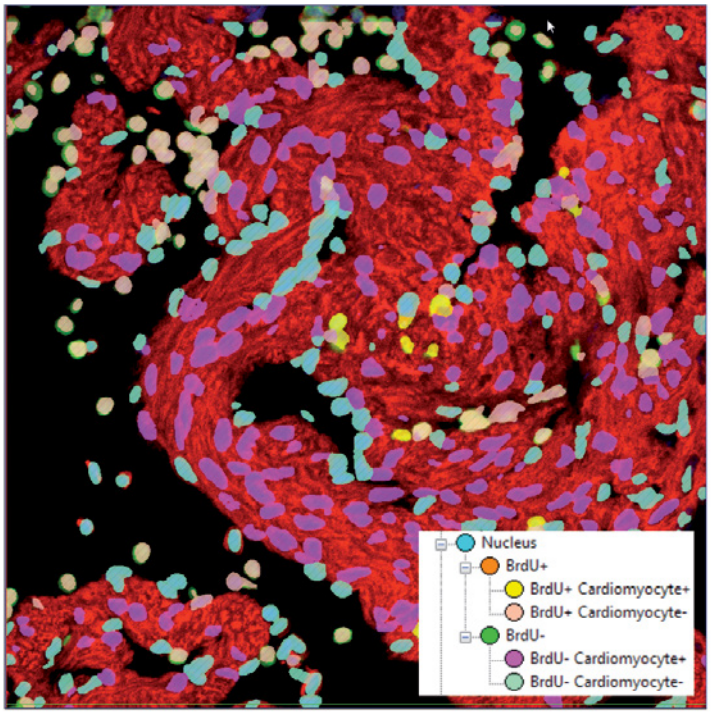
RESEARCH INTEREST

Although recent advances have overturned the old view of the human heart as an inert postmitotic organ, it is clear that the human 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.

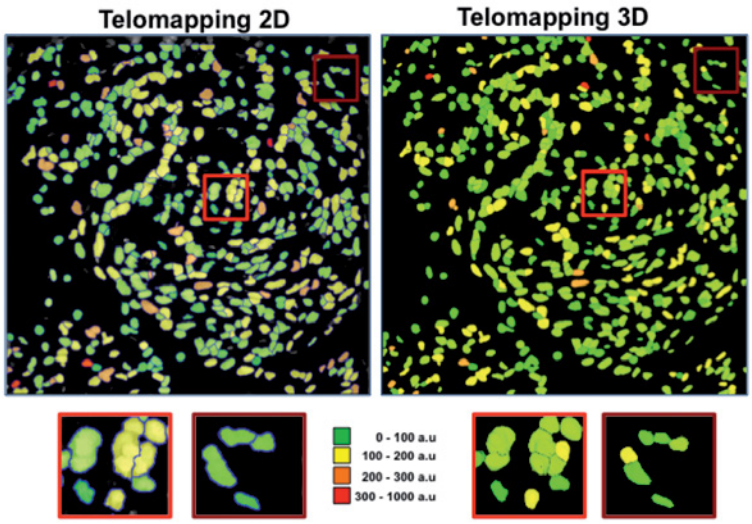
Our group aims to develop strategies to enhance cardiac regeneration. Toward this goal, we are characterizing the subpopulation of cardiac cells capable of regeneration, and using the knowledge generated to explore to promote the repair of injured hearts. We have eliminated and reactivated telomerase, 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. 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.



Telomere length measurements in cardiomyocytes. Staining of heart sections reveals telomeres (red), nuclei (blue) and the cardiomyocyte marker troponin I (green).



Quantitative analysis of cardiac proliferation. A specifically tailored image analysis program was used to segment, classify and quantify proliferation of different subtypes of cardiac cells after infarction. This work was done in collaboration with Hind Azegrouz of the Cellomics Unit.



Direct comparison of 2D and 3D telomapping. Better telomere and nuclei segmentation and more accurate results are obtained when a Z stack of confocal images is analyzed instead of the classical 2D maximum projection image. This work was done in collaboration with Hind Azegrouz of the Cellomics Unit.

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

Schneider RP, Garrobo I, Foronda M, Palacios JA, Marión RM, Flores I, Ortega S, Blasco MA. **TRF1 is a stem cell marker and is essential for the generation of induced pluripotent stem cells**. *Nat Commun* (2013) 4:1946



Development of the epicardium and its role during regeneration

RESEARCH INTEREST

Unlike adult mammals, zebrafish have the capacity to regenerate their hearts in response to several types of injury. In the laboratory, we use cryoinjury to induce a cardiac tissue damage with the aim of mimmicking 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 clinical antifibrotic strategies. We recently conducted a detailed analysis to establish whether the regeneration we observe also is accomplished at a functional level. For this, we set up echocardiography in the zebrafish in order to study ventricular pumping efficiency. Our results reveal that cryoinjury transiently impairs ventricular fractional volume shortening but that pumping efficiency was completely recovered at late stages postinjury. However, in many cases ventricular wall contraction showed long-term alterations. 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 pericardial fluid flow forces, which are triggered by the beating heart. Our current effort is dedicated to understanding the mechanosensory pathways underlying these events.



Head of Laboratory
Nadia Mercader

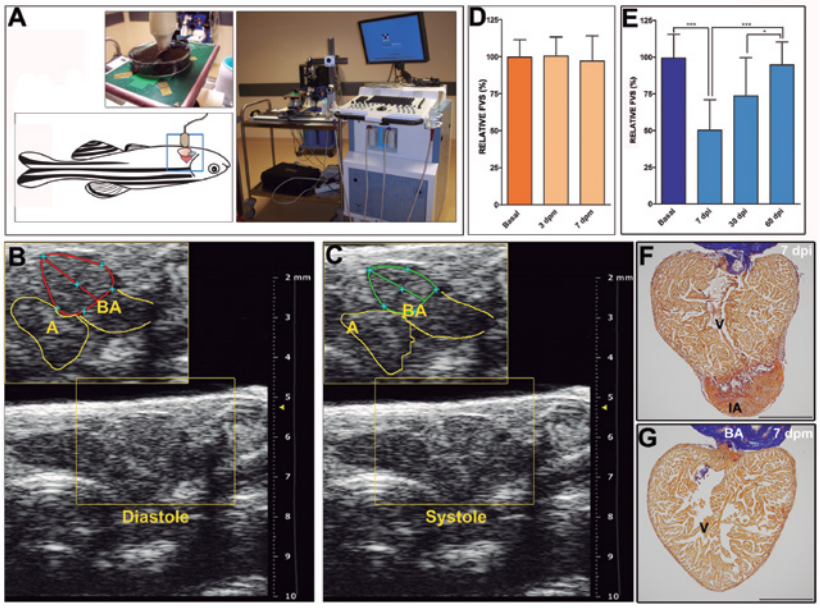
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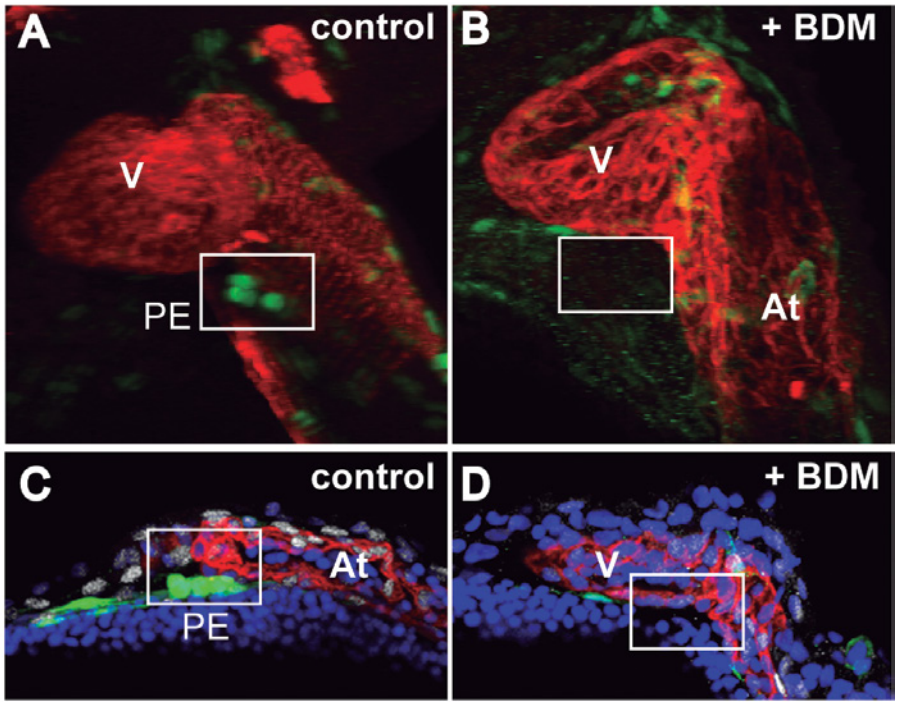
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Echocardiographic assessment of ventricular function recovery during zebrafish heart regeneration. (A) Apparatus for image acquisition in the zebrafish and scheme representing animal positioning. Animals are positioned ventrally and are immobilized in a Petri dish, and are covered with fish water containing anesthetic. This positioning allows a transducer to be placed directly over the body wall at the level of the heart. The transducer is attached to a holder to ensure a stable position during acquisition. (B,C) Details from representative 2D echocardiography images from a non-injured zebrafish heart showing maximal ventricular dilatation (B, diastole) and maximal ventricular contraction (C, systole). The diastolic (red) and systolic (green) ventricular areas are outlined and the length of the apical image long axis is indicated (L). Red and green lines in B and C highlight the ventricular border in diastole and systole, respectively. Yellow lines indicate the bulbus arteriosus (BA). (D,E) Cryoinjury transiently impairs ventricular pumping efficiency. Temporal evolution of changes in the relative FVS in sham-operated (D) and cryoinjured (E) animals in a longitudinal study. Graphs show relative means ± SD. The relative FVS is not significantly changed in sham-operated animals but cryoinjured animals show a 50% decrease in the RFVS that gradually recovers by around 60 dpi. (F,G) AFOG histological staining of ventricles from cryoinjured and sham-operated zebrafish. A, atrium; BA, bulbus arteriosus; dpi, days postinjury; dpm, days postmanipulation; FVS, fractional volume shortening; RFVS, relative fractional volume shortening.



Lack of a heartbeat impairs proepicardium formation. (A,B) 3D recontructions and (C,D) confocal sections of 60-hour-old zebrafish of the Epi:GFP line, expressing GFP in proepicardial (PE) and some pericardial cells. The myocardium is stained red (myosin heavy chain immunohistochemistry) and cell nuclei are counterstained with DAPI. Note the PE cell cluster visible in control samples but absent in larvae treated for 5 hours with 20 mM 2,3-butane monoxime (BDM), a drug that stops cardiac contraction. At, atrium; 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)

Selected Publications

Peralta M*, González-Rosa JM*, Marques IJ, Mercader N. **The epicardium in the embryonic and adult zebrafish**. *J Dev Biol* (2014) 2:101-16
*Equal contribution

Gonzalez-Rosa JM, Guzman-Martinez G, Marques IJ, Sanchez-Iranzo H, Jimenez-Borreguero LJ and Mercader N. **Use of echocardiography reveals reestablishment of ventricular pumping efficiency and partial ventricular wall motion recovery upon ventricular cryoinjury in the zebrafish.** *PLoS One* (2014) 9: e115604

Hermann M, Stillhard P, Wildner H, Seruggia D, Kapp V, Sanchez-Iranzo H, Mercader N, Montoliu L, Zeilhofer HU and Pelczar P. **Binary recombinase systems for high-resolution conditional mutagenesis.** *Nucleic Acids Res* (2014) 42: 3894-907

Peralta M, Steed E, Harlepp S, González-Rosa JM, Monduc F, Ariza-Cosano A, Cortés A, Rayón T, Gómez-Skarmeta JL, Zapata A, Vermot J, Mercader N. **Heartbeat-driven pericardiac fluid forces contribute to epicardium morphogenesis**. *Curr Biol* (2013) 23:1726-35

Espín R, Roca FJ, Candel S, Sepulcre MP, González-Rosa JM, Alcaraz-Pérez F, Meseguer J, Cayuela ML, Mercader N, Mulero V. **TNF receptors regulate vascular homeostasis through a caspase-8, caspase-2 and P53 apoptotic program that bypasses caspase-3**. *Dis Model Mech* (2013) 6:383-95



Molecular genetics of angiogenesis



Head of Laboratory
Rui Benedito

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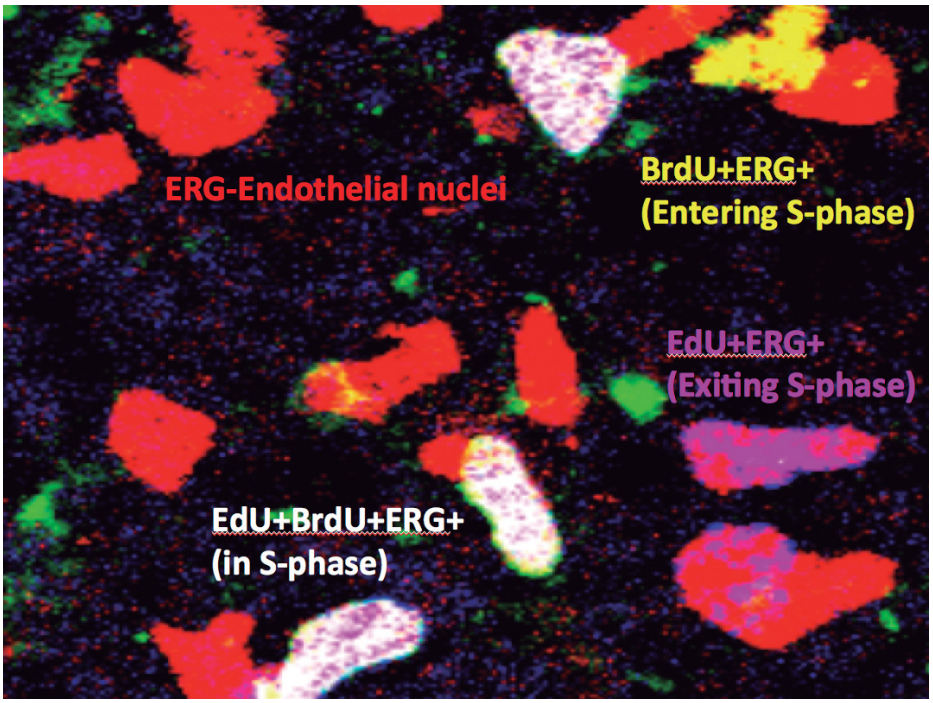
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Masters Student:
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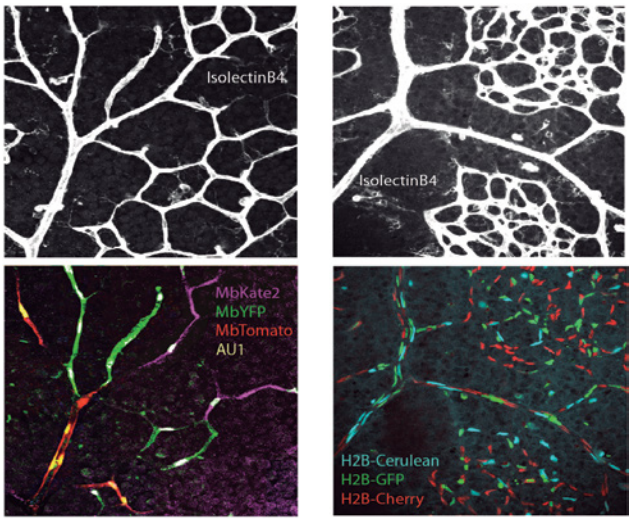
Graduate Technician:
Luis Heredia Gallego

RESEARCH INTEREST

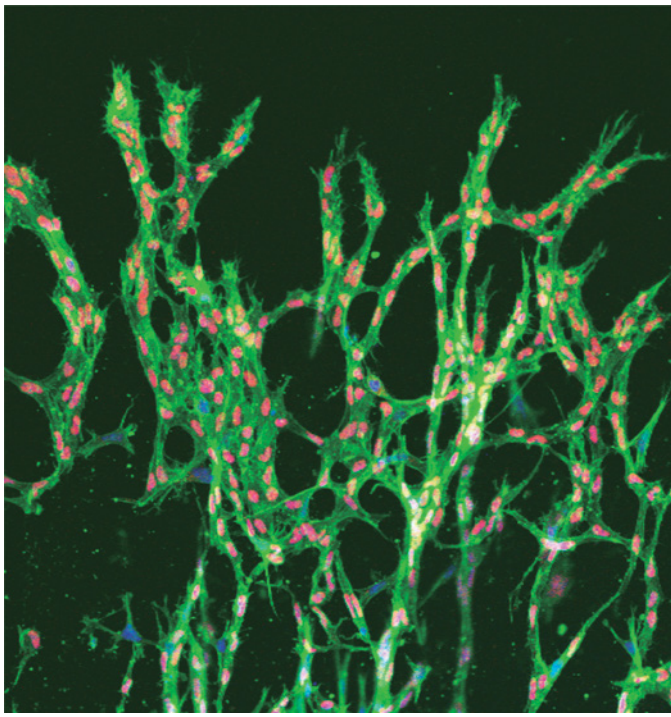
Blood vessels are an important therapeutic target in cardiovascular diseases and cancer. The knowledge accumulated during the past several years in the field of vascular biology has allowed the use of VEGF and its major endothelial receptors as targets in numerous therapies designed to stimulate or inhibit the growth of blood vessels, leading to significant improvements in the treatment of many vascular related diseases. But VEGFs and their receptors do not work alone, and their different functions can also be controlled by other mechanisms which compensate or override VEGF function. One such mechanism is the Notch signaling pathway, which is a key regulator of the initial arterial differentiation program and also plays a fundamental role during developmental and pathological angiogenesis.We are studying in high detail the role of several molecular mechanisms that lie downstream of VEGF and Notch signaling, and that are involved in specific aspects of vascular proliferation, differentiation, maturation and quiescence. In the last year we generated new mouse models, optimized several in vitro angiogenesis assays, and performed quantitative gene expression analysis *in vitro* and *in vivo* to define the molecular regulation of endothelial proliferation in different contexts.



Confocal image showing different endothelial nuclei (Erg+) with BrdU or Edu labeling, indicating the different stages of the cell-cycle.



Confocal images showing vessels (IsolectinB4+) of two mouse lines, recently generated by us at the CNIC, in which different endothelial cell clones express different membrane (left) or nuclear (right) fluorescent proteins. Up to 5 different signals can be detected and separated in the same sample.



Endothelial sprouts derived from mouse embryoid bodies *in vitro*. We use this assay to screen for the function of several genes in endothelial sprouting and proliferation.

MAJOR GRANTS

- Ministerio de Economia y Competitividad, Europa Excelencia 2013 (MIN/SAF1301)
- Ministerio de Economia y Competitividad, Plan Nacional (SAF2013-44329-P)
- Ministerio de Economia y Competitividad, Programa Ramón y Cajal (MIN/RYC1301)

Selected Publications

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

Ehling M, Adams S, Benedito R, Adams RH. **Notch controls retinal blood vessel maturation and quiescence**. *Development* (2013) 140:3051-61

Benedito R, Hellström M. **Notch as a hub for signaling in angiogenesis**. *Exp Cell Res* (2013) 319:1281-8

Wang L, Benedito R, Bixel MG, Zeuschner D, Stehling M, Sävendahl L, Haigh JJ, Snippert H, Clevers H, Breier G, Kiefer F, Adams RH. **Identification of a clonally expanding haematopoietic compartment in bone marrow**. *Embo J* (2013) 32:219-30



Intercellular signaling in cardiac development & disease



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Graduate Technician:
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Visiting Scientists:
José María Pérez-Pomares

RESEARCH INTEREST

The heart is the first organ to form and function in the developing vertebrate embryo. Understanding the molecular mechanisms that regulate the cellular proliferation, differentiation and patterning processes that give rise to the adult heart is essential for understanding cardiac disease.

In the last year we have focused our efforts on the role of various intercellular signals in chamber and valve development and disease (cardiomyopathy and valve disease) and cardiac repair. Our ultimate goal is to identify new molecular markers for cardiac disease or processes eventually amenable to therapeutic intervention. To address these questions we have used mouse and zebrafish genetics, hiPSC, biochemistry, next-generation sequencing (NGS) applied to mouse and human samples, and imaging analysis.

We find that during ventricular chamber development the Notch signaling pathway emits signals from the inner layer of the heart, the endocardium, that promote trabeculation—the formation of a network of endocardium-covered myocardial ridges that protrude into the ventricular lumen, increasing the surface area for oxygen exchange within the growing ventricles. When the chambers reach a critical size, the task of nourishing the cardiomyocytes embedded in the thick ventricular walls is taken over by the developing coronary vasculature, and the trabeculae compact and contribute to increasing the mass of the outer and highly proliferative compact myocardium layer. Trabecular compaction is also dependent on Notch signaling, as the whole process of chamber development is tightly regulated, with various Notch ligands acting at different stages to activate the Notch1 receptor in the endocardium. Alterations of these developmental processes result in cardiomyopathy (Figure 1). We are currently validating the results of an NGS analysis in familial patients with left ventricular non-compaction cardiomyopathy, performed in collaboration with clinical colleagues at the CNIC and various hospitals.

We have also studied the interplay of Notch and Bmp2 signaling in valve development. Besides its involvement in the promotion of epithelial mesenchyme transition (EMT) to give rise to the valve primordium (Figure 2), Notch is also required for the fine tuning of mesenchymal cell proliferation, extracellular-matrix (ECM) secretion and remodeling processes associated with valve sculpting. EMT is coordinated by Notch and Bmp2, and a new gain-of-function model shows that Bmp2 must be tightly regulated throughout valve development, with Bmp2 overexpression in the myocardium disrupting valve morphogenesis and chamber development (Figure 3).

Through our collaboration with clinical colleagues we have undertaken a NGS study to try to identify the gene expression profile associated with aortic valve disease in patients with calcified tricuspid or bicuspid aortic valves. We are currently carrying out functional studies to verify the role of the identified genes in this disease, using valve endothelial and interstitial cells obtained from surgical samples.

Using gain- and loss-of-function models in the regenerating zebrafish heart, we have shown that Notch plays a crucial role in the modulation of cardiomyocyte proliferation and differentiation and the inflammatory and fibrotic response associated with cardiac damage (Figure 4).

We believe that advancing knowledge of the molecular mechanisms underlying cardiac development and disease will help us to identify novel diagnostic or therapeutic strategies to treat the diseased heart.

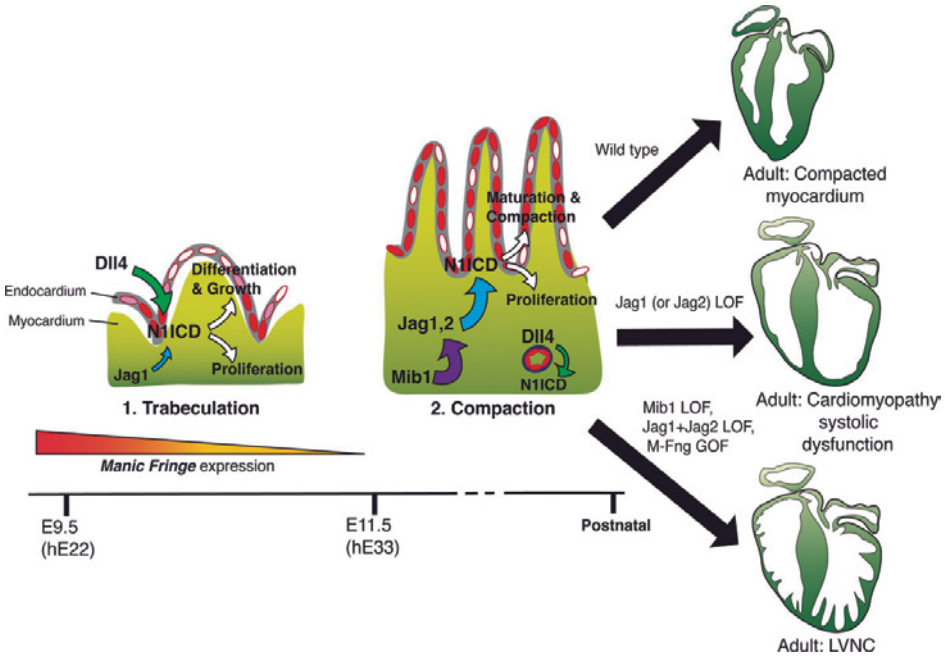


Figure 1. Working model of Notch function in murine ventricular chamber development. During an early (initiation) phase Dll4-Notch activation leads to trabeculae formation. At a later (maturation) phase, Jag1 and Jag2 are able to activate Notch from the myocardium and regulate cardiomyocyte proliferation and differentiation and myocardial compaction. M-Fng plays an important role in the temporal regulation of ligand specificity. Disruption of the signals mediated by the late-acting ligands leads to cardiomyopathy.

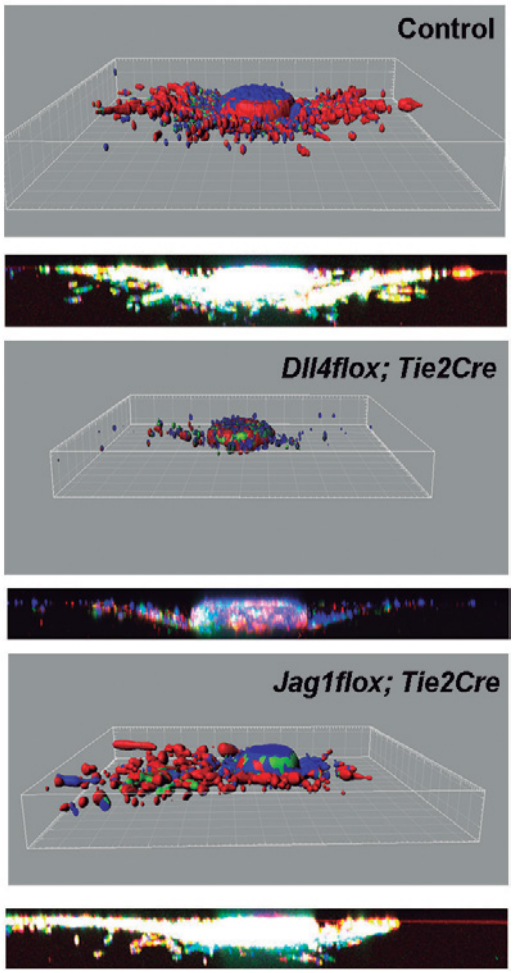


Figure 2. The Notch ligand Dll4, but not Jag1, is required for EMT. Amira 3D reconstructions and lateral views of confocal microscopy images of an atrio-ventricular canal explant, cultured on collagen for 3 days. Mesenchyme cells are labeled red, nuclei blue and cardiomyocytes green.

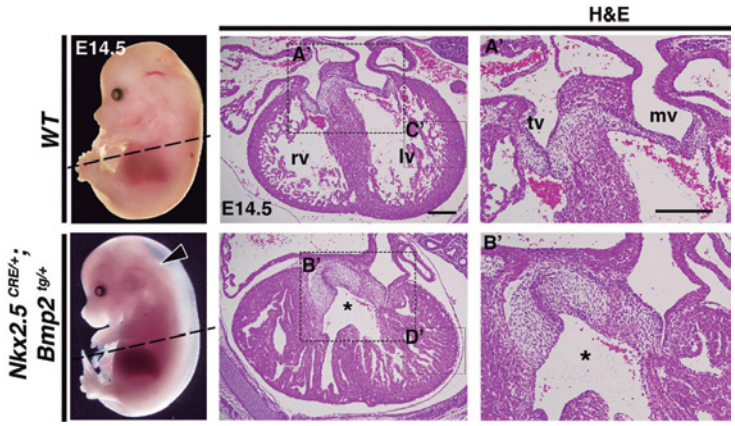


Figure 3. Bmp2 overexpression in the developing heart disrupts cardiac development. Whole mount views of E14.5 embryos and H&E stained cardiac sections of wild-type (WT, A, A') and *Nkx2.5-Cre;Bmp2* $^{tg/+}$ embryos. Note the dysmorphology and increased cellullarization of the trasngenic cushion (B'). The general views of the heart show that chamber structure is also disrupted.

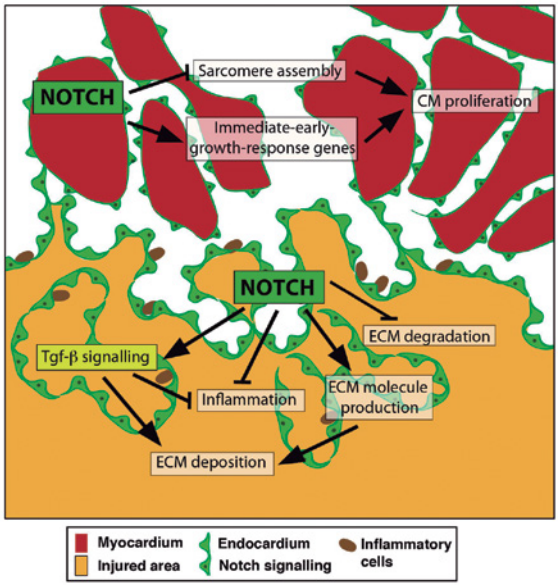


Figure 4. Notch signaling mediates cardiomyocyte proliferation and fibrotic tissue deposition in the cryoinjured zebrafish heart. Endocardial Notch signaling attenuates the expression of sarcomere assembly genes and regulates immediate-early growth gene activation, two requirements for cardiomyocyte proliferation. In the injured area Notch weakens the inflammatory response and favors ECM deposition by inducing ECM molecule expression and blocking ECM degrading proteases. Tgf-β signaling may be one mediator of these functions as its activation in endocardial cells partly depends on Notch signaling.

MAJOR GRANTS

- **Ministerio de Economía y Competitividad. FIS RETICS (TERCEL: RD12/0019/0003 and RIC: RD12/0042/0005)**
- **European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600) (Coordinador E. Lara)**
- **Ministerio de Economía y Competitividad (SAF2013-45543-R)**
- **Fundación BBVA (2015-2017)**
- **Ministerio de Economía y Competitividad, Juan de la Cierva contract (JCI-2012-12260) PI: Mauro Sbroggio**
- **Ministerio de Economía y Competitividad (SAF2010-17555)**

Selected Publications

Garcia-Pavia P, de La Pompa JL **Left ventricular noncompaction: a genetic cardiomyopathy looking for diagnostic criteria**. *J Am Coll Cardiol* (2014) 64:1981-3

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(11):a013912

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

Cortegano I, Melgar-Rojas P, Luna-Zurita L, Siguero-Álvarez M, Marcos MA, Gaspar ML, & de La Pompa JL **Notch1 regulates progenitor cell proliferation and differentiation during mouse yolk sac hematopoiesis**. ***Cell Death Differ*** (2014) 21:1081-94

MacGrogan D, Luxán G, de La Pompa JL **Genetic and functional genomics approaches targeting the Notch pathway in cardiac development and congenital heart disease**. *Brief Funct Genomics* (2014) 13:15-27



Stem cell niche pathophysiology



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Daniel Martín Pérez
Abel Sánchez-Aguilera Peño

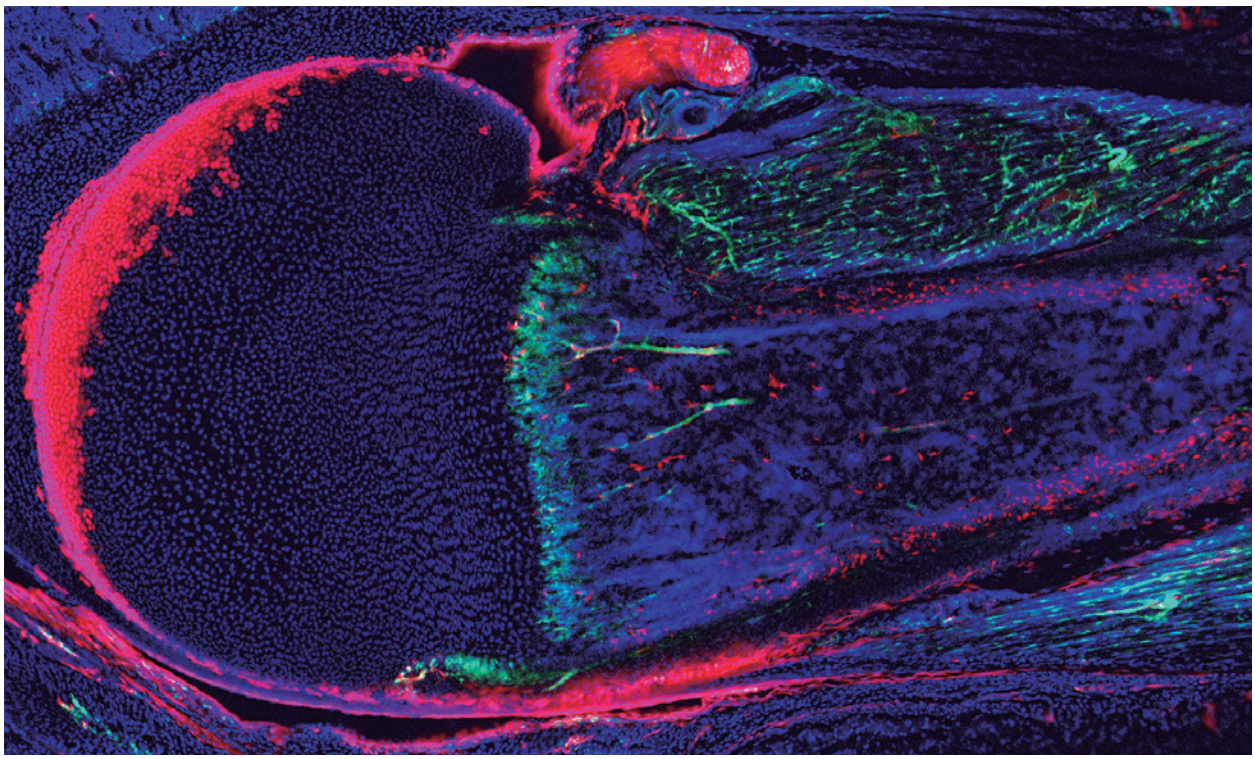
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Master Students:
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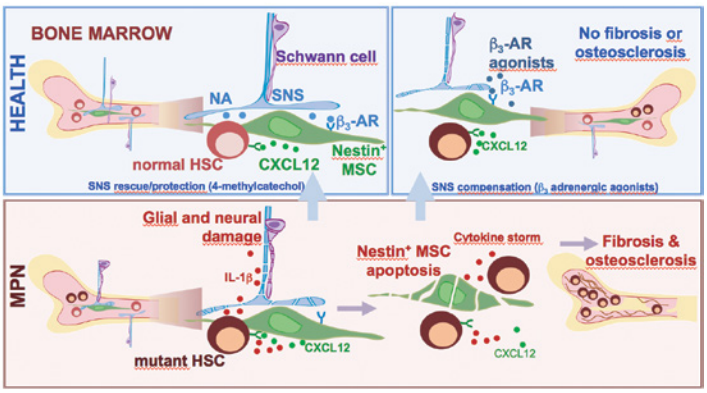
Technicians:
Javier Langa Oliva
Sandra Martín Salamanca

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).

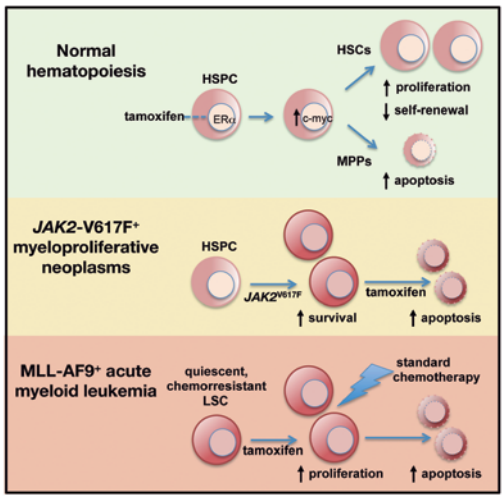


Firure 1. The neural crest is a source of mesenchymal stem cells with specialized functions in the hematopoietic stem cell niche. Neonatal bone marrow section from triple transgenic mouse in which neural crest-derived cells are labeled in red and nestin+ cells in green. Blue signal corresponds to cell nuclei (Isern J et al. eLife 2014).



Firure 2. 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)

Graphical Abstract



Firure 3. 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)

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) PI: Joan Isern**
- **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**

Selected Publications

Sánchez-Aguilera A, Arranz L, Martín-Pérez D, García-García A, Stavropoulou V, Kubovcakova L, Isern J, Martín-Salamanca S, Langa X, Skoda RC, Schwaller J and Méndez-Ferrer S. **Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis**. *Cell Stem Cell* (2014) 15:791-80

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

Zaidi M, Méndez-Ferrer S. **Cell biology: tumour stem cells in bone.** *Nature* (2013) 499:414-6

Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, Del Toro R, Sánchez-Aguilera A, Arranz L, Martín-Pérez D, Suárez-Lledó M, Marín P, Van Pel M, Fibbe WE, Vázquez J, Scheding S, Urbano-Ispizúa A, Méndez-Ferrer S. **Self-Renewing Human Bone Marrow Mesenspheres Promote Hematopoietic Stem Cell Expansion.** *Cell Rep* (2013) 3:1714-24



Pathophysiology of adipose and cardiac tissues



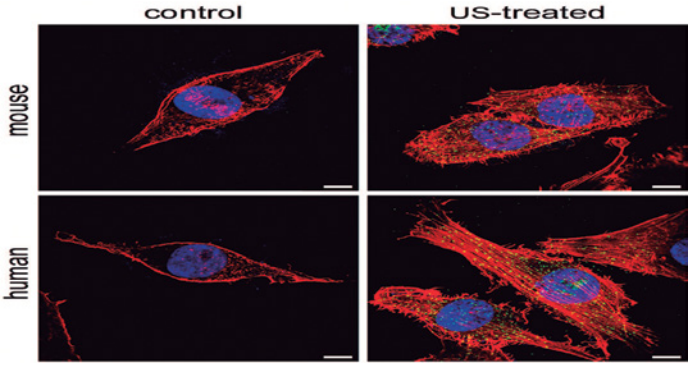
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Laura Martín

Visiting Scientist:
Javier Suárez

RESEARCH INTEREST

Cell-based therapy is a promising approach for many diseases, including ischemic heart disease. Our work focuses on cardiac mesoangioblasts, committed vessel-associated progenitors that can restore heart structure and function to a significant, albeit partial, extent in a mouse model of myocardial infarction. Low-intensity pulsed ultrasound (LIPUS) is a non-invasive form of mechanical energy that can be delivered into biological tissues as acoustic pressure waves, and is widely used for clinical applications including bone fracture healing. We hypothesized that the positive effects of LIPUS on bone and soft tissue, which include increased cell differentiation and cytoskeleton reorganization, could be applied to increase the therapeutic potential of mesoangioblasts for heart repair. During this year, we showed that LIPUS stimulation of cardiac mesoangioblasts isolated from mouse and human heart results in significant cellular modifications that provide beneficial effects to cells, including increased malleability and improved motility and invasiveness. Additionally, LIPUS stimulation increased the number of binucleated mesoangioblasts and induced cardiac differentiation to an extent comparable with 5-azacytidine treatment. Administration of LIPUS-stimulated mesoangioblasts *in vivo* resulted in greater retention and incorporation into cardiotoxin-damaged hearts. Taken together, these results provide functional evidence for the potential of LIPUS as a useful tool in heart cell therapy



Cardiac mesoangioblasts after treatment with LIPUS

Top Canonical Pathways
Cardiomyocyte differentiation via BMP Receptors
Chemokine Signaling
Regulation of Actin-based Motility by Rho
Factors Promoting Cardiogenesis in vertebrates
PAK Signaling

Main canonical pathways activated after treatment with LIPUS

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2010-15239)

Selected Publications

McCreath KJ, Espada S, Gálvez BG, Benito M, de Molina A, Sepúlveda P, Cervera AM. **Targeted disruption of the SUCNR1 metabolic receptor leads to dichotomous effects on obesity.** *Diabetes* (2014) Oct 28

Giannotta M, Benedetti S, Tedesco FS, Corada M, Trani M, D'Antuono R, Millet Q, Orsenigo F, Gálvez BG, Cossu G, Dejana E. **Targeting endothelial junctional adhesion molecule-A/ EPAC/ Rap-1 axis as a novel strategy to increase stem cell engraftment in dystrophic muscles.** *EMBO Mol Med* (2014) 6:239-58

Bernal A, Gálvez BG. **The potential of stem cells in the treatment of cardiovascular diseases.** *Stem Cell Rev* (2013) 9:814-32

Pérez LM, Bernal A, San Martín N, Gálvez BG. **Obese-derived ASCs show impaired migration and angiogenesis properties.** *Arch Physiol Biochem* (2013) 119:195-201

Pérez LM, Bernal A, San Martín N, Lorenzo M, Fernández-Veledo S, Gálvez BG. **Metabolic rescue of obese adipose-derived stem cells by Lin28/Let7 pathway.** *Diabetes* (2013) 62:2368-79



Functional genomics of embryonic pluripotency and heart development



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Mª Elena López Jiménez

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Julio González Sainz de Aja
Sergio Menchero Fernández
Teresa Rayón
Raquel Rouco García

Masters Student:
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Claudio Badía Careaga
Eva Fernández Cáceres

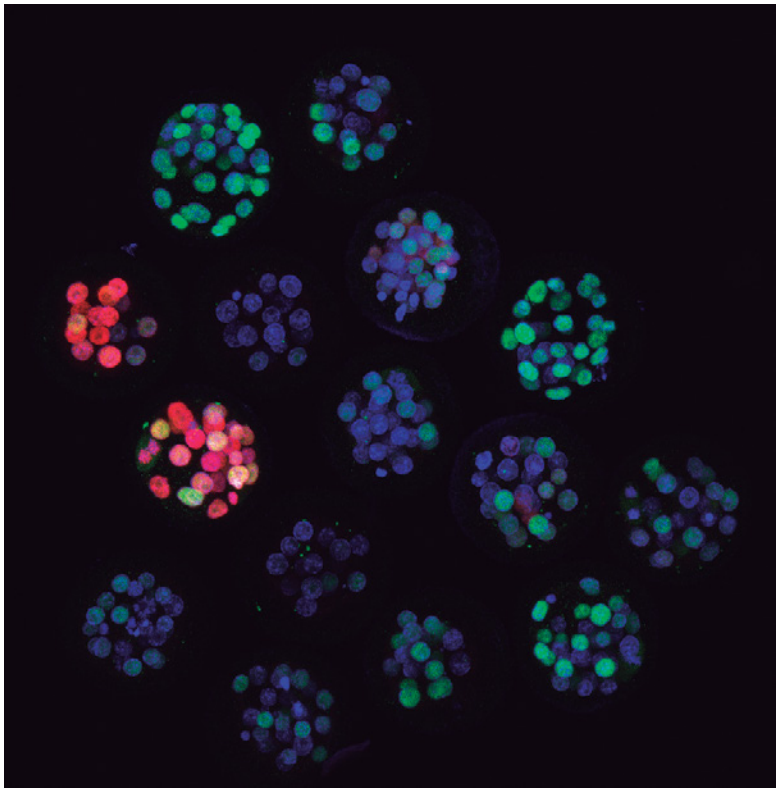
Visiting Scientis:
Gonzalo Carreño Gómez-Tarragona

RESEARCH INTEREST

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 these elements are the target of variation that results in increased risk of human disease. Uncovering the regulatory basis of cardiovascular diseases is one of our major goals.

We are exploring how initial decisions and lineage choices occur in the mammalian embryo, before it implants in the maternal uterus and when pluripotent cell fate is established. We have shown how different signaling pathways act together to activate gene expression in the outer layer at the blastocyst stage, thus distinguishing the embryonic from the extraembryonic lineage. A critical event in this process is the regulation of the expression of CDX2 by the Notch and Hippo pathways through a specific enhancer.

By exploring the findings of genome-wide association studies, we have found that regulatory elements distal to the *PITX2* gene lie in a genomic region associated with an increased risk of atrial fibrillation. We are exploring the genomic architecture of this and other atrial fibrillation associated loci, finding unsuspected interactions with other genes in these regions. Using mouse genetic models, we are conducting a genome-wide study of how chromatin structure crucially regulates proper gene expression in the heart, and how this could underlie certain cases of human cardiovascular disease.



Collection of mouse blastocysts showing expression of a fluorescent reporter driven by a *Cdx2* enhancer (red), endogenous CDX2 expression (green), and nuclei (blue).

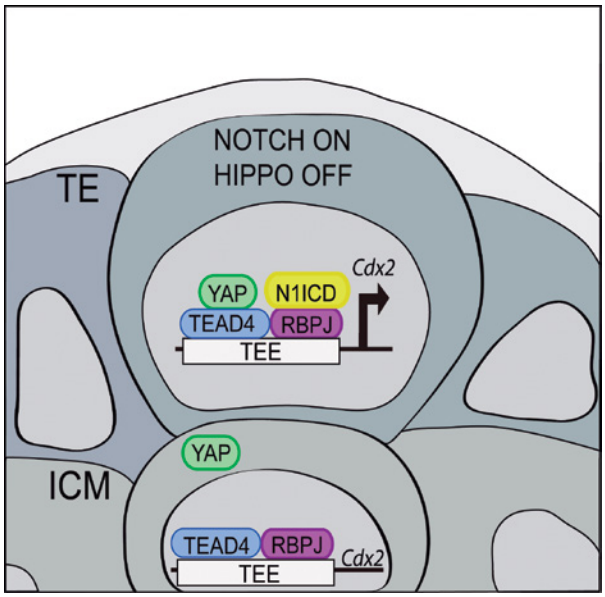
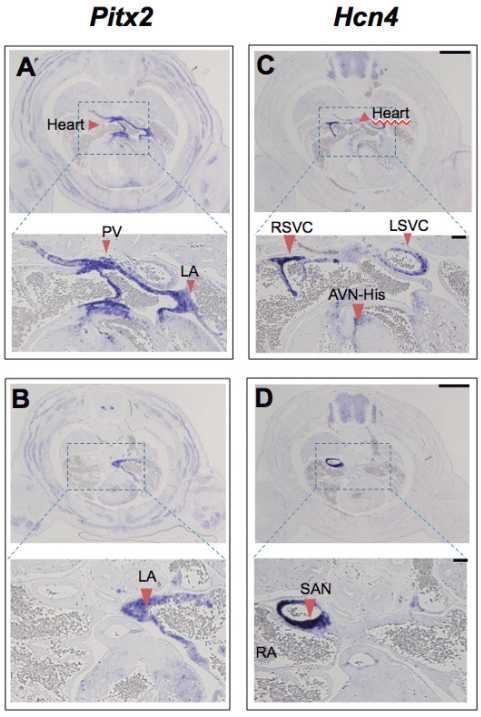


Diagram showing how different states of the Notch and Hippo pathways result in differential expression of the *Cdx2* gene between the outer blastocyst cells, which will form the trophectoderm (TE, the precursor of the placenta), and the cells of the inner cell mass (ICM), whcih will give raise to the embryo proper and later to all adult lineages.



Differential expression of the Pitx2 and Hcn4 genes in arrhythmogenic regions of the developing 14.5 dpc mouse heart. While *Pitx2*, which encodes a transcription factor that has been linked to atrial fibrillation, is expressed in the pulmonary veins (PV) and the left atrium (LA), the ion-channel-encoding gene *Hcn4* is expressed in the right and left superior vena cava (RSVC, LSVC), the atrioventricular node and His bundle (AVN-His), and the sinoatrial node (SAN). Mis-expression of both of these genes has been linked to the occurrence of atrial fibrillation

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (BFU2011-23083)
- Centro Nacional de Investigaciones Cardiovasculares. CNIC Translational Projects (CNIC-08-2009),
- Comunidad Autónoma de Madrid (CELLDD-CM: S2010/BMD-2315)
- Ministerio de Economía y Competitividad (BFU2014-57703-REDC)

Selected Publications

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

Pernaute B, Spruce T, Smith KM, Sanchez-Nieto JM, Manzanares M, Cobb B and Rodriguez TA. **microRNAs control the apoptotic threshold in primed pluripotent stem cells through regulation of BIM.** *Genes Dev* (2014) 28: 1873-8

Piazzolla D, Palla AR, Pantoja C, Cañamero M, de Castro IP, Ortega S, Gómez-López G, Dominguez O, Megías D, Roncador G, Luque-Garcia JL, Fernandez-Tresguerres B, Fernandez AF, Fraga MF, Rodriguez-Justo M, Manzanares M, Sánchez-Carbayo M, García-Pedrero JM, Rodrigo JP, Malumbres M, Serrano M. **Lineage-restricted function of the pluripotency factor NANOG in stratified epithelia.** *Nat Commun* (2014) 30:4226

Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gomez-Marin 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 and Nobrega MA. **Obesity-associated variants within FTO form long-range functional connections with IRX3.** *Nature* (2014) 507: 371-5

Abad M, Mosteiro L, Pantoja C, Cañamero M, Rayon T, Ors I, Graña O, Megías D, Domínguez O, Martínez D, Manzanares M, Ortega S, Serrano M. **Reprogramming in vivo produces teratomas and iPS cells with totipotency features.** *Nature* (2013) 502:340-5



Epigenetic regulation in cardiac aging and disease

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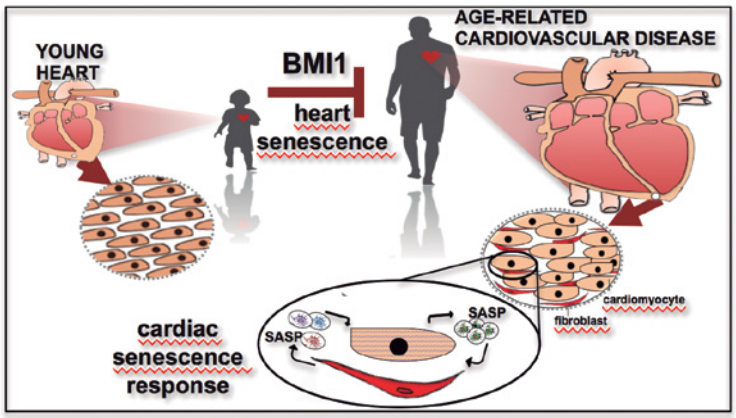
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Rebeca Diges López

Visiting Scientist:
José Manuel Garrido Jiménez

RESEARCH INTEREST

Adult stem cells participate in the natural homeostasis of adult tissues through their ability to both self-renew and differentiate into multiple lineages to regenerate tissue in response to injury signals. During aging, the proliferation and differentiation capacity of tissue-specific stem cells decreases, and they lose their potential to regenerate tissues after damage. PcG-mediated alteration of the epigenetic status of hematopoietic stem cells (HSCs) is proposed as one of the driving forces behind many age-related HSC changes and is often found to be misregulated in human malignancies. Protection of the transcriptional "stemness" network is thus essential for maintenance of a healthy HSC compartment throughout life. A key unanswered question in the field is whether the functional decline in adult stem cells is related to reversible chromatin modifications. 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, a key adult stem cell population with diverse regenerative capacities. Understanding molecular mechanisms by which Polycomb members control stem cell fate will provide new insights into hematopoietic stem cell biology and will also increase understanding of neoplastic transformation.

We are also interested 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. Dilated cardiomyopathy (DCM) represents the third most common cause of heart failure but has been 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.



Impact of the cardiac-specific action of Bmi1. Aging is the greatest risk factor for cardiovascular disease, and the cardiac action of Bmi1 might be the key to limiting the cardiac senescence response. Our data establish the idea that the non-proliferative cardiomyocyte-related senescence phenotype can be locally propagated through the senescence-associated secretory phenotype (SASP).

MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2010-15386)

Selected Publications

Sousa-Victor P, Gutarra S, García-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, Jardí M, Ballestar E, González S, Serrano AL, Perdiguero E, Muñoz-Cánoves P. **Geriatric muscle stem cells switch reversible quiescence into senescence**. *Nature* (2014) 506:316-21

Hidalgo I, Gonzalez S. **New epigenetic pathway for stemness maintenance mediated by the histone methyltransferase Ezh1.** *Cell Cycle* (2013) 12:383-4



Nuclear receptor signaling



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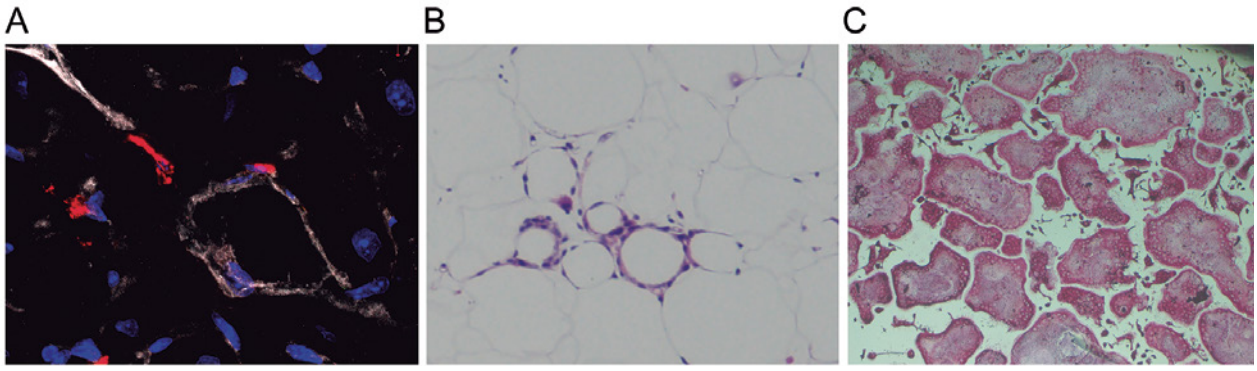
Masters Student:
Laura Alonso Herranz

Technician:
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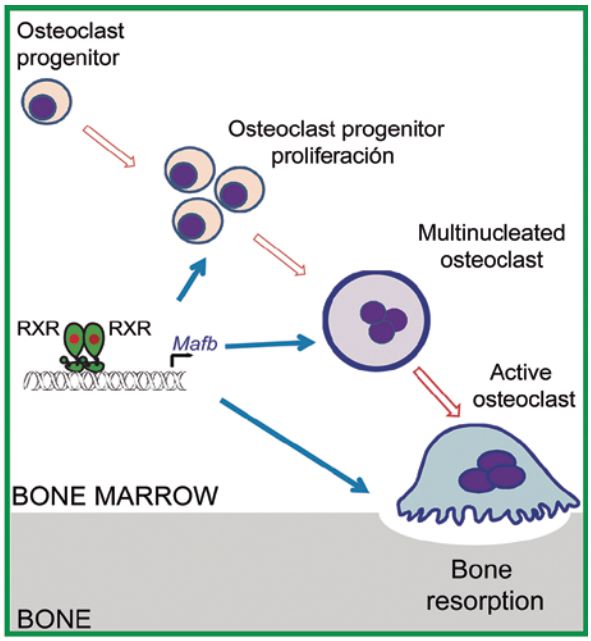
RESEARCH INTEREST

Macrophages are myeloid cells that can be found in almost all tissues, making important contributions to homeostasis and protection against injury. Projects in our laboratory focus on elucidating the transcriptional control of macrophages in different tissues, especially in the heart, adipose tissue and bone, with special emphasis on possible medical applications in the treatment of metabolic and cardiovascular diseases.

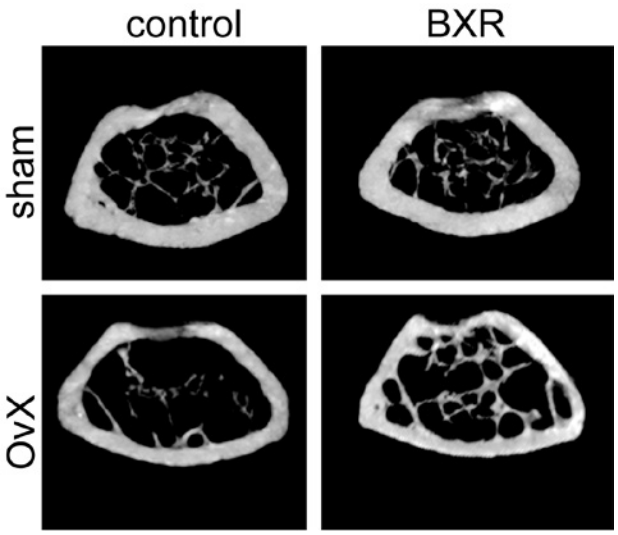
A special interest of our laboratory is the study of the transcriptional regulation of macrophage functions by nuclear hormone receptors, especially retinoid X receptors (RXRs). Our most recent studies have underscored the importance of RXR signaling in the development of macrophage-associated pathologies, such as insulin resistance and osteoporosis. We have discovered that RXRs are involved in adipose tissue and bone homeostasis. Macrophage RXR deficiency exacerbates inflammation and insulin resistance *in vivo*, and leads to increased bone mass due to the formation of non-resorbing osteoclasts. Our results demonstrate that RXRs control osteoclast progenitor proliferation and their differentiation into active osteoclasts through dual mechanisms that converge on the upregulation of the transcription factor MAFB. Since RXRs are druggable targets for human diseases, our basic findings open new perspectives on the use of RXR ligands as potential regulators of pathologies where metabolic and bone homeostasis is disrupted due to altered macrophage differentiation and function. We are now extending these studies to the role of RXR macrophages in tissue homeostasis and injury in the heart.



Tissue-resident macrophages. (A) Heart macrophages: Cross sectional image of C57BL/6 mouse heart left ventricle stained for nuclei (blue), CD68 (red), and CD31 (white). **(B) Adipose tissue macrophages**: H&E stained V-WAT sections from high-fat-diet-fed mice, showing crown-like structures formed by macrophages. **(C) Bone osteoclasts**: TRAP-positive osteoclasts *in vitro* differentiated from bone marrow cells.



RXR/MAFB signaling in osteoclastogenesis. RXR homodimers sustain *Mafb* transcription in osteoclast progenitors, which allows a proper proliferative response to M-CSF and activation of mature osteoclasts, leading to bone resorption.



The RXR ligand bexarotene protects against post-ovariectomy bone loss. Representative µCT scans of the distal femur in control and bexarotene (BXR)-treated female mice that underwent sham surgery or ovariectomy (OvX).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2012-31483)
- Fundación TV3 Marató
- European Union. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2013-ITN, "CardioNext" 608027)

Selected Publications

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

Röszer T, Menéndez-Gutiérrez MP, Cedenilla M, Ricote M. **Retinoid X receptors in macrophage biology.** *Trends Endocrin Met* (2013) 24:460-8



Molecular regulation of heart development and disease



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Masters Student:
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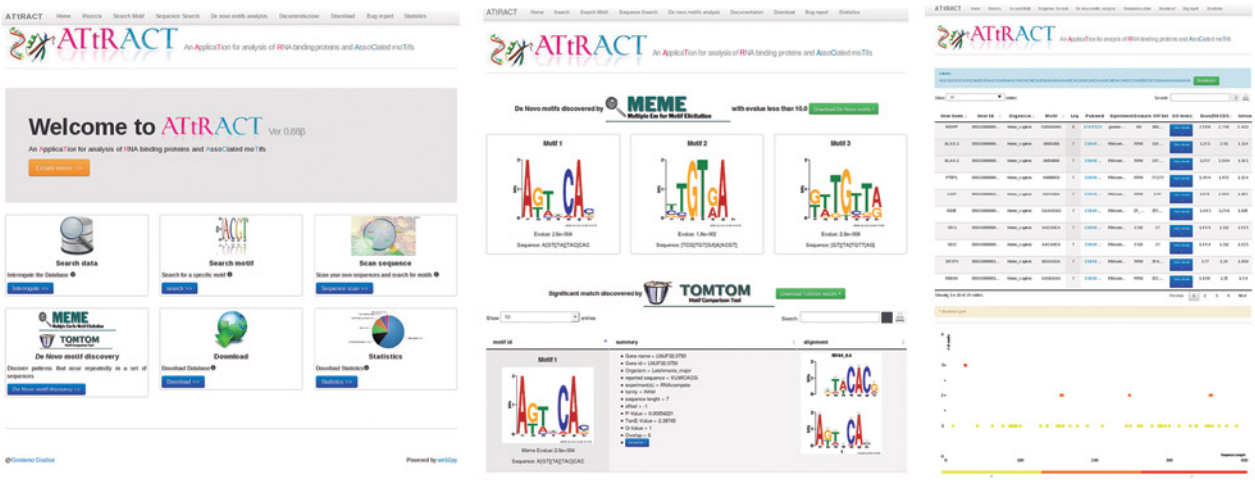
Visiting Scientist:
Pablo García Pavía
Raquel San José Martín-Albo

RESEARCH INTEREST

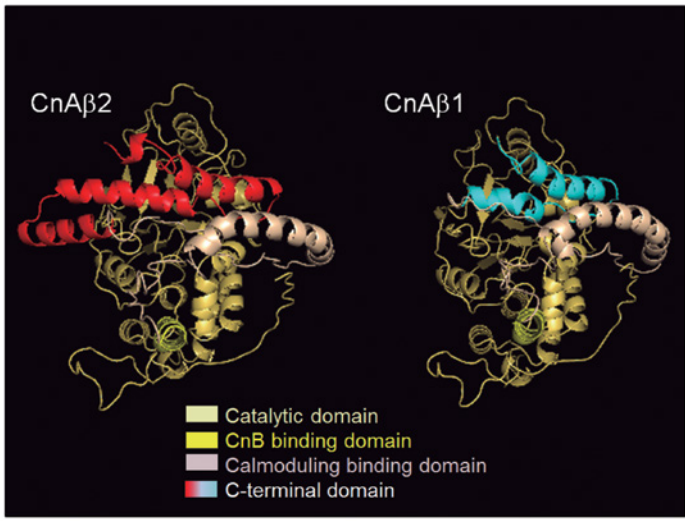
Heart failure is a major cause of death and hospitalization worldwide, especially among the elderly. Despite advances in medical management, there is no cure for heart failure and prognosis is still very poor. A major cause of heart failure is myocardial infarction, in which blockade of a coronary artery causes massive cardiomyocyte death due to lack of oxygen and nutrients. Due to the very limited regenerative capacity of the mammalian heart, dead cardiomyocytes are not substituted by new contractile cells, but by a collagen scar that prevents cardiac rupture. Although initially effective, this response progressively leads to dilatation of the left ventricle, remodeling of the heart, and, eventually, heart failure and death.

The molecular mechanisms underlying heart remodeling are poorly understood. In particular, the role of alternative splicing in this pathological response is almost unknown. Alternative splicing is the main mechanism driving protein diversity and is the main research interest in our lab. The study of alternative splicing at a global level requires the development of advanced bioinformatic pipelines. Our bioinformaticians have developed new analysis tools that considerably increase the precision with which we detect alternatively spliced genes in RNA-Seq experiments. We have also developed a new database of RNA binding proteins (ATtRACT), the largest to date. Using these tools, we have identified splicing factors that play regulatory roles in the infarcted heart. We are now analyzing the role of these factors through different gain- and loss-of-function approaches in mice.

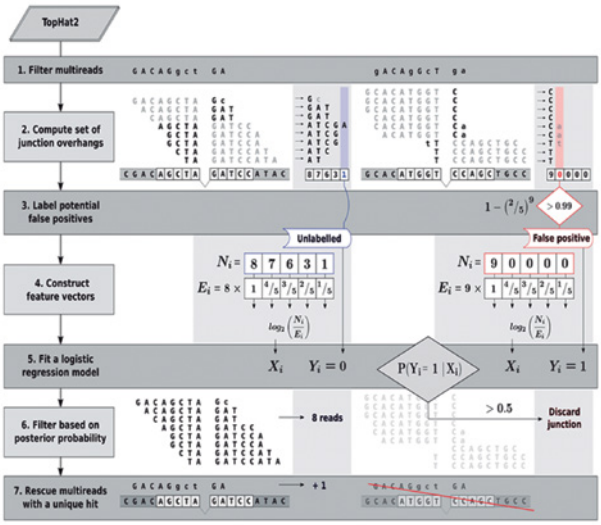
The calcineurin splicing variant CnAβ1 represents a good example of how alternative splicing can dramatically change the function of a protein. Due to retention of an intron, CnAβ1 has a unique C-terminal domain that has no similarity with any other known protein. This unique domain drives CnAβ1 to the Golgi apparatus and is necessary for the activation of the Akt signaling pathway. Unlike other calcineurin isoforms, which play a pathological role in the heart, CnAβ1 improves cardiac function after myocardial infarction by reducing infarct expansion. In embryonic stem cells, where CnAβ1 is highly expressed, it regulates differentiation towards the mesodermal lineage. We are now investigating its antifibrotic effects. As part of a new European Network (CardioNext), also involving other CNIC groups, we are exploring the therapeutic potential of CnAβ1 in a preclinical myocardial infarction model in the pig.



ATtRACT database. AtRACT is the largest database of RNA binding proteins and their associated nucleotide motifs. It incorporates search engines for RNA binding proteins and identification of motif enrichment in a series of sequences. *Left*, Home page of the ATtRACT database. *Center*, ATtRACT can run MEME and TOMTOM programs to search and identify new and known motifs. *Right*, Visualization of search results in ATtRACT.



Protein structure of CnAβ1 and CnAβ2. CnAβ1 and CnAβ2 are produced from the same gene. However, they have opposite functions due to their different C-terminal domains. The figure shows a 3D prediction of the CnAβ1 and CnAβ2 structures. CnAβ2 has 3 alpha helices that act as an autoinhibitory domain (red) by preventing exposure of the catalytic domain (light yellow) in the absence of intracellular calcium increases. CnAβ1 has two alpha helices (cyan) that act as an autoinhibitory domain and in addition promote localization of CnAβ1 in the Golgi apparatus and activation of the Akt signaling pathway.



FineSplice analysis pipeline. FineSplice is a program developed in our group to reduce the number of false-positive exon-exon junctions detected by RNA-Seq read aligners like TopHat. FineSplice increases precision when aligning a read and reduces the detection of false positive junctions from 10% to 1%. The figure and additional details can be found in Gatto et al., Nucl. Acids Res. 2014.

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)**
- **Ministerio de Ciencia e Innovación. FIS (CP08/00144)**

Selected Publications

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.

Lara-Pezzi E, Gómez-Salinero J, Gatto A, García-Pavía P. **The alternative heart: impact of alternative splicing in heart disease.** *J Cardiovasc Transl Res* (2013) 6: 945-55

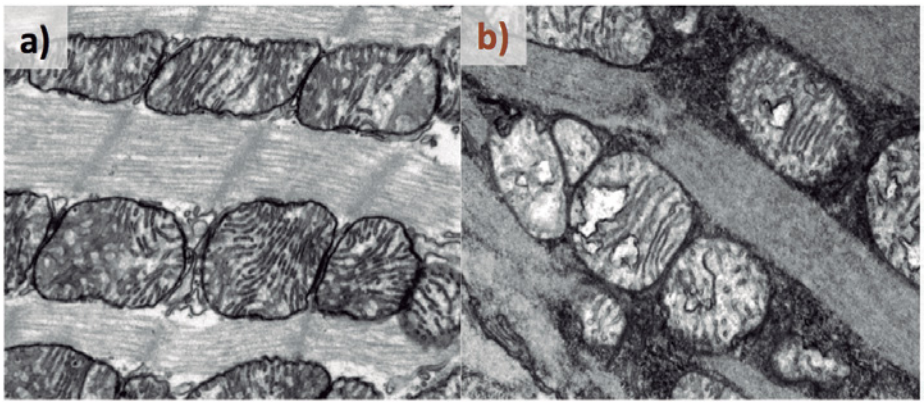


Functional genetics of the oxidative phosphorylation system

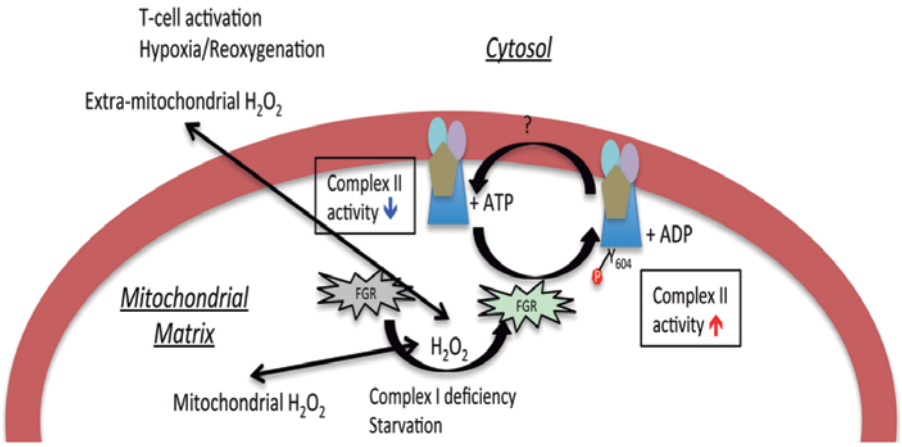
RESEARCH INTEREST

We study of the mammalian oxidative phosphorylation (OXPHOS) system, including its role in metabolic integration at cellular and organismal levels and its homeostatic response in health and disease. Part of our work is dedicated to cataloguing the set of nuclear encoded genes required for OXPHOS system homeostasis. We are also using high-throughput proteomics to define the mitochondrial protein interactome and posttranslational modifications in healthy, heart-stressed and metabolically altered animals.

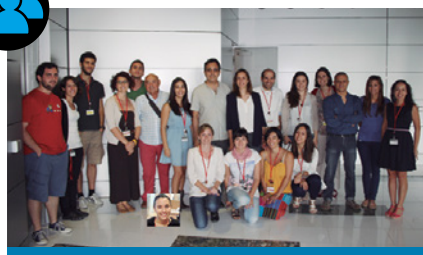
A major program in the lab is directed at defining the role of genetic variability in mitochondrial function and in lifelong physiology, longevity, predisposition to disease, and the response to clinically relevant drugs. For this program we use conplastic mice—animals with the same nuclear genome but different non-pathological mtDNA haplotypes—as well as a variety of genetically defined healthy animals. Using these models, we are investigating phenotypic variations in angiogenesis regulation and vessel morphology and function (Fig1); deciphering adaptation of mitochondrial fuel use to suit the cell type or functional status; analyzing ROS-dependent activation of the Fgr pathway (Fig2); and defining supercomplex reorganization in the respiratory chain (Fig3). We are also developing noninvasive *in vivo* imaging methodologies to evaluate mitochondrial function upon heart disfunction. The long-term aim is to define new targets for the diagnosis or treatment of cardiovascular diseases.



Firure 1. Cardiac mitochondria. Transmission elecron micrographs of C57BL6 mouse ventricular myocytes (x 40000). a) Intermyofibrillar mitochondria—longitudinal rows of mitochondria located within myofibrils. b) Cardiac muscle damaged by 27 days of administration with isoproterenol; vacuolated and disrupted intermyofibrillar mitochondria are evident.



Firure 2. ROS dependent activation of mito-Fgr pathway
Acin-Perez et al., 2014, Cell Metab



Head of Laboratory
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Rebeca Acín Pérez

Support Scientist:
Mª Concepción Jiménez Gómez

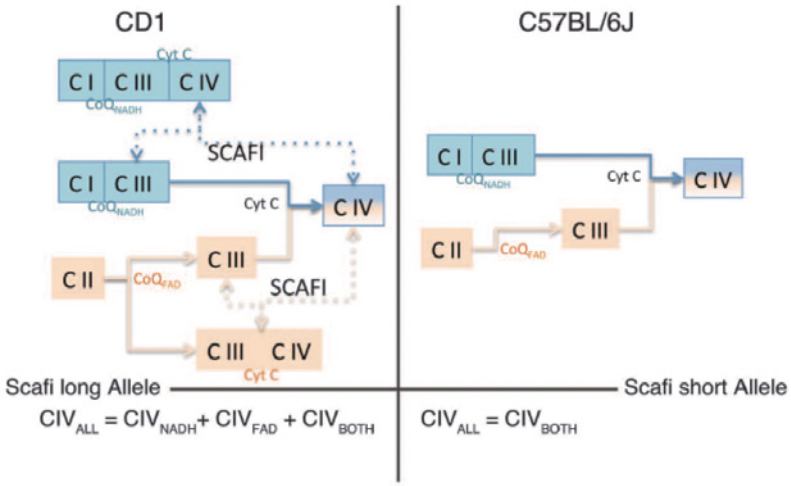
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Sarah Dadon
Romuald Loutre
Anne-Marie Heckel
Annette Hofer
Marta Gómez de Cedrón Cardeñiosa
Manar Aour



Firure 3. Plasticity model of the mitochondrial electron transport chain
Lapuente et al., 2013, Science

MAJOR GRANTS

- **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**

Selected Publications

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

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

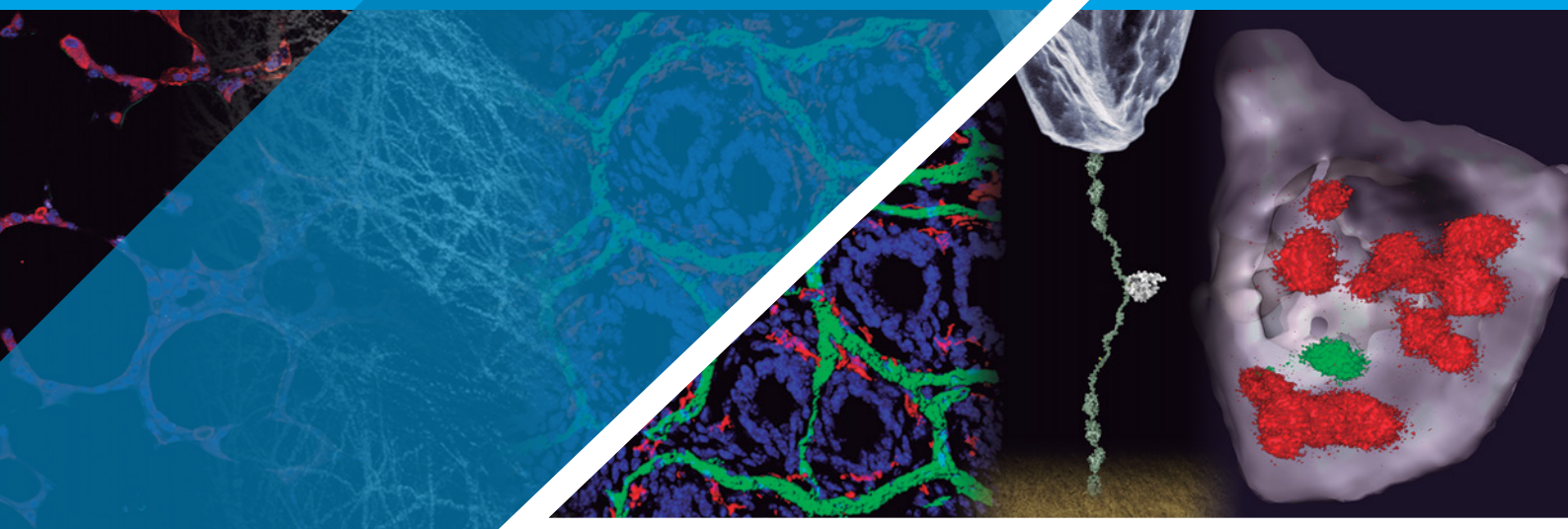
Acín-Pérez R, Enríquez JA. **The function of the respiratory supercomplexes: the plasticity model**. *Biochim Biophys Acta* (2014) 1837:444-50

Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, Perales-Clemente E, Salviati L, Fernandez-Silva P, Enríquez JA*, Scorrano L*. **Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency**. *Cell* (2013) 155:160-71.
*JAE and LS co-corresponding authors

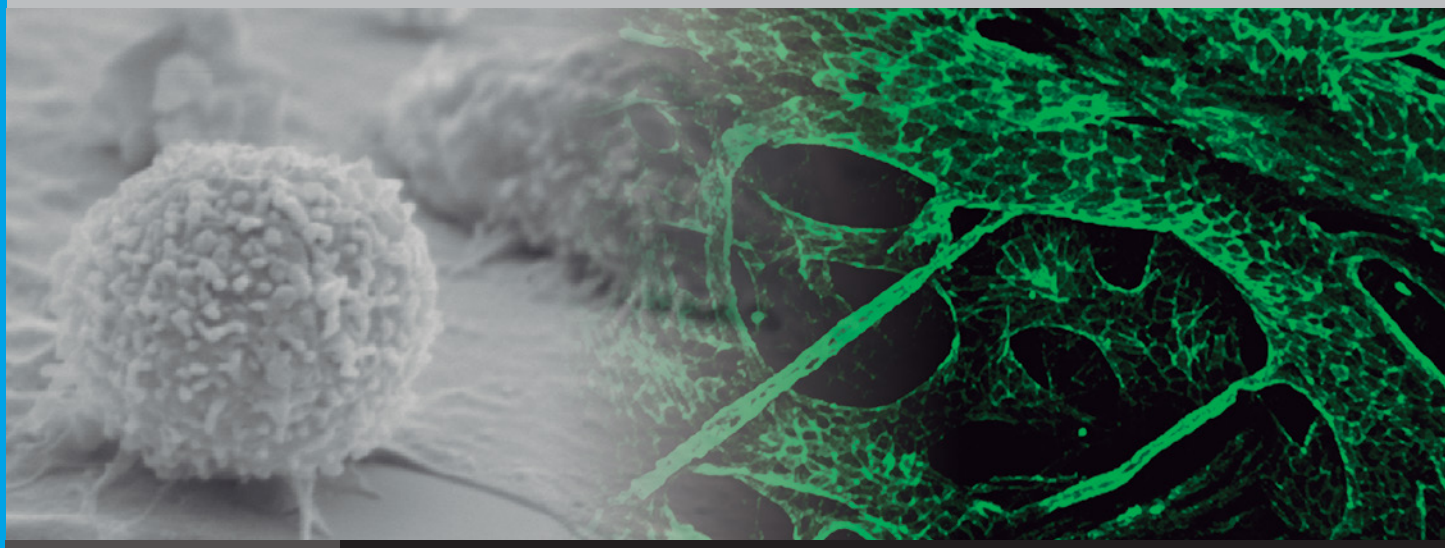
Lapuente-Brun E, Moreno-Loshuertos R, Acín-Pérez R, Latorre-Pellicer A, Colás C, Balsa E, Perales-Clemente E, Quirós PM, Calvo E, Rodríguez-Hernández MA, Navas P, Cruz R, Carracedo Á, López-Otín C, Pérez-Martos A, Fernández-Silva P, Fernández-Vizarra E, Enríquez JA. **Supercomplex assembly determines electron flux in the mitochondrial electron transport chain.** *Science* (2013) 40:1567-70

RESEARCH DEPARTMENTS

Vascular Biology and Inflammation



RESEARCH DEPARTMENTS



2. Vascular Biology and Inflammation

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Administrative Support:
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Eduardo Bieger

Research in the **Department of Vascular Biology and Inflammation** (DVBI) is focused on the interactions between cells of the vascular system. The groups in the department work along the following strategic research lines: 1) Vascular Wall Remodeling; 2) Inflammation and Autoimmunity; and 3) Cell Biology and Signaling in Metabolism and Disease. DVBI research groups use a wide variety of techniques, including animal, tissue, cellular and molecular models, to investigate normal vascular function and the key steps in the vascular alterations that underlie cardiovascular diseases. Cardiovascular proteomics is also a major interest.



Gene regulation in cardiovascular remodelling and inflammation



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Beatriz Carolina Ornés
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Visiting Scientist:
Ángel Luis Armesilla

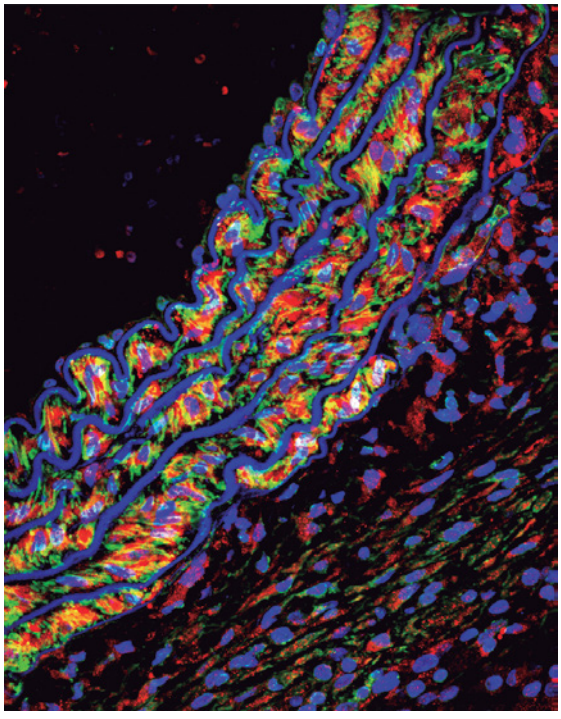
RESEARCH INTEREST

Many important biological processes, including the regulation and development of the immune and cardiovascular systems, are regulated by the calcineurin (CN)/NFAT pathway. Much of our previous work relates to molecular interactions of CN with substrates. We are now studying the regulation and function of this pathway in inflammation, cardiovascular and inflammatory diseases.

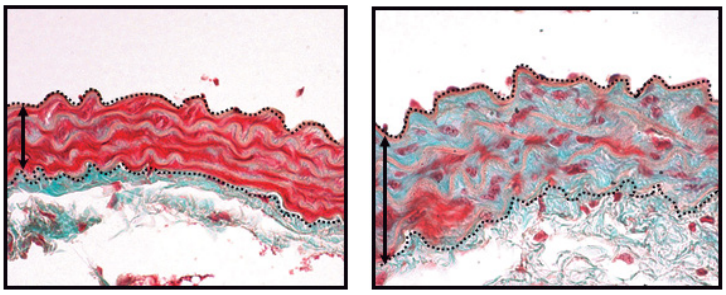
We are also analyzing gene expression triggered by angiotensin II (AngII) in cardiomyocytes and vascular smooth muscle (VSM). This work is aimed at identifying molecular mediators of cardiac hypertrophy. We have found several genes regulated by CN in two mouse models of cardiac hypertrophy and plan to characterize their roles in this pathology.

Through in vivo infection with lentiviral vectors encoding motifs important for CN-NFAT interactions, we can prevent or retard the development of arthritis in mice. In our system, inflammation is curtailed by the infection in vivo of macrophages at distinct locations and the subsequent migration of these cells to inflammation sites.

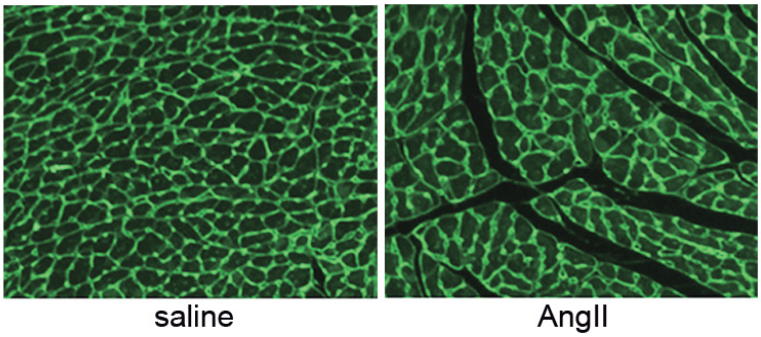
We are dissecting signaling pathways involved in vascular wall remodeling, a major feature of vascular diseases such as atherosclerosis, aneurysm and restenosis. We have set up animal models of these pathologies, and have generated mice deficient for AngII-target molecules that are regulated by CN. Some of these animals are totally resistant to these diseases and we are working to elucidate the molecular and cellular mechanisms underlying this protection.



Merged confocal microscopy immunostaining images of Rcan1 (red) and SMA (green) together with nuclear staining (blue) in abdominal aorta cross-sections from AngII-treated Apoe-/- mice. Images are maximal projections of a complete z-series.



Histological analysis of aortas before (left) and after (right) treating mice with pathological stimuli. Masson's Trichrome staining shows fibrosis in blue, cellular cytoplasm in red and nuclei in purple.



Cross-sectional images of myocardium from mice treated with saline (left) and AngII (right) stained with wheat germ agglutinin-FITC, which stains cardiomyocyte cell membranes.

MAJOR GRANTS

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

Selected Publications

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

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Méndez-Barbero N, Esteban V, Villahoz S, Escolano A, Urso K, Alfranca A, Rodríguez C, Sánchez SA, Osawa T, Andrés V, Martínez-González J, Minami T, Redondo JM* Campanero MR*. **A major role for RCAN1 in atherosclerosis progression**. *EMBO Mol Med* (2013) 5:1901-17
**Co-corresponding authors*

Garaulet G, Alfranca A, Torrente M, Escolano A, López-Fontal R, Hortelano S, Redondo JM, Rodríguez A. **IL10 released by a new inflammation-regulated lentiviral system efficiently attenuates zymosan-induced arthritis**. *Mol Ther* (2013) 21:119-30



CNIC-UAM COLLABORATIVE PROGRAM
Intercellular communication in the inflammatory response



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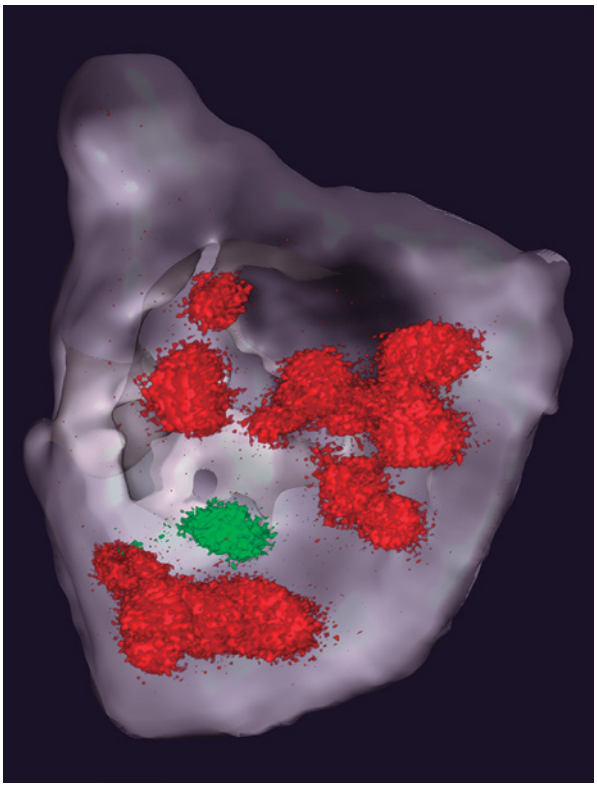
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RESEARCH INTEREST

Lymphocytes exert their functions by communicating with other cells. They form specific immune synapses with antigen-presenting cells (APCs) and also secrete soluble mediators. During synapsis, exosomes—nanovesicles produced by multivesicular bodies (MVBs)—act as "shuttles" that transfer specific mediators, including microRNAs (miRNA), to the target cell. Formation of the immune synapse is triggered by T cell receptor (TCR) stimulation. Its downstream signaling pathways engage actin and tubulin-based cytoskeletal networks that control the spatial and molecular architecture of the IS. One of these elements is the centrosome or MTOC (microtubule-organizing center). The MTOC governs directed secretion toward the APC by juxtaposing the Golgi and endosomal compartments at the T:APC contact zone. MVBs form part of this assembly for intracellular transport and sorting. Our group aims to define the connection between TCR activation and the reorganization of the tubulin cytoskeleton and organelle transport, the delivery of specific mediators to the APC and other target cells, and the functional consequences of this mode of cell-cell communication in the regulation of the immune inflammatory response.

The group pursues three main lines of investigation. 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 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 different 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



Miro-1 controls polarity of mitochondria and the microtubule organizing center during lymphocyte chemotaxis

MAJOR GRANTS

- **European Commission. ERC Advanced Investigators Grant (ERC-2011-AdG 20110310) (GENTRIS)**
- **Ministerio de Economía y Competitividad (SAF2011-25834)**
- **Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)**
- **Redes de Excelencia de la Comunidad de Madrid (P2010/BMD-2332)**
- **Ministerio de Economía y Competitividad. FIS (PI11/00939) PI: Gloria Martínez del Hoyo**

Selected Publications

Rocha-Perugini V, Gordon-Alonso M, Sánchez-Madrid F. **PIP2: choreographer of actin-adaptor proteins in the HIV-1 dance.** *Trends Microbiol* (2014) 22:379-88

de la Fuente H, Cruz-Adalia A, Martinez Del Hoyo G, Cibrián-Vera D, Bonay P, Pérez-Hernández D, Vázquez J, Navarro P, Gutierrez-Gallego R, Ramirez-Huesca M, Martín P, Sánchez-Madrid F. **The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1.** *Mol Cell Biol* (2014) 34:2479-87

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

Martín-Cófreces NB, Baixauli F, Sánchez-Madrid F. **Immune synapse: conductor of orchestrated organelle movement.** *Trends Cell Biol* (2014) 24:61-72

Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofreces N, Martinez-Herrera DJ, Pascual-Montano A, Mittelbrunn M, Sánchez-Madrid F. **Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs.** *Nat Commun* (2013) 4:2980



Integrin signaling



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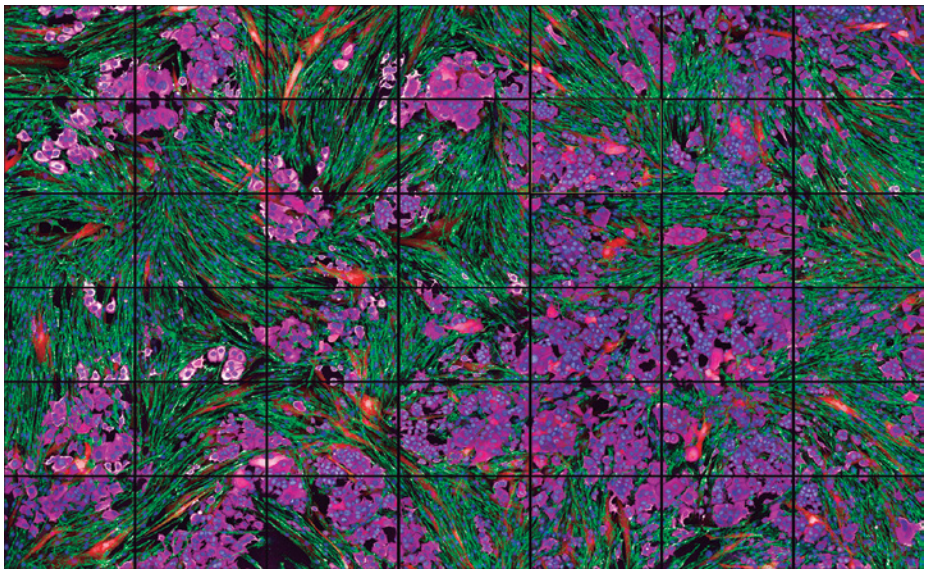
RESEARCH INTEREST

Caveolae are actin-linked plasma membrane nano-invaginations, abundant in mechanically stressed tissues. They are involved in signaling, viral entry, membrane trafficking and lipid metabolism; however, controversy surrounds these actions, and the precise functions of caveolae and their main proteins caveolins (Cav 1-3) and cavins (1-4) remain unresolved. Cav-deficient mice show tissue abnormalities, and caveolar disorders are associated with lipodystrophy and muscular dystrophy, cardiovascular disease and cancer, suggesting a role as homeostatic regulators. Preliminary evidence suggests that caveolae sense mechanical cues. We have shown 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, promoting local invasion and subsequently distant metastasis. By performing image-and-RNAi-based high content screening in co-cultures of tumor cells and CAFs (Figure 1), we aim to identify neworks of stromal genes involved in biomechanical matrix stiffening.

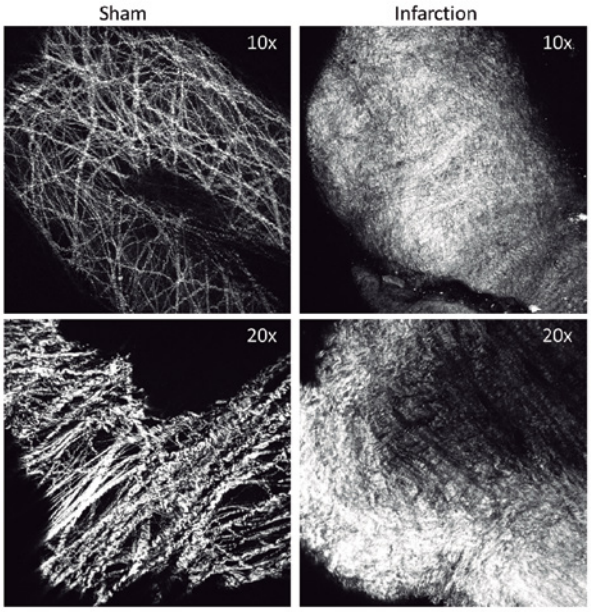
Our works shows that stromal-Cav1 drives not only pathological remodeling of the tumor microenvironment, but also physiological remodeling, for example in the mammary gland and the skin. We are now addressing the role of Cav1 and the phosphorylation of its Tyr14 in cardiac remodeling after acute myocardial infarction using the LAD (left anterior descending) artery permanent ligation model (Figure 2).

Our recent work also establishes Cav1, through the suppression of a MEK-ERK1/2-Snail1 pathway, as a major checkpoint in the transition from an epithelial to a mesenchymal identity in the peritoneum. The efficacy of a MEK pharmacological inhibitor in counteracting the EMT/fibrosis developed in Cav1$^{-/-}$ mice during peritoneal dialysis (PD) warrants further translational studies in other chronic inflammatory diseases.

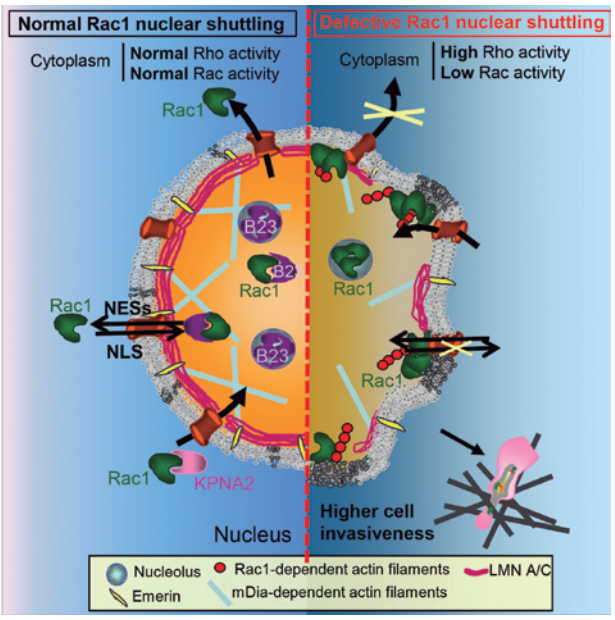
We have previously established links between caveolae-dependent membrane trafficking and directional cell migration and invasion through Rho family GTPases, including RhoA and Rac1. Rac1 has been detected in the nucleus, but the function of nuclear Rac1 remains elusive. We now provide insight into the 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 (Figure 3).



Coculture of breast cancer cells and cancer-associated fibroblasts (CAFs). Cav1 (green) is silenced upon lentiviral infection (marked by CherryFP, in red). Silencing and CherryFP expression are restricted to the fibroblast cell line (1069sk) via the integrin alpha-11 promoter. Breast cancer cells (MCF7 cell line) are labeled with a pan-cytokeratin antibody (magenta).



Collagen fiber remodeling after myocardial infarction imaged by second harmonic generation (SHG). Native (non-fixed) hearts from wild-type mice were imaged for SHG. Whereas collagen fibers are highly ordered under basal conditions (left), after permanent ligation of the LAD strong, disordered collagen deposition is observed (right).



Scheme showing that Rac1 nucleocytoplasmic shuttling drives nuclear shape changes. Dysregulation of this mechanism leads to Rac1 nuclear accumulation, deformation, and cell invasion through narrow spaces.

MAJOR GRANTS

- European Commission. Marie Curie Actions Initial Training Network (ITN) (Horizon 2020, "BIOPOL")
- European Union. Marie Curie Actions Intra-European Fellowships (FP7-PEOPLE-2013-IEF)
- -WorldWide Cancer Research (UK) (formerly known as AICR) (AICR 15 – 0404)
- Ministerio de Economía y Competitividad (SAF2011-25047)
- Ministerio de Economía y Competitividad. Consolider COAT (CSD2009-00016)
- Fundació La Marató TV3 (674/C/2013)

Selected Publications

Navarro-Lérida I, Pellinen T, Sanchez 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* (accepted: doi:10.1016/j.devcel.2014.12.019)

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* (2014) 7:102-23

Rey-Barroso J, Alvarez-Barrientos A, Rico-Leo E, Contador-Troca M, Carvajal-Gonzalez JM, Echarri A, Del Pozo MA, Fernandez-Salguero PM. **The dioxin receptor modulates caveolin-1 mobilization during directional migration: role of cholesterol.** *Cell Commun Signal* (2014) 12:57

Moreno-Càceres J, Caja L, Mainez J, Mayoral R, Martín-Sanz P, Moreno-Vicente R, Del Pozo MÁ, Dooley S, Egea G, Fabregat I. **Caveolin-1 is required for TGF-β-induced transactivation of the EGF receptor pathway in hepatocytes through the activation of the metalloprotease TACE/ADAM17.** *Cell Death Dis.* 5: e1326

Parton RG, Del Pozo MA. **Caveolae as plasma membrane sensors, protectors and organizers.** *Nat Rev Mol Cell Biol* (2013) 14: 98-112



Cardiovascular proteomics



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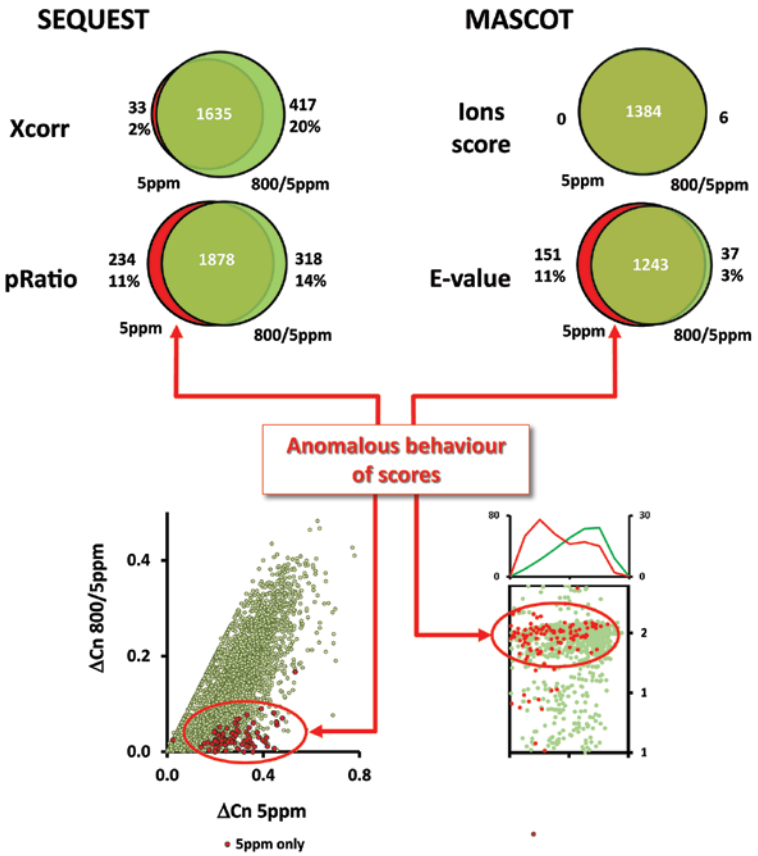
Masters Student:
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Technician:
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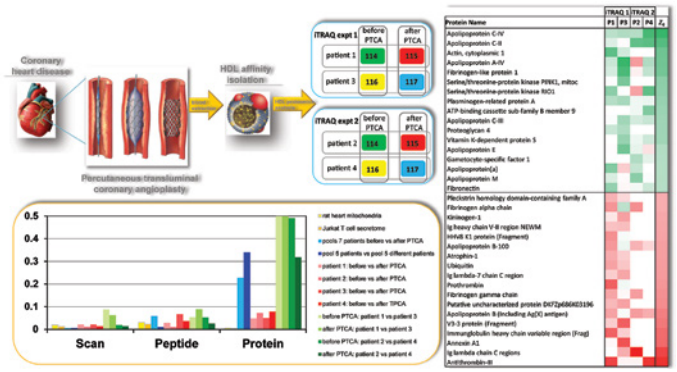
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RESEARCH INTEREST

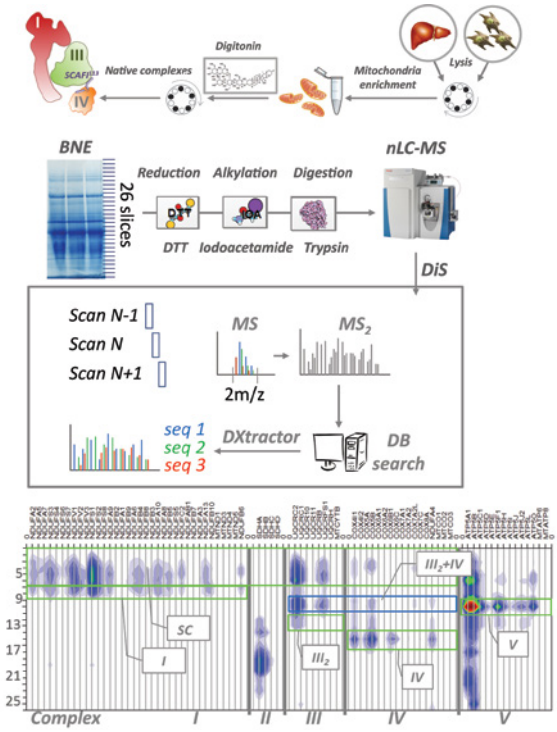
Our group works on the development of high-throughput quantitative approaches for the dynamic analysis of the deep proteome and their application to cardiovascular projects. We have recently developed a generic integration algorithm (SanXoT) that serves as the basis for systems biology analysis of high-throughput quantitative proteomics experiments. By using a novel redox proteomics technology (GELSILOX) recently developed in our laboratory, we have also demonstrated that ischemia-reperfusion increases oxidation of Cys sites in mitochondrial proteins and that this effect is inhibited by ischemic or pharmacological preconditioning. These studies are being extended to models of aging, hypertrophy and animal models of deletion or overexpression of several protein factors. 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 post-translationally-modified species (PTM). 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 PTMs that may act as molecular determinants of assembly. We have also used advanced interactomics to study the supramolecular structure of human T-cell-derived exosomes. We have found evidence that the network of intramolecular interactions with tetraspanins may act as a sorting machinery that determines the protein composition of exosomes. In an extension of these studies, we have contributed to the discovery of a novel molecular mechanism by which miRNAs are selectively transported from cell to cell within exosomes.



Our peptide identification workflow uses postscoring filtering schemes to take full advantage of the high mass resolution provided by modern orbitraps, avoiding identification artifacts due to anomalous behavior of scores in conventional approaches.



Our layered statistical model reveals that HDL proteome composition is highly variable among different individuals, but remains stable in the same individual, allowing the systematic detection of protein alterations resulting from atheroma plaque rupture.



Scheme of the Blue-DiS technology for the systematic analysis of the protein composition of mitochondrial electron transport chain complexes and supercomplexes.

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)**

Selected Publications

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

Jorge I, Burillo E, Mesa R, Baila-Rueda L, Moreno M, Trevisan-Herraz M, Silla-Castro JC, Camafeita E, Ortega-Muñoz M, Bonzon-Kulichenko E, Calvo I, Cenarro A, Civeira F, Vázquez J. The human HDL proteome displays high inter-individual variability and is altered dynamically in response to angioplasty-induced atheroma plaque rupture. *J Proteomics* (2014) 106:61-73

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Perez-Hernandez D, Gutiérrez-Vázquez C, Jorge I, López-Martín S, Ursa A, Sánchez-Madrid F, Vázquez J*, Yáñez-Mó M*. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. *J Biol Chem* (2013) 288:11649-61
*Co-corresponding authors



Matrix metalloproteinases in angiogenesis and inflammation

RESEARCH INTEREST

In adults, angiogenesis, the formation of new vessels from pre-existing ones, is often coupled to inflammation, and its deregulation can result in failed tissue repair after acute injury or in the progression of chronic inflammatory disorders. One of the first steps in the transition from a quiescent to an angiogenic vasculature is the remodeling of the basement membrane and the perivascular extracellular matrix, a process involving the action of matrix metalloproteinases (MMPs). We have lately identified i) a combinatorial proteolytic program driven by the protease MT1-MMP in TNFα-activated endothelial tip cells; ii) a molecular complex formed by EMMPRIN (an MT1-MMP substrate) that provides local ATP for actomyosin contractility and endothelial cell junction stability; and iii) a requirement for MT4-MMP in aortic vessel wall development and function.

Our interests include the initiation events of angiogenesis in inflammation, the therapeutic potential of MT1-MMP and MT4-MMP in animal models of inflammatory pathologies, the visualization of the dynamics and cell-cell interactions during inflammation-induced angiogenesis, and the intimate nature of macrophage/endothelial cell crosstalk in inflammatory angiogenesis. Our studies in these areas are conducted with 2D and 3D angiogenic models, genetically modified mouse lines, and animal models of inflammatory disease. Cutting edge technologies employed in the lab include high-resolution and super-resolution confocal microscopy, 3D reconstruction and image analysis, bioinformatics and biomathematics, and novel lentivirus-based gene therapy strategies. These approaches are being applied to gain greater understanding of how angiogenesis occurs in the inflammatory context and to identify new molecules or cell subsets whose modulation might impact the angiogenic process.

In this way we aim to contribute to the goals of improving physiological wound healing and tissue repair in diseases such as myocardial infarction and ameliorating the symptoms and progression of chronic disorders, such as inflammatory bowel disease, that involve alterations to vascular function.



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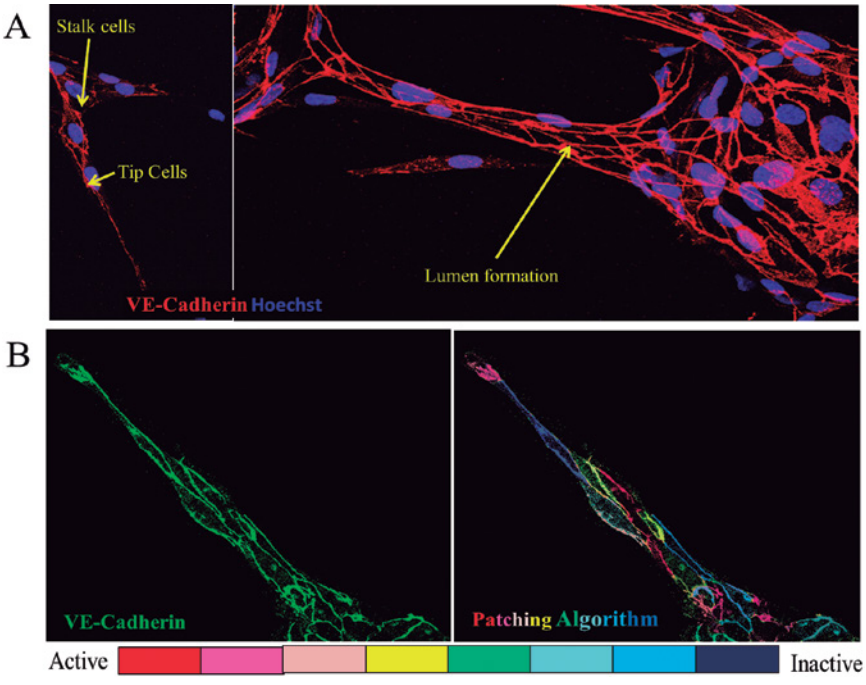
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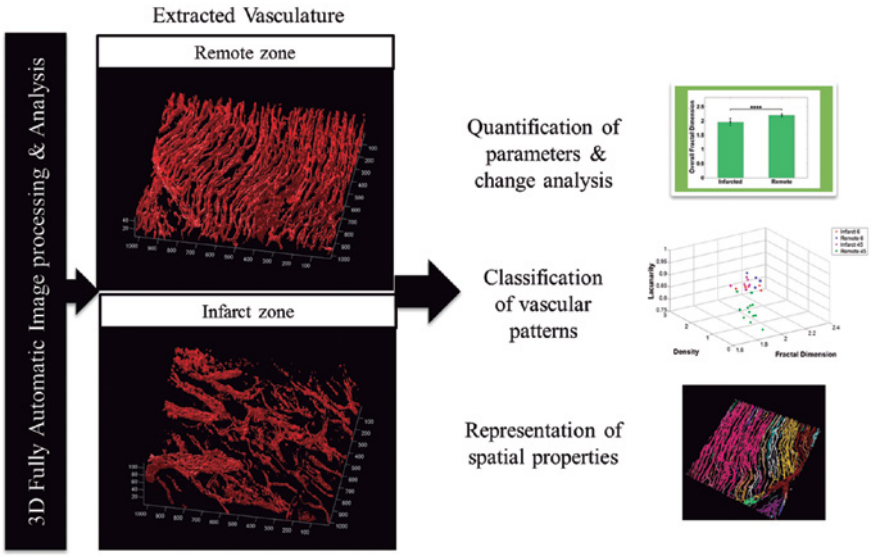
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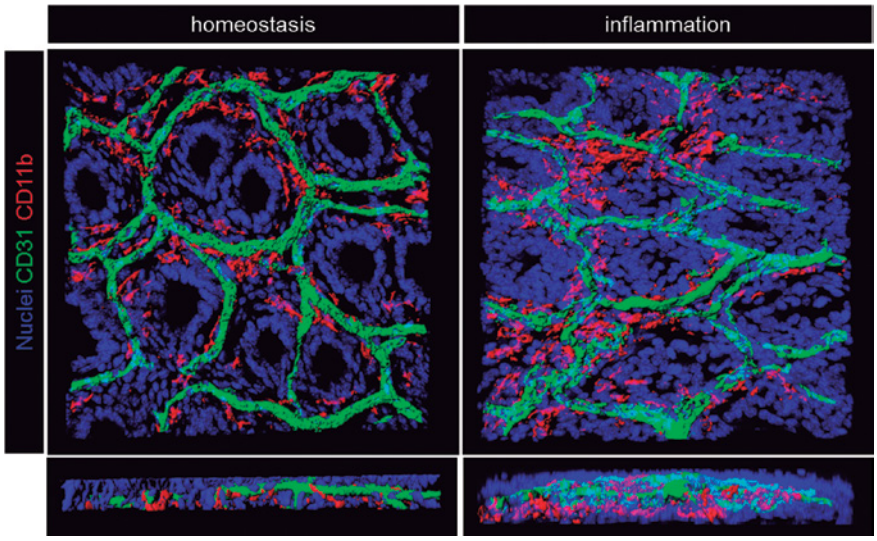
Masters Student
Álvaro Sahún



Analysis of endothelial cell junction dynamics during capillary formation. (A) Staining of endothelial cells (VE-cadherin, red) coating a Cytodex bead and embedded in a fibrin gel allows investigation of endothelial junctions during the formation (left) and maturation (right) of 3D capillaries in response to angiogenic factors. (B) The 'Patching Algorithm' (Bentley et al., Nat Cell Biol 16, 309, 2014) is a new image analysis tool that allows the classification of single endothelial cell junctions (VE-cadherin, green; left) depending on their stage of remodeling (active to inactive; right).



Ex vivo image analysis of the vasculture in the infarcted heart. Confocal microscopy imaging of thick sections from the pig heart are used for 3D image analysis with MatLab to extract parameters of the whole vasculature (geometrical, fractal) and single vessels (diameter, length) in ischemic versus non-ischemic tissue. This analysis allows the classification and visualization of vascular responses to ischemia.



Imaging vascular responses and cellular crosstalk in inflammation-driven angiogenesis. Confocal microscopy imaging of whole-mount intestine and 3D reconstruction with Imaris software show the changes in organization and structure of the mucosal vascular plexus (green) in a mouse model of chemical-induced colitis (inflammation: 7 days of treatment with 1% DSS) compared with control (homeostasis). Note also the altered association of vessels (green) with monocytes/macrophages (red) in the inflamed tissue. Nuclei in blue; lower panels show orthogonal views.

MAJOR GRANTS

- **Ministerio de Economía y Competitividad (SAF2011-25619)**
- **FIS RETICS (Red de Investigación Cardiovascular: RD12/0042/0023)**
- **Comunidad Autónoma de Madrid. ANGIOBODIES 2.0 (S2010/BMD-2312)**
- **Fundació La Marató TV3 (165/C/2012)**
- **European Union (PITN-GA-2013-608027) (CardioNext) (Coordinator)**

Selected Publications

Moreno V, Gonzalo P, Gómez-Escudero J, Pollán Á, Acín-Pérez R, Breckenridge M, Yáñez-Mó M, Barreiro O, Orsenigo F, Kadomatsu K, Chen CS, Enríquez JA, Dejana E, Sánchez-Madrid F, Arroyo AG. **An EMMPRIN-γ-catenin-Nm23 complex drives ATP production and actomyosin contractility at endothelial junctions**. *J Cell Sci* (2014) 127:3768-81

Udi Y, Grossman M, Solomonov I, Dym O, Rozenberg H, Moreno V, Cuniasse P, Dive V, Arroyo AG, Sagi I. **Inhibition mechanism of membrane metalloprotease by an exosite-swiveling conformational antibody**. *Structure*. 2015 Jan 6;23(I): 104-15



B lymphocyte biology

RESEARCH INTEREST

B lymphocytes protect the organism against infection by producing highly specific antibodies. Misregulation of B lymphocytes and antibody generation can lead to health conditions such as immune deficiencies, autoimmunity and cancer. Our lab focuses mostly on the biology of B cells in germinal centers, the site of secondary antibody diversification. Somatic remodeling of antibody genes in germinal centers involves two key molecular events, somatic hypermutation and class switch recombination. Both of these events are initiated by the enzyme activation-induced deaminase (AID). However, when AID targets non-antibody genes its activity can damage DNA, causing mutations and chromosome translocations.

We are interested in understanding the regulation of germinal center events, above all the function of AID and the role of microRNAs in this environment. Our work over the past few years has highlighted the importance of microRNAs in autoimmunity and the link between germinal centers and cancer and AID regulation (J Exp Med 2008, Immunity 2010, Curr Opin Immunol 2011, Immunol Rev 2013, Blood 2014). In addition, we have deciphered some other molecular events that regulate AID activity (PloS One 2008, J Exp Med 2012). We are currently exploring the molecular regulation of germinal center B cells in health and disease.



Head of Laboratory
Almudena R Ramiro

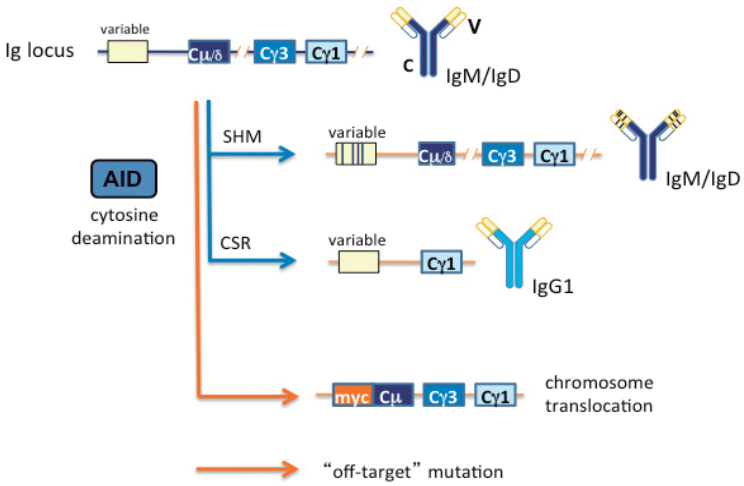
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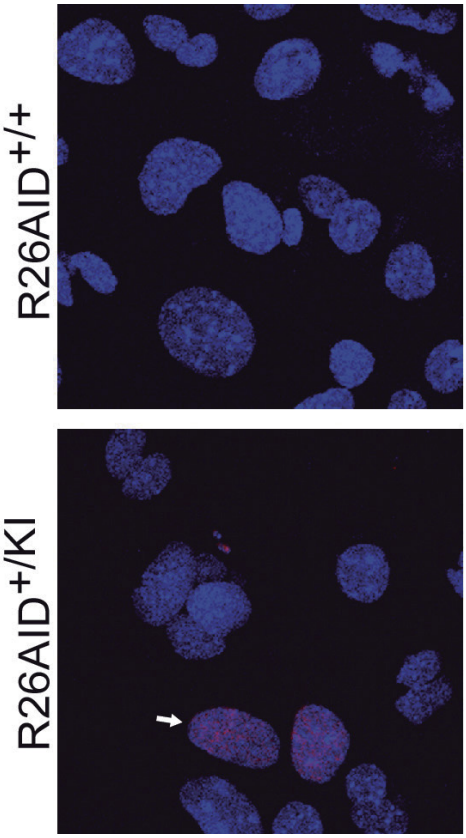
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Ester Marina

Technician:
Sonia Mur



AID activity promotes antibody diversification and oncogenic lesions. The antibody genes of B cells in germinal centers undergo somatic diversification through somatic hypermutation (SHM) and class switch recombination (CSR), thus allowing the generation of high-affinity and effector-versatile antibodies. SHM and CSR are initiated by activationInduced deaminase, an enzyme that deaminates cytosines on the DNA of antibody genes. However, this same activity can target other genes, promoting mutations and chromosome translocations with lymphomagenic potential.



AID promotes genotoxic damage in pancreas. We have generated a mouse model to express AID in pancreas and address its oncogenic potential in a non-B-cell context. We introduced an AID-GFP-encoding cassette preceded by a transcriptional stop flanked by two loxP sites into the endogenous Rosa26 locus and crossed these mice with p48-$Cre^{Ki/+}$ mice to promote specific expression of AID in pancreas. Pancreatic explants from AID+ ($R26AID^{Ki/+}$) and control ($R26AID^{+/+}$) mice were stained with anti γ-H2AX antibody to detect double-strand breaks.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2013-42767-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-BCLYM 2007)

Selected Publications

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.

de Yébenes VG, Bartolomé-Izquierdo N, Ramiro AR. **Regulation of B-cell development and function by microRNAs**. *Immunol Rev* (2013) 253:25-39



Immunobiology of inflammation



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Sarai Martínez Cano

RESEARCH INTEREST

We use innovative approaches to study the immune and inflammatory responses to infection and tissue damage. The general research question addressed in the lab is how myeloid cells initiate and modulate immunity and inflammation by sensing damaged-self and non-self. We believe that this research has the potential to lead to the development of new vaccines and immunotherapy strategies. We are interested in how damaged-self and non-self is detected by dendritic cells (DCs) and macrophages, how this sensing modulates the function of myeloid cells, and what relevance this interaction this has in vivo in models of infection, inflammation and cancer (Fig. 1).

Infection is frequently associated with tissue damage, but knowledge is limited about how the immune and inflammatory response to infection are affected by concomitant sensing of cell death by myeloid cells. Some myeloid C-type lectin receptors (CLRs) have been identified as receptors for necrotic cells that couple to Syk signaling, potentially triggering innate and adaptive immune responses. DNGR-1 (CLEC9A), expressed on DCs, and Mincle (CLEC4E), expressed broadly in myeloid cells, detect ligands exposed upon necrosis and potentially modulate signals from other pattern-recognition receptors during infection.

We preferentially focus on the following specific areas of research (Fig. 1):

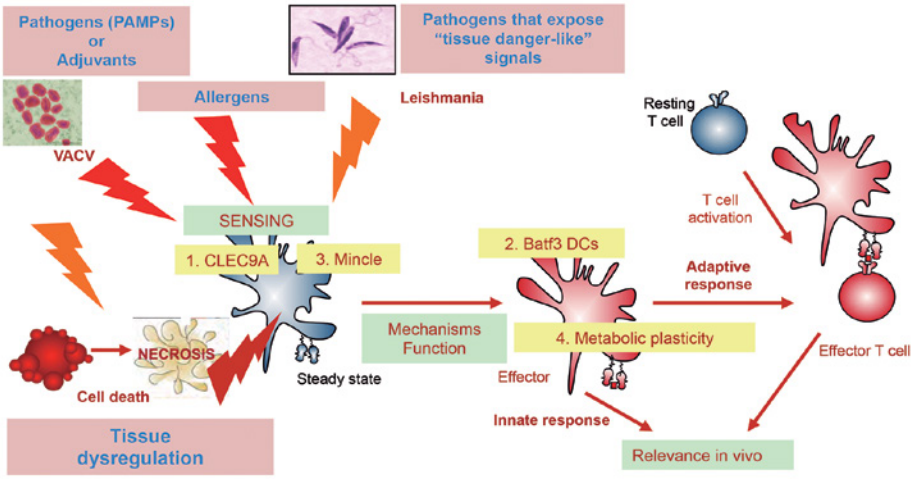
1. CLEC9A as a model CLR that detects tissue damage and modulates immunity.

2. The specialized functions of Batf3-dependent DCs.

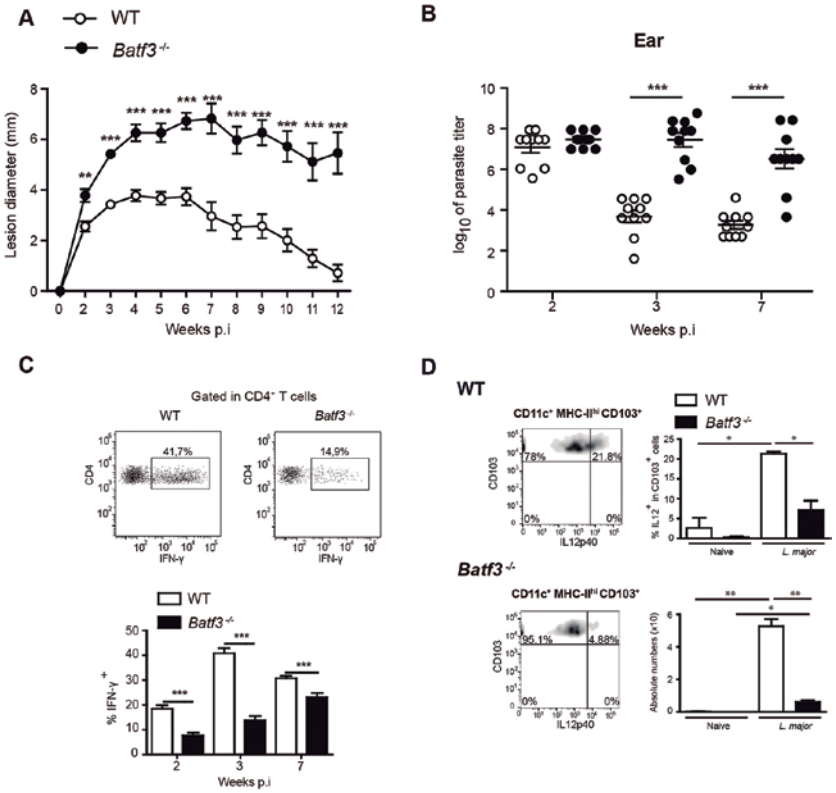
3. Mincle as a model CLR that senses ligands both in damaged-self and in non-self.

4. New avenues of research into additional functional effects of sensing by myeloid cells: we are interested in the effects of sensing non-self and damaged-self on the metabolism of myeloid cells, and particularly the role of oxidative phosphorylation activity in myeloid cells in inflammation and immunity.

Our recent results reveal that Batf3-dependent DCs are crucial for generation of Th1 immunity through the production of IL-12 (Fig. 2 and Martínez-López et al. 2014). This has been dissected in the gold standard model of infection by the eukaryote parasite parasite Leishmania major, which induces tissue damage and also mimics many tissue-derived danger signals. Using Batf3-/- mice, we are currently analyzing the role of this DC subset in models of atherosclerosis, asthma and cancer.



Current areas of research in the Immunobiology of Inflammation laboratory. Our research focuses on how infection and tissue damage interact to generate signals that can impact myeloid cell metabolism. Sensing infection and cell death can influence the development of inflammation and immunity in models of disease that we are analyzing to explore the translational potential of our findings.



Batf3-dependent DCs are crucial for generation of Th1 immunity against *Leishmania major*. (A, B) Batf3-deficient mice develop an exacerbated *L.major* cutaneous pathology (A) Lesion diameter (measured with a digital calliper) in WT and *Batf3*$^{-/-}$ mice was tracked for 12 weeks after intradermal infection in the ear pinnae with *L. major*. (B) Parasite load in the ears of WT and Batf3$^{-/-}$ mice at different times after infection with *L. major* (each circle represents one sample). (C) Batf3 deficiency impairs local Th1 immunity to *L. major*. Ear cells were obtained 2, 3 and 7 weeks after infection, restimulated with anti-CD3 and anti-CD28 antibodies, and stained for IFN-γ. (D) Draining lymph node cells from infected ears were stained for CD11c, MHC-class-II, CD8α, CD103 and intracellular IL-12p40. Plots show frequency (top) and absolute numbers (bottom) of IL-12p40 staining in CD103^{+} CD11c^{+} MHC class II^{hi} DCs. Data are presented as mean + SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (unpaired two-tailed Student's *t* test).

MAJOR GRANTS

- **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)**
- **Ministerio de Economía y Competitividad (RYC2009-04235)**
- **ERS/EU Marie Curie Post-doctoral Research Fellowships (RESPIRE 2 - 3708-2013).**

Selected Publications

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* doi: 10.1002/eji.201444651

Iborra S, Sancho D. **Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors.** *Immunobiology* doi: 10.1016/j.imbio.2014.09.013

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

del Fresno C, Soulat D, Roth S, Blazek K, Udalova I, Sancho D, Ruland J, Ardavín C. **Interferon-β production via Dectin-1-Syk-IRF5 signaling in dendritic cells is crucial for immunity to C. albicans**. *Immunity* (2013) 38(6):1176-86.

Sancho D, Reis e Sousa C. **Sensing of cell death by myeloid C-type lectin receptors**. *Curr Opin Immunol* (2013) 25:46-52



Stress kinases in diabetes, cancer and cardiovascular disease



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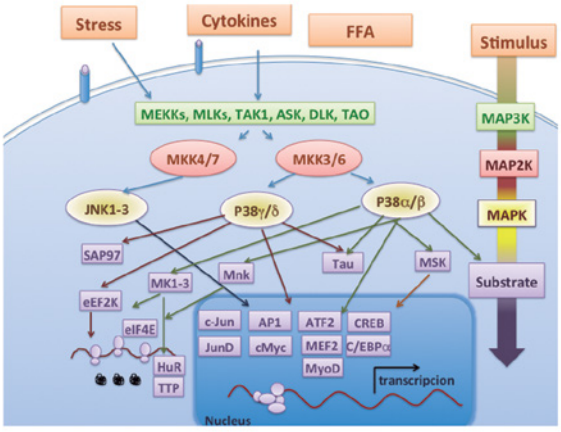
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Beatriz Caballero

RESEARCH INTEREST

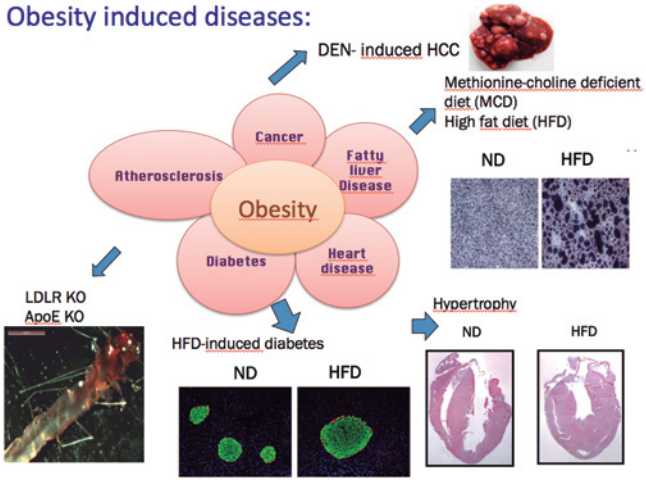
Metabolic syndrome is a medical disorder defined by the co-occurrence of obesity, impaired glucose tolerance, dyslipidemia and hypertension. The condition is associated with proinflammatory and prothrombotic states, and clinical outcomes include cardiovascular disease and type 2 diabetes. Moreover, metabolic 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.

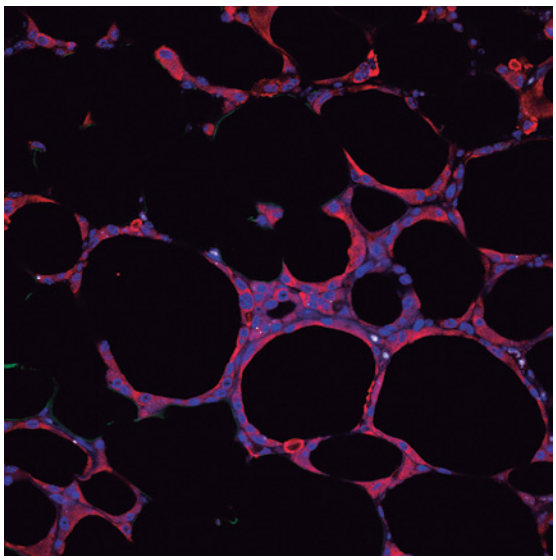
Our group investigates the involvement of SAPKs in the development of cancer, diabetes, cardiac hypertrophy and atherosclerosis induced by obesity. Our research is conducted with a number of disease models in combination with whole-body and tissue-specific knockout mice, and has shown that the p38γ/δ 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).



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 in 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)**
- **Ministerio de Economía y Competitividad (RYC-2009-04972)**

Selected Publications

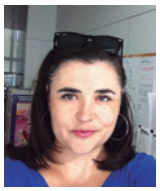
Vernia S, Cavanagh-Kyros J, Garcia-Haro L, Sabio G, Barrett T, Jung DY, Kim JK, Xu J, Shulha HP, Garber M, Gao G, Davis RJ. **The PPARα-FGF21 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway.** *Cell Metab* (2014) 20:512-25

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

González-Terán B, Cortés JR, Manieri E, Matesanz N, Verdugo A, Rodríguez ME, González-Rodríguez A, Valverde AM, Martín P, Davis RJ, Sabio G. **Eukaryotic elongation factor 2 controls TNF-α translation in LPS-induced hepatitis.** *J Clin Invest* (2013) 123:164-78

Imbernon M, Beiroa D, Vázquez MJ, Morgan DA, Veyrat-Durebex C, Porteiro B, Díaz-Arteaga A, Senra A, Busquets S, Velásquez DA, Al-Massadi O, Varela L, Gándara M, López-Soriano FJ, Gallego R, Seoane LM, Argiles JM, López M, Davis RJ, Sabio G, Rohner-Jeanrenaud F, Rahmouni K, Dieguez C, Nogueiras R. **Central melanin-concentrating hormone influences liver and adipose metabolism via specific hypothalamic nuclei and efferent autonomic/JNK1 pathways.** *Gastroenterol* (2013) 144:636-649



Regulatory molecules of inflammatory processes



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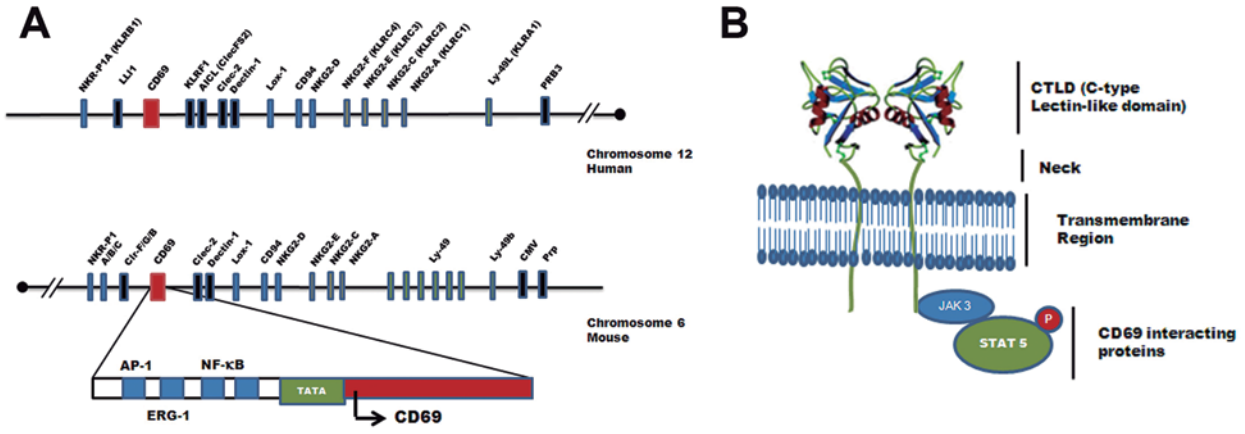
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Technician:
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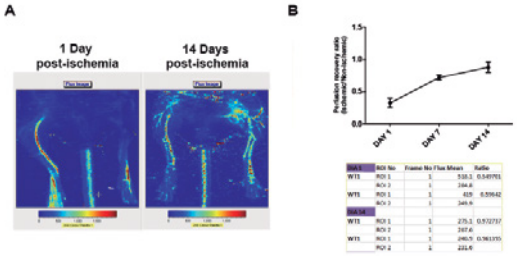
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Guadalupe González Tirma
Miguel Fernández de la Torre

RESEARCH INTEREST

Innate and adaptive immune responses are implicated in immune-mediated damage and repair of the heart. Although a moderate immune response is needed for tissue repair after an insult, an exacerbated immune response or autoimmunity against heart-derived antigens induce irreparable damage to the myocardium. Elucidation of the factors that determine the balance of the immune response is therefore critical for efforts to control the severity of cardiovascular diseases (CVD). MicroRNAs (miRNAs) have emerged as one of the most innovative tools for the development of strategies to stop both, inflammation and CVD. Several emerging studies link pro-inflammatory helper 17 T (Th17) cells, and anti-inflammatory regulatory T (Treg) cells with acute coronary syndrome, congestive heart failure and other CVDs. Other studies, in which ischemia is induced by right femoral artery ligation, show that Treg and Th17 cells modulate postischemic neovascularization in an antagonistic manner, revealing a possible role of these cell types in vascular diseases. However, the role of miRNAs derived from Th17 or Treg cells has received scant attention, leaving an open window for the development of novel diagnostic and therapeutic tools. Our group has revealed the role of the leukocyte antigen CD69 in the control of cardiomyopathy. CD69 is a master regulator of Th17/Tregs balance, necessary for the control of Th17-mediated heart inflammation and failure. We are now investigating Th17/Treg-related miRNAs in murine models of CVDs and in patients with the aim of identifying their putative target genes, an essential step toward elucidating their potential as novel therapeutic molecules and diagnostic tools in these diseases.



Main characteristics of CD69. **(A)** The gene encoding CD69 belongs to a family of receptors that modulate the immune response, the NK gene complex. The CD69 gene is located on chromosome 12 in humans and chromosome 6 in mice, and the promoter sequence contains a potential TATA box with a number of putative binding sites for inducible transcription factors (NF-κB, ERG-1, AP-1). **(B)** CD69 protein structure and interacting proteins. CD69 belongs to the C-type lectin receptor family. 3D analysis of human CD69 reveals that it exists as a homodimer, formed by two identical polypeptide chains with different degrees of glycosylation (yielding molecular weights of 33 and 27 kDa), linked by a disulfide bridge. The cytoplasmic tail of mouse and human CD69 associates with Jak3/Stat5 signaling proteins, regulating the transcription of RORγt/RORC2 and therefore the differentiation of Th17 lymphocytes.



Neovascularization after hindlimb ischemia. **(A)** Blood perfusion after femoral aorta surgery was measured using a laser Doppler perfusion imaging system. **(B)** Quantitative analysis of the blood flow ratio between ischemic and non-ischemic hindlimbs.

MAJOR GRANTS

- **Ministerio de Economía y Competitividad (SAF2011-27330)**
- **Comunidad de Madrid. Redes de Excelencia (S2010/BMD-2332)**
- **Ministerio de Economía y Competitividad. FIS RETICS (RIC: RD12/0042/0056)**

Selected Publications

Cortés JR; Sánchez-Díaz R; Bovolenta ER; Barreiro O; Lasarte S; Matesanz-Marin A; Toribio ML; Sanchez-Madrid F; Martin P. **Maintenance of immune tolerance by Foxp3(+) regulatory T cells requires CD69 expression**. *J Autoimmun* (2014) 55:51-62

De la Fuente H; Cruz-Adalia A; Martínez del Hoyo G; Cebrian D; Bonay P; Pérez-Hernández D; Vázquez J; Navarro P; Gutierrez-Gallego R; Ramirez-Huesca M; Martín P; Sánchez-Madrid F. **The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1**. *Mol Cell Biol* (2014) 34: 2479-87

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López-Bravo M, Minguito de la Escalera M, Domínguez PM, González-Cintado L, Del Fresno C, Martín P, Martínez Del Hoyo G, Ardavín C. **IL-4 blocks TH1-polarizing/ inflammatory cytokine gene expression during monocyte-derived dendritic cell differentiation through histone hypoacetylation**. *J Allergy Clin Immunol*. (2013) 132:1409-19

González-Terán B, Cortés JR, Manieri E, Matesanz N, Verdugo A, Rodríguez ME, González-Rodríguez A, Valverde A, Martín P, Davis RJ, Sabio G. **Eukaryotic elongation factor 2 controls TNF-α translation in LPS-induced hepatitis**. *J Clin Invest* (2013) 123:164-78



Single-molecule mechanobiochemistry



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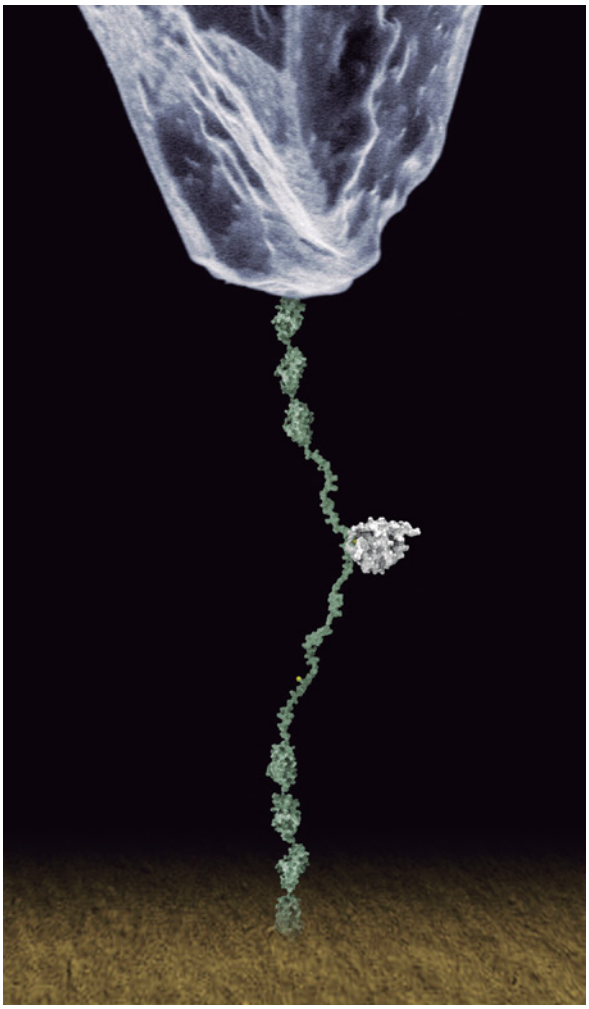
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Visiting Student:
Carmen Suay Corredera

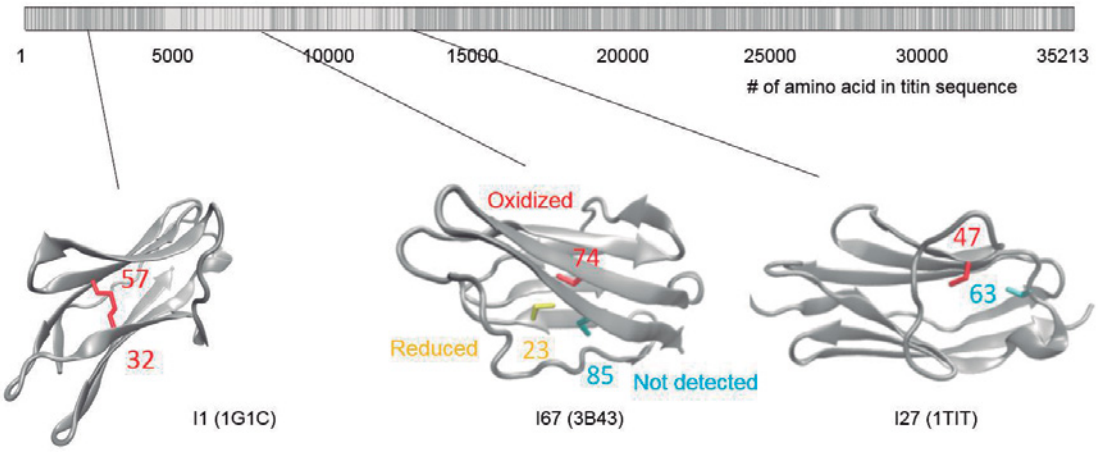
Technician:
Diana Velázquez Carreras

RESEARCH INTEREST

In our laboratory, we generate new knowledge about the mechanical properties of the myocardium and its regulation, with the potential to uncover new targets for the treatment of heart disease. Contractility of cardiac muscle depends on the concerted action of sarcomeric proteins with a mechanical function—actin, myosin, titin and several other associated proteins. Mutations in these proteins lead to different forms of life-threatening cardiomyopathy. However, the molecular mechanisms leading from genotype to pathogenic phenotype remain unknown. We are currently testing the hypothesis that missense mutations in elastic sarcomeric proteins can induce mechanical phenotypes that result in the development of disease. We also want to understand how the elasticity of the myocardium is tuned by posttranslational modifications of sarcomeric proteins. To bridge the gap between mechanics and biochemical regulation of sarcomeric proteins, we apply our double expertise in protein biochemistry and single-molecule force-clamp spectroscopy by atomic force microscopy (AFM). The mechanisms of cardiac regulation we examine cannot be studied by classic biochemistry techniques alone, and have remained unexplored. By implementing advanced single-molecule manipulation techniques, we are helping to establish mechanobiochemistry as a new field of scientific research.



A single molecule experiment by AFM. A single polyprotein made from 8 repetitions of a titin domain is pulled using an AFM tip. In the picture, one of the domains has been mechanically unfolded, exposing buried cysteines that are being posttranslationally modified by an oxidorreductase enzyme.



Detection of redox posttranslational modifications in titin. Preliminary mass spectrometry results showing the redox state of cysteine residues in titin domains (in collaboration with the CNIC Cardiovascular Proteomics group). Top: Gray lines represent identified peptides in the primary sequence of full- length titin. We obtain 70% coverage of the titin sequence. Bottom: Three domains are highlighted (PDB codes are indicated). The paired cysteines detected in domain I1 are oxidized, suggesting that they form a disulphide bond in native tissue. Results with domain I67 suggest that a disulphide bond is formed between residues 74 and 85. Oxidized cysteines are also found at positions that are incompatible with disulphide bond formation, suggesting that they are the target of other redox posttranslational modifications, such as S-glutathionylation.

MAJOR GRANTS

- European Union / CNIC: CNIC IIF (International Incoming Fellowships for Young Group Leaders) (FP7-PEOPLE-2010-COFUND-267149)

Selected Publications

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

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* (accepted: doi: 10.1002/mame.201400271)

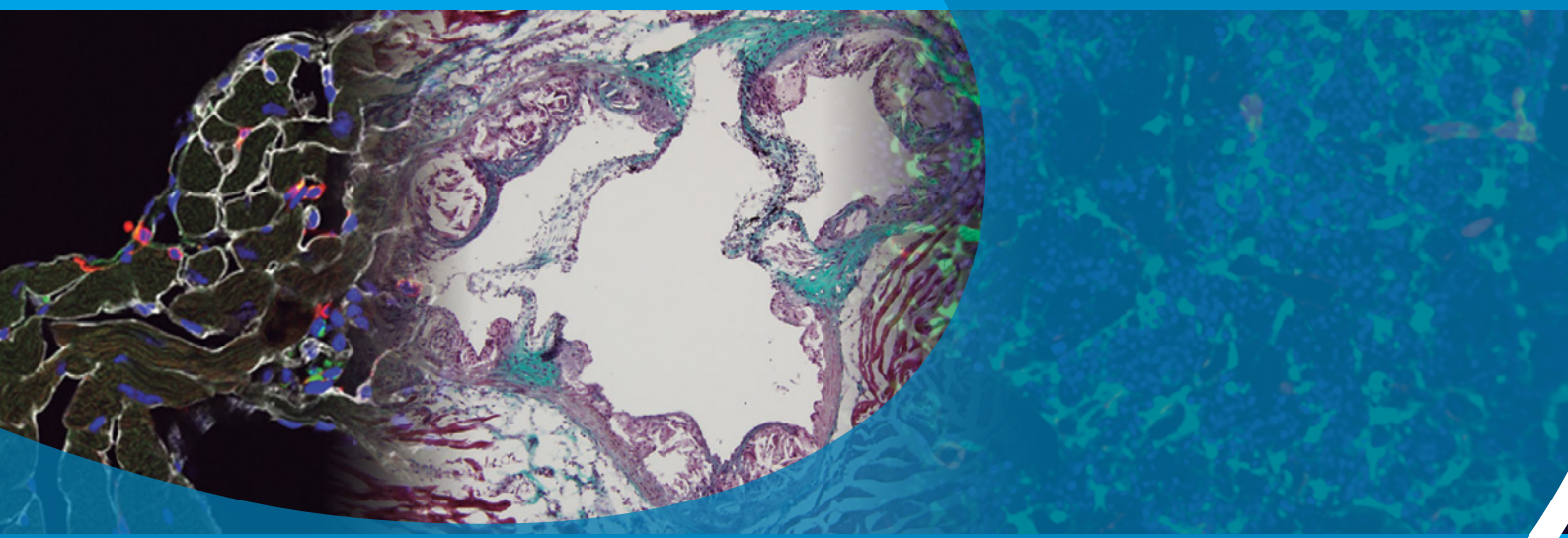
Solsona C, Kahn TB, Badilla CL, Álvarez-Zaldiernas C, Blasi J, Fernandez JM, Alegre-Cebollada J. **Altered thiol chemistry in human amyotrophic lateral sclerosis-linked mutants of superoxide dismutase 1**. *J Biol Chem* (2014) 289:26722-32

Popa I, Berkovich R, Alegre-Cebollada J, Badilla CL, Rivas-Pardo JA, Taniguchi Y, Kawakami M, Fernandez JM. **Nanomechanics of HaloTag tethers**. *J Am Chem Soc* (2013) 135:12762-71

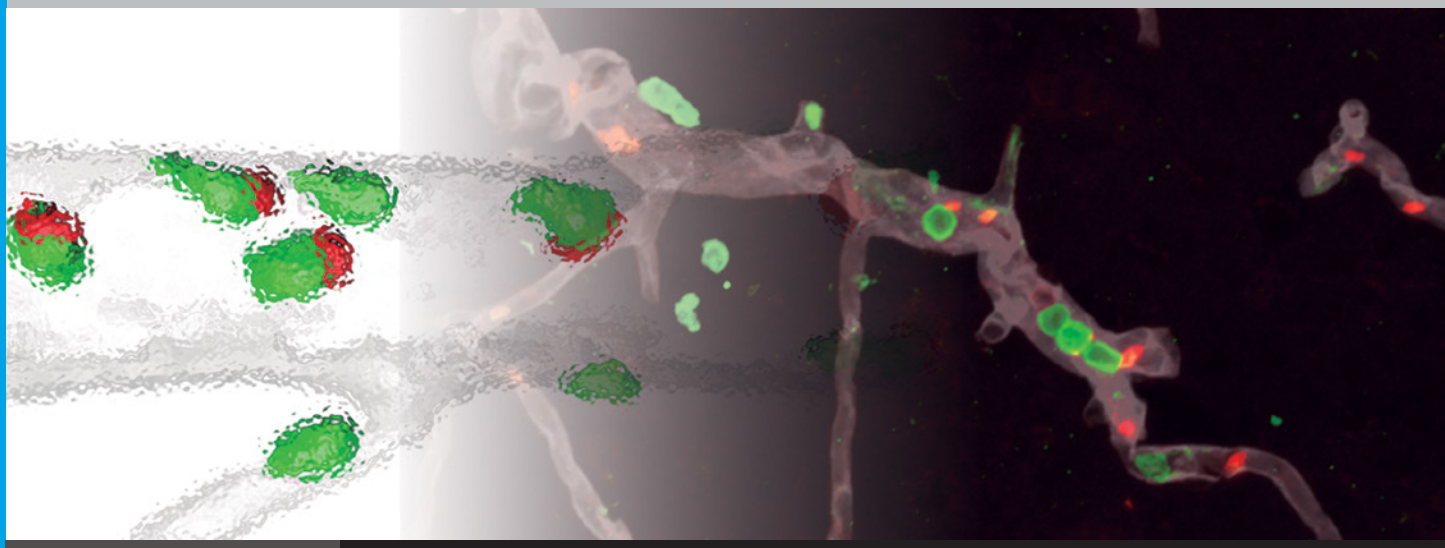
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RESEARCH DEPARTMENTS

Atherothrombosis, Imaging and Epidemiology



RESEARCH DEPARTMENTS



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Valentín Fuster

3. Atherothrombosis, Imaging and Epidemiology

Department Manager:
Ana Isabel Castillo

Project Manager:
Eeva Inari Soininen

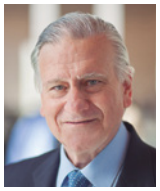
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Our department combines basic science, clinical data, and population-level analysis in order to better understand the occurrence, natural history and prognosis of cardiovascular disease, and to develop therapeutic alternatives. Our programs include studies into the molecular and cellular mechanisms underlying atherosclerosis, restenosis and aging; the role of neutrophils and other myeloid leukocytes in various aspects of the inflammatory response; cardioprotection during myocardial infarction; and complex cardiac arrhythmias including studies in animal models and humans using latest-generation advanced imaging techniques.

The department works closely with the Advanced Imaging Unit, bringing in expertise in imaging, nanomedicine, radiochemistry and metabolomics. The department also coordinates epidemiolgical studies on the distribution and progression of atherosclerosis and the genetic, environmental, lifestyle, and social determinants in human populations.



Cardiovascular imaging



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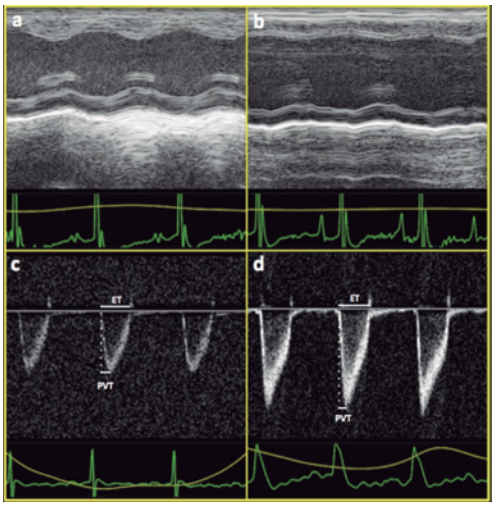
RESEARCH INTEREST

Our group has developed research applications for noninvasive, high-resolution and high-sensitivity imaging technologies to support translational research and population studies in preclinical atherosclerosis. We collaborate with other CNIC investigators, offering support in translational research in the use of basic cardiovascular imaging techniques (Figure 1). Our main noninvasive bioimaging population studies (Figure 2) for early detection and progression of atherosclerosis, PESA and AWHS (see Multidepartamental Projects) are clinical trials designed to identify new imaging, lifestyle habits and omics factors associated with the presence and progression of atherosclerosis in middle aged adult populations.

In 2014 our group performed more than 4000 preclinical echocardiogram procedures in mouse and zebrafish models to support research projects from all CNIC departments. This work has contributed to more than 40 studies and preclinical sub-studies to elucidate pathological phenotypes by basic CV imaging. Our collaborations include work on several mouse models of cardiovascular disease, ischemia-reperfusion in acute myocardial infarction, myocardial regeneration in zebrafish, mouse models of atherosclerosis and aging, models of systolic, diastolic and diabetic heart failure, and models of non-compacted cardiomyopathy, left ventricular hypertrophy, aortic aneurysm, degenerative aortic valve, aortic annular ectasia, mitral insufficiency, coronary aneurysm, acute pulmonary hypertension, and chronic pulmonary hypertension associated with smoking or scleroderma. In many of these projects our basic CV imaging analysis and our clinical expertise have been crucial to identifying the preclinical cardiovascular phenotype and have been the key to identifying the focus of clinical translation to human disease.

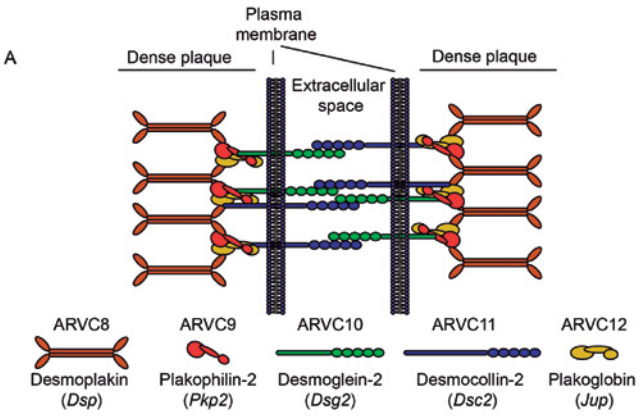
The PESA study has moved into Phase II follow-up at 3 years for 4000 participants aged 40-54 years. In this second phase, we look for changes in the systemic extent of atherosclerosis and its association with factors that were detected in the recruitment phase, three years earlier. The studies include the analysis of systemic subclinical atherosclerosis through multi-territory 2D/3D ultrasound and the detection of coronary calcium CT. We also continue to analyze the influence of lifestyle, omics, known risk factors, analytical blood and urine, as well as changes in timing. To analyze the prognostic value of local plaque inflammatory markers, a group nearly 1000 participants with atherosclerosis at baseline has been evaluated by ^{18}FDG PET/MRI. Follow-up at 3 and 6 years enables us to determine which factors predict the progression of atherosclorosis in a middle aged population, and long-term monitoring will reveal new prognostic markers for events.

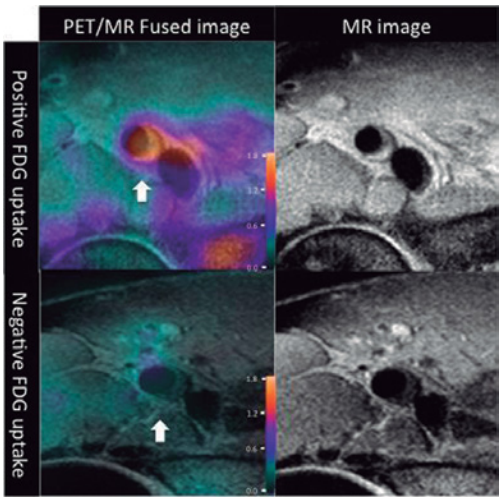
Our research into cardiovascular disease is based on a simple principle: create to understand, create to treat.



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 recommendation in carriers of this mutation.

Preclinical imaging for translational research in mouse models of left ventricular dysfunction and of pulmonary hypertension. Upper panels show M-mode echocardiogram of (a) control and (b) depressed left ventricular systolic dysfunction. Lower panels show pulsed Doppler of pulmonary flow in (c) control mice, with relative symmetric peak velocity time (PVT) compared with ejection time (ET), and (d) in mice with pulmonary hypertension, with relative shorter peak velocity time.





^{18}F-FDG PET/MR imaging of femoral atherosclerosis in PESA participants. Upper panels show positive FDG uptake, indicating active inflammation, whereas the lower panels show a plaque negative for FDG uptake.

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.

MAJOR GRANTS

- **European Commission FP7 (241559 FOCUS).**
- **Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS).**
- **AHA Grant (14SFRN20490315). PI: Fuster V.**
- **NIH Grant Programs in Excellence in Nanotechnology. (29820100045-C-0-1). Fuster V Co-Inv**
- **NIH Grant Project: (U01HL-114200-02)**
- **Grenada Heart Project (Louis Mayer FDTN/W. Eisenberg)**
- **Project Astra Zeneca. (ISSBR1L0246/ D5130L00081)**

Selected Publications

Tahara N, Mukherjee J, de Haas HJ, Petrov AD, Tawakol A, Haider N, Tahara A, Constantinescu CC, Zhou J, Boersma HH, Imaizumi T, Nakano M, Finn A, Fayad Z, Virmani R, Fuster V, Bosca L and Narula J. **2-deoxy-2-[F]fluoro-d-mannose positron emission tomography imaging in atherosclerosis.** *Nat Med* (2014) 20: 215-9

Fuster V and Kovacic JC. **Acute coronary syndromes: pathology, diagnosis, genetics, prevention and treatment.** *Circ Res* (2014) 114: 1847-51

de Haas HJ, Narula J and Fuster V. **From molecular imaging to pathogenesis and vice versa.** *Circ Cardiovasc Imaging* (2014) 7: 581-5

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Bucerius J, Mani V, Wong S, Moncrieff C, Izquierdo-Garcia D, Machac J, Fuster V, Farkouh ME, Rudd JH, Fayad ZA. **Arterial and fat tissue inflammation are highly correlated: a prospective ^{18}F-FDG PET/CT study**. Eur J Nucl Med Mol Imaging. (2014) 41:934-45

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 arrhythmogenic right ventricular cardiomyopathy phenotype in mice expressing a disease-causing mutated version of human plakophilin-2 after single adeno-associated virus-mediated gene transfer**. *J Am Coll Cardiol* (accepted)



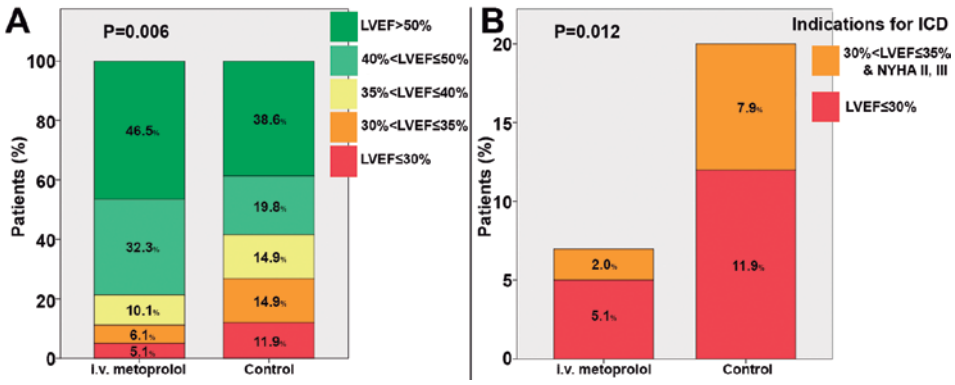
Imaging in experimental cardiology

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 we are using these to study the mechanisms underlying the beneficial effects of various cardioprotective strategies, mainly related to modulation of the adrenergic system. 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.

In the clinical setting, our team is a key participant in the METOCARD-CNIC trial, which uses magnetic resonance imaging to evaluate the effectiveness of a cardioprotective strategy based on beta adrenergic modulation in patients suffering a myocardial infarction. Last year we reported the primary outcome of the trial, which shows that this strategy can reduce infarct size in patients undergoing primary angioplasty. During 2014 we analyzed the follow-up data of this trial to evaluate the effect of the therapy on long term myocardial performance and clinical events. The METOCARD-CNIC trial serves as the platform for a future large trial in myocardial infarction patients that will test the effect of the therapy on hard clinical endpoints. This trial will be coordinated by us and will have more than six European partners. Finally, we are evaluating diffuse fibrosis within the myocardium by novel magnetic resonance imaging sequences, and are currently recruiting patients with different cardiomyopathies for this endeavour.



Impact of early i.v. metoprolol administration during myocardial infarction on long term left ventricular performance. Results from the METOCARD-CNIC trial. Follow-up left ventricular ejection fraction (LVEF) categories (A) and indications for implantable cardioverter defibrillators according to treatment allocation (B).



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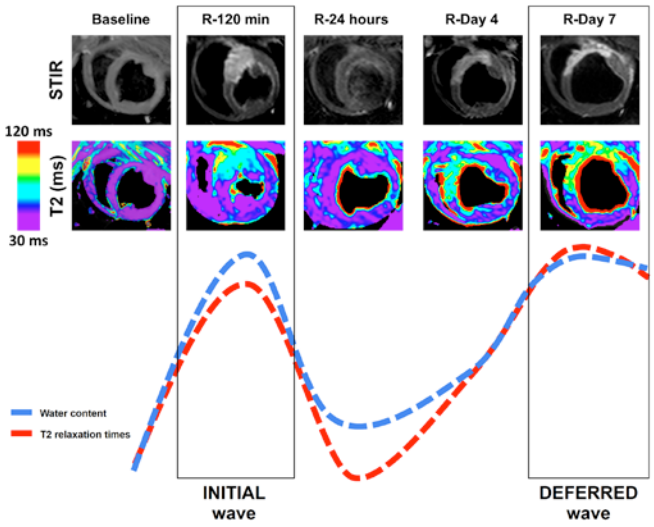
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Edematous Reaction after Ischemia/Reperfusion follows a Bimodal Pattern



Edematous reaction after myocardial infarction follows a bimodal pattern. The development of myocardial edema is a well-known phenomenon occurring after ischemia/reperfusion (myocardial infarction). This edematous reaction was long assumed to be stable for at least 1 week. Using advanced cardiac magnetic resonance and histopathology, we have challenged this classical dogma, demonstrating that post-ischemia/reperfusion edema is bimodal. An initial wave of edema abruptly appears upon reperfusion and almost completely disappears at 24 hours. A deferred wave appears later and increases progressively until day 7.

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)**
- **Maratón, Fundación TV3 (REF: 70/C/2012)**
- **CNIC Translational Grants (01-2009)**
- **European Commision FP7-PEOPLE-2013-ITN (CARDIONEXT).**

Selected Publications

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* (accepted)

Pizarro G, Fernández-Friera L, Fuster V, Fernández-Jiménez R, García-Ruiz JM, García-Álvarez A, Mateos A, Barreiro MV, Escalera N, Rodriguez MD, de Miguel A, García-Lunar I, Parra-Fuertes JJ, Sánchez-González J, Pardillos L, Nieto B, Jiménez A, Abejón R, Bastante T, Martínez de Vega V, Cabrera JA, López-Melgar B, Guzman G, García-Prieto J, Mirelis JG, Zamorano JL, Albarrán A, Goicolea J, Escaned J, Pocock S, Iñiguez A, Fernández-Ortiz A, Sánchez-Brunete V, Macaya C, Ibanez B. **Long term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial.** *J Am Coll Cardiol.* (2014) 63:2356-62

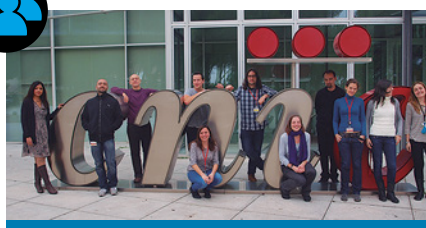
García-Álvarez A, García-Lunar I, Pereda D, Fernández-Jimenez R, Sánchez-González J, Mirelis JG, Nuño-Ayala M, Sánchez-Quintana D, Fernández-Friera L, García-Ruiz JM, Pizarro G, Agüero J, Campelos P, Castellá M, Sabaté M, Fuster V, Sanz J, Ibañez B. **Association of myocardial T1-mapping CMR with hemodynamics and right ventricular performance in pulmonary hypertension.** *JACC Cardiovasc Imaging* (accepted)

García-Prieto J, García-Ruiz JM, Sanz-Rosa D, Pun A, García-Alvarez A, Davidson SM, Fernández-Friera L, Nuno-Ayala M, Fernández-Jiménez R, Bernal JA, Izquierdo-Garcia JL, Jimenez-Borreguero J, Pizarro G, Ruiz-Cabello J, Macaya C, Fuster V, Yellon DM, Ibanez B. **β3 adrenergic receptor selective stimulation during ischemia/reperfusion improves cardiac function in translational models through inhibition of mPTP opening in cardiomyocytes.** *Basic Res Cardiol* (2014) 109:422-37

Pereda D, García-Alvarez A, Sánchez-Quintana D, Nuño M, Fernández-Friera L, Fernández-Jiménez R, García-Ruíz JM, Sandoval E, Agüero J, Castellá M, Hajjar RJ, Fuster V, Ibáñez B. **Swine model of chronic postcapillary pulmonary hypertension with right ventricular remodeling: long-term characterization by cardiac catheterization, magnetic resonance and pathology.** *J Cardiovasc Transl Res* (2014) 7:494-506



Imaging cardiovascular inflammation and the immune response



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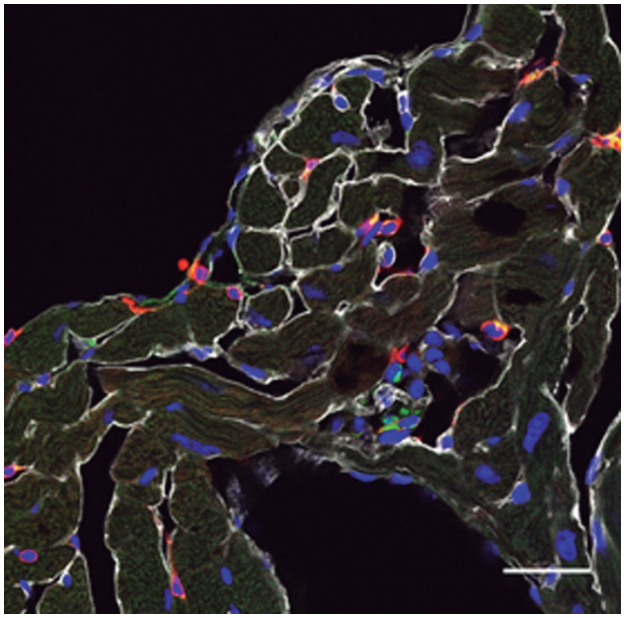
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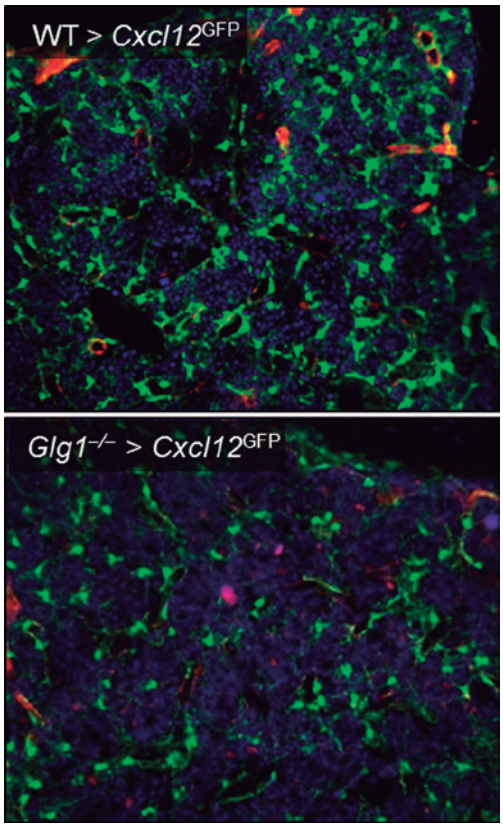
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RESEARCH INTEREST

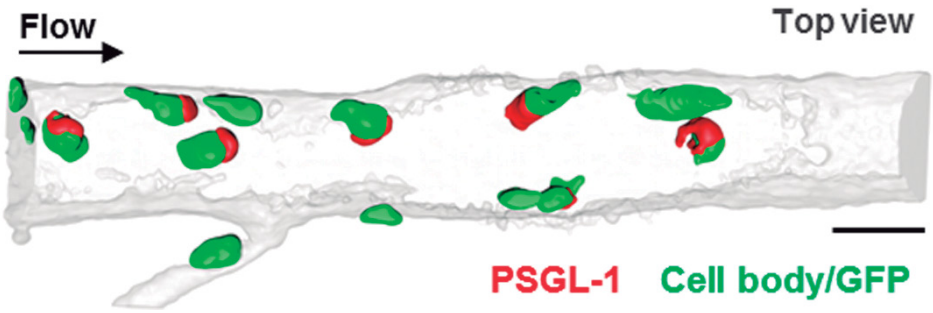
Our group studies various aspects of the biology of leukocytes, the cells that mediate organismal immunity but that are also the major culprits of vascular disease. We begin our studies with the cells that form the basis of the immune system, the hematopoietic stem cells, and search for the factors and cells that regulate their maintenance in the bone marrow. We currently focus on how two such molecules, ESL-1 and TGFβ, work together to ensure normal proliferation of stem cells. We are also working out the processes inside blood vessels in which leukocytes and platelets work together to promote inflammation. We have identified a surprising mechanism by which neutrophils (the leukocyte subset that underlies most cases of vascular disease) actively scan and search for circulating platelets to initiate inflammation. We continue to make progress in our understanding of the basic biological behavior of neutrophils in the absence of inflammation, in an attempt to identify new functions for these leukocytes. Finally, we are interested in the functions of a population of macrophages that reside within the healthy myocardium, and which may play an important role in supporting heart function.



Macrophages residing within the healthy myocardium
Sectional image of the heart of a mouse showing macrophages (red and green) located between myocardial fibers delimited by laminin (white).



Regulation of the niche by hematopoieteic cells
Whole-mount images of the sternal bone marrow, showing the distribution of CXCL12-producing cells (green), which are strongly inhibited by ESL-1-deficient hematopoietic cells.



Neutrophils scan the circulation to initiate inflammation
3D reconstruction of a live inflamed venule showing the distribution of neutrophils (green) and a specific receptor (red) that traps activated platelets and relays signals to the cells, which then initiate inflammation.

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)**

Selected Publications

Chèvre R, González-Granado JM, Megens RT, Sreeramkumar V, Silvestre-Roig C, Molina-Sánchez P, Weber C, Soehnlein O, Hidalgo A*, Andrés V*. **High-resolution imaging of intravascular atherogenic inflammation in live mice.** *Circulation Research* (2014) 114:770-79
*Equal contribution

Casanova-Acebes M, A-González N, Weiss LA, Hidalgo A. **Innate immune cells as homeostatic regulators of the hematopoietic niche.** *International Journal of Hematology* (2014) 99:685-94

Leon-Rico D, Aldea M, Sanchez R, Segovia JC, Weiss LA, Hidalgo A, Bueren JA, Almarza E. **Reduced Expression of CD18 Leads to the In Vivo Expansion of Hematopoietic Cells in Mouse Bone Marrow**. *Stem Cells* (2014) 32:2794-8

A-Gonzalez N, Hidalgo A. **Nuclear receptors and clearance of apoptotic cells: stimulating the macrophage's appetite**. *Frontiers in Immunology* (2014) 5:211. doi: 10.3389/fimmu.2014.00211

Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, Nácher M, Pitaval C, Radovanovic I, Fukui Y, McEver RP, Filippi MD, Lizasoain I, Ruiz-Cabello J, Zarbock A, Moro MA and Hidalgo A. **Neutrophils scan for activated platelets to initiate inflammation.** *Science* (2014) 346:1234-8



Molecular and genetic cardiovascular pathophysiology



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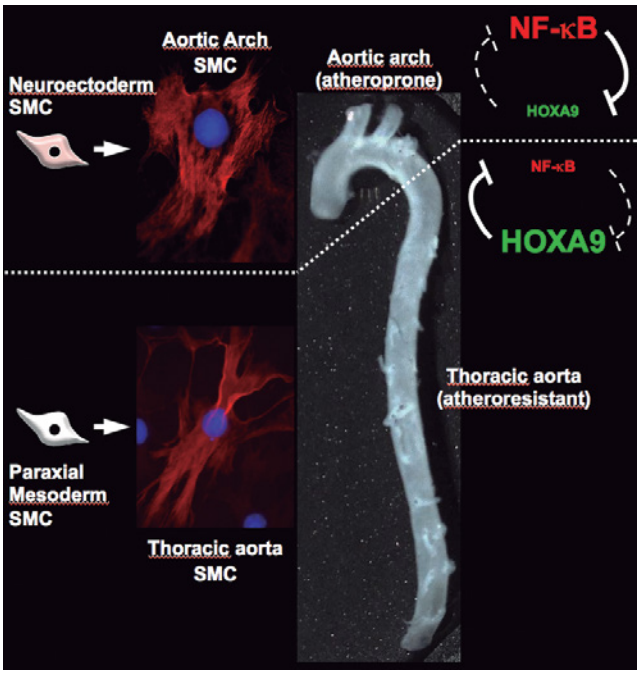
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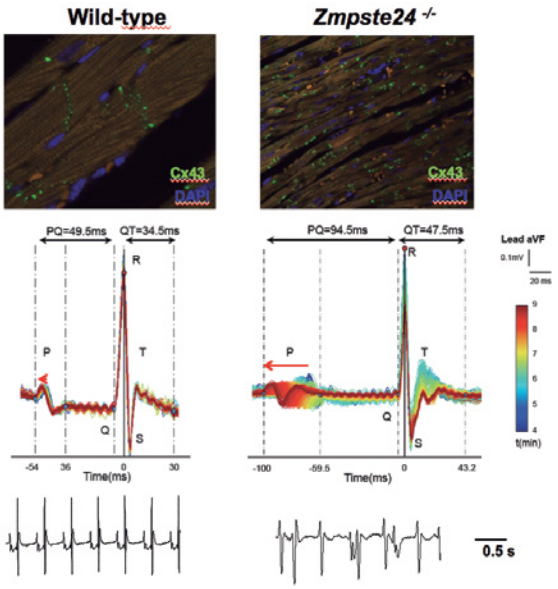
RESEARCH INTEREST

The World Health Organization estimates that cardiovascular disease (CVD) will by 2020 be the main health and socio-economic problem worldwide, in part due to the progressive aging of the world population. Atherosclerosis, vascular calcification (VC) and heart failure contribute significantly to CVD-related morbimortality in the elderly. These anomalies, and the aging process itself, are much 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, VC and cardiac electrophysiological alterations which are associated with early death (average lifespan: 13.5 yr, range: 8-21 yr), predominantly as a result of 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.

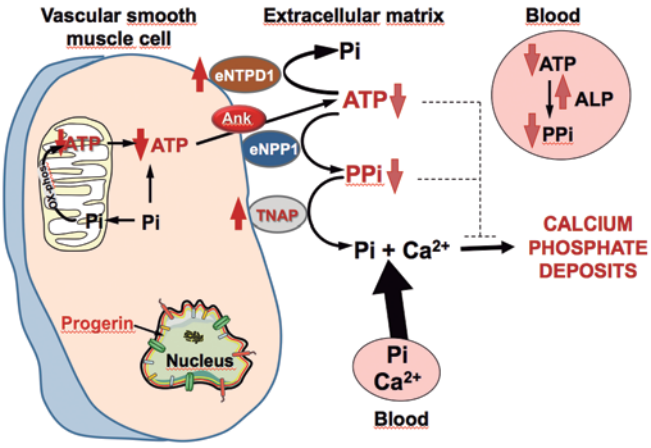
Our research is currently focused on 5 areas: 1. Identifying mechanisms through which wild-type lamin A/C regulate CVD; 2. Unraveling mechanisms of VC in the setting of normal and premature aging; 3. Identifying tissue-specific and systemic mechanisms through which progerin promotes atherosclerosis, VC, cardiac alterations, and aging; 4. Generating a porcine model of HGPS using CRISPR/Cas9 technology; and 5. Unraveling gender- and tissue-specific molecular mechanisms common to premature and physiological aging and specific to each process.



Differential Hox expression contributes to the maintenance of phenotypic differences between smooth muscle cells (SMCs) from atheroprone (aortic arch) and atheroresistant (thoracic aorta) regions through regulatory feedback mechanisms involving inflammatory mediators (e.g. reciprocal inhibition between HOXA9 and NF-κB).



Progeroid *Zmpste24*$^{-/-}$ mice exhibit mislocalization of conexin 43 (Cx43) and severe cardiac conduction abnormalities, and develop arrhythmias.



Reduced systemic and local levels of ATP and pyrophosphate (PPi) in a mouse model of HGPS accelerate vascular calcification, and this effect is ameliorated by treatment with PPi.

MAJOR GRANTS

- **European Commission FP7-ICT-2011-8 (LIPHOS-317916)**
- **Progeria Research Foundation (Innovator Award PRF 2012-42)**
- **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 (SAF2010-16044)**
- **Ministerio de Economía y Competitividad. FIS RETICS (RECAVA, RD06/0014/0021)**
- **Ministerio de Economía y Competitividad. FIS (CP11/00145) PI: J.M. González Granado**

Selected Publications

Chèvre R, González-Granado JM, Megens RTA, Sreeramkumar V, Silvestre-Roig C, Molina-Sánchez P, Weber C, Soehnlein O, Hidalgo A, Andrés V. **High-resolution imaging of intravascular atherogenic inflammation in live mice**. *Circ Res* (2014) 114:770-9

Silvestre-Roig C, Fernández P, Mansego, ML, van Tiel CM, Viana R, Anselmi CV, Condorelli G, de Winter RJ, Martín-Fuentes P, Solanas-Barca M, Civeira F, Focaccio A, de Vries CJM, Chaves FJ, Andrés V. **Genetic variants in CCNB1 associated with differential gene transcription and risk of coronary in-stent restenosis**. *Circ Cardiovasc Genet* (2014) 7:59-70

González-Granado JM, Silvestre-Roig C, Rocha-Perugini V, Trigueros-Motos L, Cibrian D, Morlino G, Blanco-Berrocal M, Osorio FG, Freije JMP, López-Otín C, Sánchez-Madrid F, Andrés V. **Nuclear envelope lamin-A couples actin dynamics with immunological synapse architecture and T cell activation**. *Science Signal* (2014) 7:ra37 (issue cover)

Andrés V. **Vitamin D puts the brakes on angiotensin II-induced oxidative stress and vascular smooth muscle cell senescence**. *Atherosclerosis* (2014) 236:444-7

Villa-Bellosta R, Rivera-Torres J, Osorio FG, Acín-Pérez R, Enriquez JA, López-Otín,C, Andrés V. **Defective extracellular pyrophosphate metabolism promotes vascular calcification in a mouse model of Hutchinson-Gilford progeria syndrome that is ameliorated on pyrophosphate treatment**. *Circulation* (2013) 127: 2442-51



Advanced development in arrhythmia mechanisms and therapy



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Visiting Student:
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Jose Manuel Alfonso Almazán

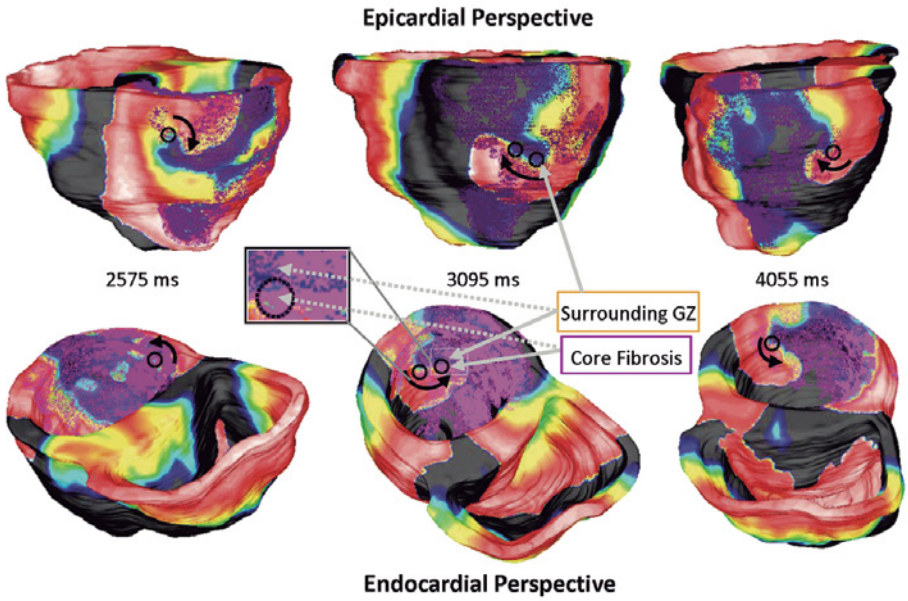
RESEARCH INTEREST

The laboratory focuses on investigating the mechanisms underlying complex cardiac arrhythmias that occur in highly prevalent cardiovascular diseases in the general population, as well as in specific subsets at particular risk of sudden cardiac death. Atrial fibrillation (AF), ventricular fibrillation (VF) and infarct scar-related ventricular tachycardia (VT) represent 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 three 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.

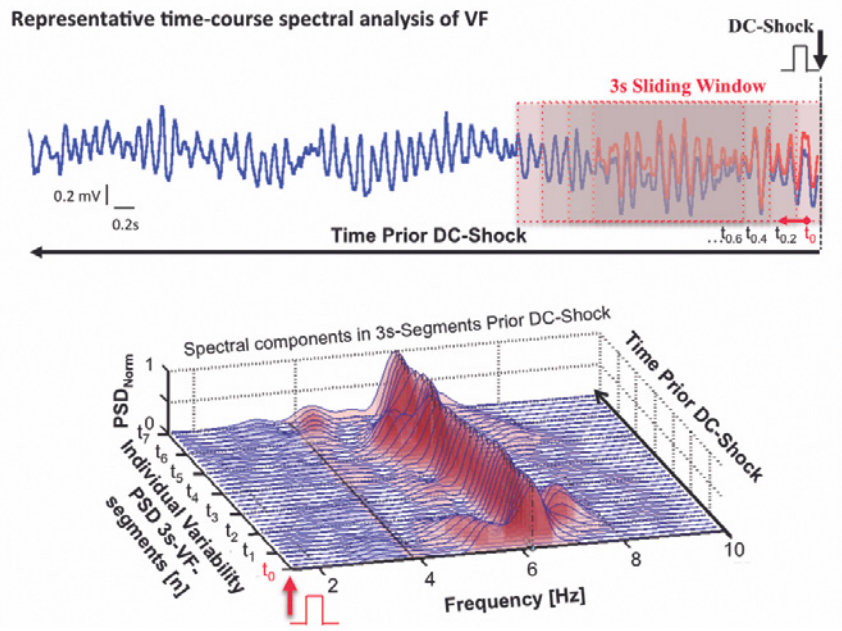
We use a translational approach to study infarct scar-related VT in pigs and clinical infarct-related reentrant VT. High-resolution MRI images, both in humans (in vivo) and animals (ex vivo) provide the structural details to construct patient and animal-specific 3D anatomical models of the ventricles. 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 are currently involved in developing a reliable risk-score to enable early prediction of cerebral performance and survival.

In AF, we aim to characterize differences in the transcriptome, ion channel density and function, and molecular and gross anatomical structure of relevant regions of the atria of pigs with paroxysmal, persistent and long-standing persistent AF. Such differences provide information essential for the understanding of AF maintenance and patterns of electrical activation observed during the arrhythmia.



Sample of ventricular tachycardia reentry patterns on one patient-derived model with dilated pathology with high gray zone. Loose anchoring around regions of high gray zone causes the focal activation to shift throughout simulation. Beginning on the inferior wall, the main spiral shifts further along the lateral wall as simulation progresses.



Upper panel. VF tracing with superimposed 3-s sliding windows that were used to obtain spectral components from the DC shock up to the available signal length. The VF spectrum was obtained for each segment and the spectral components were estimated. **Bottom panel**. Sequential spectral components along 3-s segments ('t' seconds prior to the DC shock) in a patient with predicted and observed favorable neurological performance using a spectral based predictive algorithm.

MAJOR GRANTS

- Eugenio Rodríguez Pascual Foundation.
- Spanish Society of Cardiology (Electrophysiology & Arrhythmia Division).
- Salud 2000 Foundation.
- Spanish Society of Cardiology (General Division – not CNIC)

Selected Publications

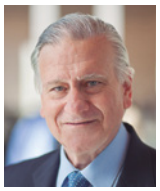
Martins RP, Kaur K, Hwang E, Ramirez RJ, Cicero Willis B, Filgueiras-Rama D, Ennis SR, Takemoto Y, Ponce-Balbuena D, Zarzoso M, O'Connell RP, Musa H, Guerrero-Serna G, Avula UMR, Swartz MF, Bhushal S, Deo M, Pandit SV, Berenfeld O, Jalife J. **Dominant frequency increase rate predicts transition from paroxysmal to long-term persistent atrial fibrillation**. *Circulation* (2014) 129:1472-82

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.* Aug 11. doi: 10.1111/echo.12714. [Epub ahead of print]

Ringenberg J, Deo M, Filgueiras-Rama D, Pizarro G, Ibañez B, Peinado R, Merino JL, Berenfeld O, Devabhaktuni V. **Effects of fibrosis morphology on reentrant ventricular tachycardia inducibility and simulation fidelity in patient-derived models**. *Clin Med Insights Cardiol* (2014) 8 (Suppl 1):1-13

Berenfeld O, Yamazaki M, Filgueiras-Rama D, Kalifa J. **Surface and intramural reentrant patterns during atrial fibrillation in the sheep**. *Methods Inf Med*. (2014) 53:314-9

Moreno J, Quintanilla JG, Molina-Morúa R, García-Torrent MJ, Angulo-Hernández MJ, Curiel-Llamazares C, Ramiro-Bargueño J, González P, Caamaño AJ, Pérez-Castellano N, Rojo-Álvarez JL, Macaya C, Pérez-Villacastín J. **Morphological and thermodynamic comparison of the lesions created by 4 open-irrigated catheters in 2 experimental models**. *J Cardiovasc Electrophysiol* (2014) 25:1391-9



Atherothrombosis and cardiovascular epidemiology



RESEARCH INTEREST

We are a multidisciplinary group pursuing highly innovative research that covers the major risk factors for CVD, including diet, exercise, genetics and epigenetics, metabolic factors, the environment, and psychosocial factors. We are also developing expertise in the analysis of high-throughput data and in the evaluation of novel and established cardiovascular risk factors in studies of populations with subclinical measures of atherosclerosis. Through these approaches and by conducting and coordinating various high-quality and high-impact research studies both in primary and secondary prevention, we are making significant contributions to the understanding and control of the current epidemic of CVD.

The CNIC's major population studies include 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), IMJOVEN, and the Fuster-CNIC-Ferrer Polypill project. The polypill study was finalized in 2014 and showed that the polypill strategy met the primary endpoint for adherence to secondary prevention following an AMI—increased self-reported and directly measured medication.

We are also developing educational programs from early in life to adulthood to promote healthy habits for cardiovascular prevention. The Program SI! (in collaboration with the SHE Foundation) works with children from the ages of 3 to 16 and their proximal environment (family and school) with the aim of instilling appropriate lifestyle behaviors for CVD prevention. The 50/50 Project (in collaboration with the Spanish Observatory of Nutrition and the Study of Obesity) works with adults on peer-to-peer motivation to improve physical activity, healthy diet, smoking cessation, self-controlled blood pressure, and weight management.

We also continue to make significant contributions to leading international studies such as the Framingham Heart Study (FHS), the Atherosclerosis Risk in Communities (ARIC) Study, the Multiethnic Study of Atherosclerosis (MESA), the High Risk Plaque (HRP) Study, the US National Health and Nutrition Examination Survey, and the UK National Diet and Nutrition Survey.

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Julia Díez Escudero
(University of Alcalá, Madrid)
Rachel Emily Anderson
(St. Louis University, USA)

MAJOR GRANTS

- **European Commission FP7 (241559 FOCUS).**
- **Instituto de Salud Carlos III (CP08/112). PI: M Laclaustra**
- **Comunidad de Madrid (P2009/AGR-1469). PI: JL Peñalvo**
- **Instituto de Salud Carlos III (PI10/00021). PI: M Laclaustra**
- **Instituto de Salud Carlos III (PI11/00403). PI: JL Peñalvo**
- **Departamento de Salud y Consumo of the regional government of Aragon, General Motors Spain and CNIC (AWHS).**
- **NIH Grant (5U01 HL-114200-03). PI: Fuster V.**
- **AHA Grant (14SFRN20490315). PI: Fuster V.**
- **Astra Zeneca. (ISSBR1L0246/ D5130L00081) PI: Fuster V.**

Selected Publications

Castellano JM, Sanz G, Garrido E, Bansilal S, Fuster V. **A polypill strategy to improve global secondary cardiovascular prevention: from concept to reality**. *J Am Coll Cardiol* (2014) 64:613-21

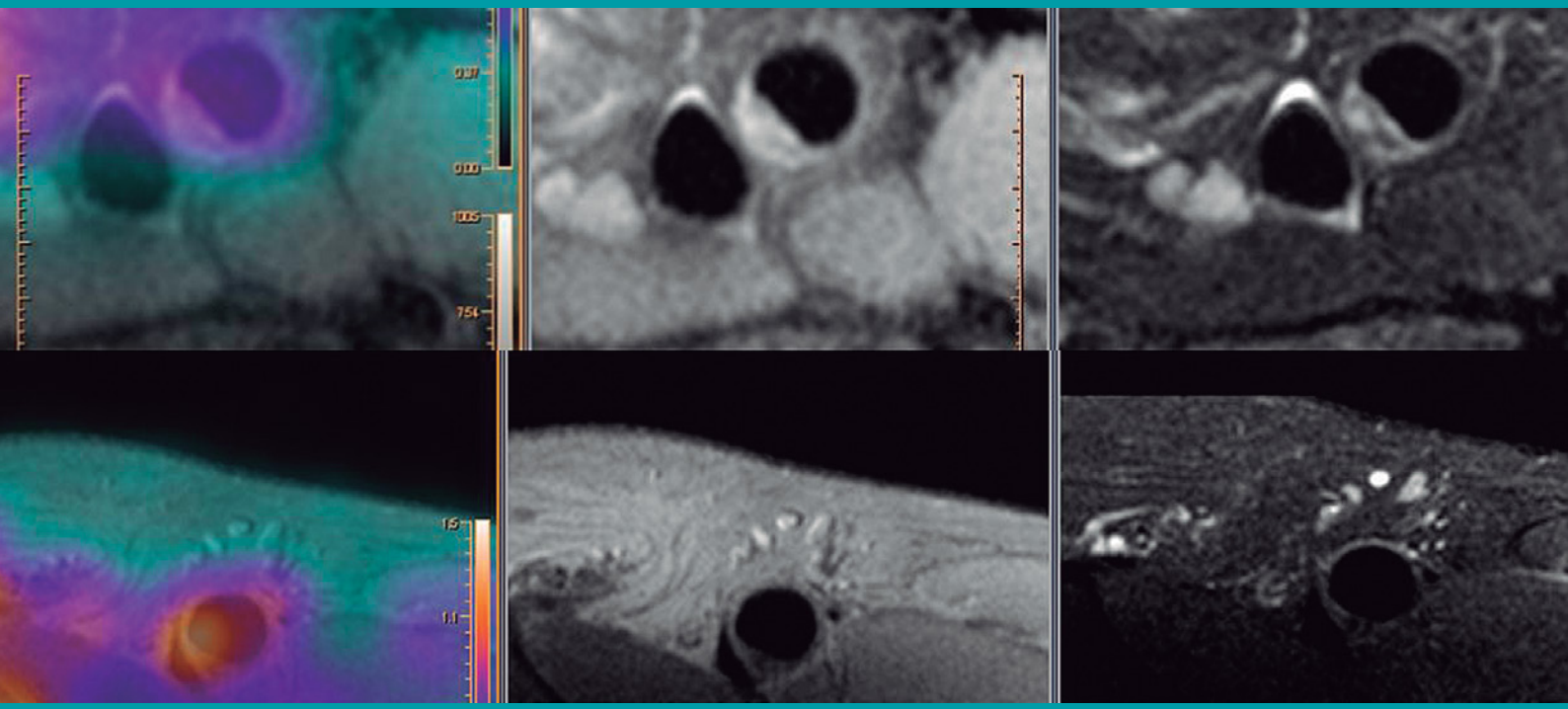
Castellano JM, Sanz G, Peñalvo JL, Fernández-Ortiz A, Alvarez L, Guzmán L, Linares JC, García F, D'Aniello F, Arnáiz JA, Varea S, Martínez F, Lorenzatti A, Imaz I, Sánchez-Gómez LM, Roncaglioni MC, Baviera M, Smith S, Taubert K, Pocock S, Brotons C, Fuster V. **A polypill strategy to improve adherence: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

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Dangas GD, Farkouh ME, Sleeper LA, Yang M, Schoos MM, Macaya C, Abizaid A,
Buller CE, Devlin G, Rodriguez AE, Lansky AJ, Siami FS, Domanski M, Fuster V. **FREEDOM Investigators. Long-term outcome of PCI versus CABG in insulin and non-insulin-treated diabetic patients: results from the FREEDOM trial**. *J Am Coll Cardiol* (2014) 64:1189-97

Vedanthan R, Choi BG, Baber U, Narula J, Fuster V. **Bioimaging and subclinical cardiovascular disease in low- and middle-income countries**. *J Cardiovasc Transl Res* (2014) 7:701-10

TRANSLATIONAL PROJECTS



AWHS
PESA, Grupo Santander and Fundación Botín
Polypill/CNIC-Ferrer
METOCARD-CNIC trial
TANSNIP

AWHS

The Aragon Workers Health Study (AWHS) is an ongoing project conducted in collaboration with the Instituto Aragonés de Ciencias de la Salud (IACS) and the General Motors factory in Zaragoza. The AWHS has been 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. Current planned follow-up will continue to 2018.

The initial visit consisted of a clinical examination, biochemical and hematologic tests and sample collection. Sample aliquots of serum, plasma, whole blood, DNA, and urine have been frozen and stored. All laboratory procedures conform to the ISO9001:2008 quality standard. After inclusion, workers' health data and biochemistry tests are collected at each annual health check-up.

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* in an open access journal to support a more focused future publication of the on-going research subprojects and to provide a clear description of the study to support fund-attracting strategies.

* 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) Jun 19: 12:45.

Additional external funding has been raised for the following sub-studies on the cohort, which are being conducted by CNIC-based researchers:

- Insulin resistance and inflammatory response to oxidative stress: Study of determinants and interactions (ISCIII CP08/112)
- Identification of the genetic determinants of mitochondrial DNA content in a working population, and its relationship with oxidative stress and subclinical atherosclerosis (ISCIII PI10/21)
- Cadmium exposure, metallothionein levels, and kidney disease in a General Motors assembly plant (Johns Hopkins NIOSH Education and Research Center Research Project Award)
- DNA methylation and the association of cadmium exposure with chronic kidney disease in a population-based occupational study (Johns Hopkins NIEHS Center in Urban Environmental Health Award)
- Polymorphism APOA2 -265T>C in relation to dietary patterns and cardiovascular risk factors (ISCIII PI11/403)

Selected Publications

Moreno Franco B, León Latre M, Andrés Esteban EM, Ordovás JM, Casasnovas JA, Peñalvo JL. **Soluble and insoluble dietary fibre intake and risk factors for metabolic syndrome and cardiovascular disease in middle-aged adults: the AWHS cohort.** *Nutr Hosp* (2014) 30:1279-1288

Sotos-Prieto M, Moreno-Franco B, Ordovás JM, León M, Casasnovas JA, Peñalvo JL. **Design and development of an instrument to measure overall lifestyle habits for epidemiological research: the Mediterranean Lifestyle (MEDLIFE) index.** *Public Health Nutr* (2014) 15:1-9

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De Castro-Orós I, Pérez-López J, Mateo-Gallego R, Rebollar S, Ledesma M, León M, Cofán M, Casasnovas JA, Ros E, Rodríguez-Rey JC, Civeira F, Pocoví M. **A genetic variant in the LDLR promoter is responsible for part of the LDL-cholesterol variability in primary hypercholesterolemia.** *BMC Med Genomics* (2014) 7:17

AWHS

Prevalence of subclinal atherosclerosis in the initial 587 AWHS participants completing all imaging procedures

	Age 40 - 50	Age 50 - 56	Overall
Age, years	47.0 (2.5)	52.9 (2.5)	50.0 (3.6)
Carotid plaque	46 (18.5)	194 (35.7)	240 (30.3)
Femoral plaque	101 (39.6)	317 (56.0)	418 (50.9)
Coronary calcium			
Agatston score >1 to 100	48 (20.0)	159 (30.5)	207 (27.2)
Agatston score >100	8 (3.3)	59 (11.3)	67 (8.8)

From Casasnovas et al. BMC Cardiovascular Disorders 2012 12: 45

PESA CNIC- GRUPO SANTANDER AND FUNDACIÓN BOTÍN

(Progression of Early Subclinical Atherosclerosis)

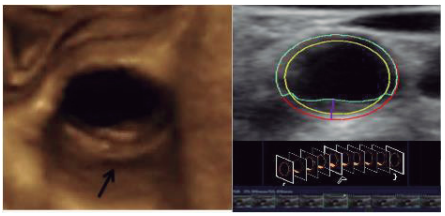
Strategies to identify individuals with subclinical alterations indicating increased risk of cardiovascular events have been boosted by the development of basic noninvasive imaging techniques (2D/3D vascular ultrasound and coronary calcium score by computed tomography) and advanced imaging techniques (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. Atherosclerotic disease in this group has already several decades of evolution and may be too advanced for prevention of future events.

The PESA CNIC-Grupo Santander and Fundación Botín is an ambitious clinical trial 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 (Figure 1), together with coronary artery calcium score by computed tomography (Figure 2). 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, advanced imaging assessment by ^{18}FDG PET/MRI technology was performed at the CNIC Advanced Imaging Unit during 2013 and 2014 in 938 individuals in whom a significant plaque burden was detected by ultrasound and CT. The study has also received approval for research into the association between atherosclerosis initiation/progression and telomere dysfunction and progerin expression in circulating leukocytes, and leukocyte samples have been collected from a subgroup of 1456 PESA participants for this analysis.

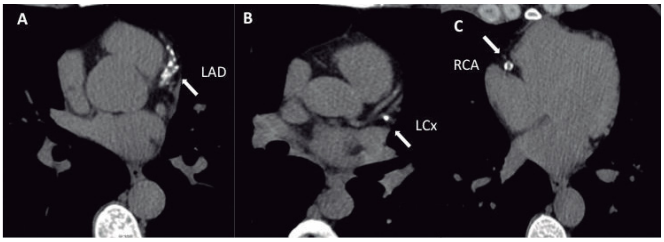
All PESA participants are followed-up at 3 and 6 years to assess the evolution of early phases of atherosclerosis and to determine how the detection of subclinical disease may impact the risk of future cardiovascular events. By the end of 2014, more than 1500 participants had already undergone the 3-year follow up visit (visit 2).

In May 2014 the PESA CNIC-Santander project received renewed ISO 9001:2008 certification from Bureau Veritas. This certification gives external recognition of the management quality and of data control and traceability.

In September 2014, the analysis and initial results from the baseline imaging studies were concluded and the article entitled "Prevalence, Vascular Distribution and Multi-territorial Extent of Subclinical Atherosclerosis in a Middle-Aged Population: The PESA (Progression of Early Subclinical Atherosclerosis) Study" was submitted for publication.



Novel 3D vascular ultrasound technology used in the PESA study to detect atherosclerotic plaques in the carotid artery, allowing volumetric quantification of plaque burden.



Non-contrast computed tomography images, showing coronary artery calcification in (A) the left anterior descending artery, (B) the left circumflex artery, and (C) the right coronary artery in a PESA participant.

Selected Publications

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

Polypill/CNIC-Ferrer

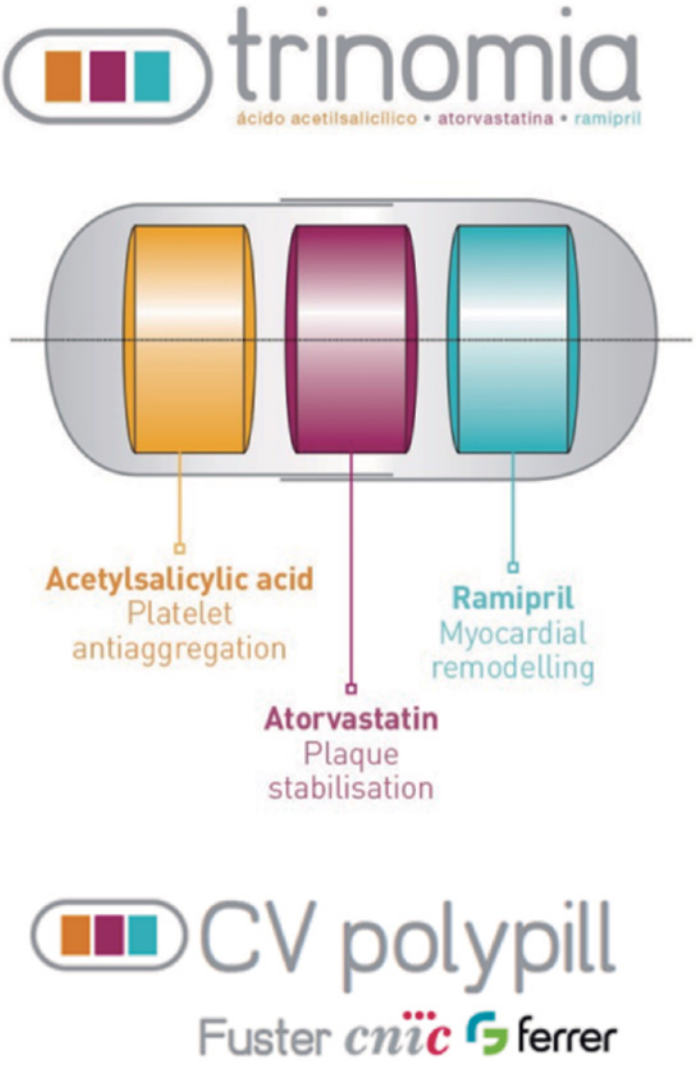
FOCUS Project

The Focus Project was supported by a grant from the Seventh European Framework program. Finalized in 2014, the results of the FOCUS Project were presented by Dr. Fuster at the European Society of Cardiology meeting in Barcelona and published in the Journal of the American College of Cardiology.

The FOCUS (Fixed Dose Combination Drug for Secondary Cardiovascular Prevention) project consisted of a cross-sectional study (phase 1) aimed at elucidating the factors that interfere with appropriate adherence to cardiovascular medication for secondary prevention after an acute myocardial infarction (AMI). Additionally, 695 patients from phase 1 were randomized into a controlled clinical trial (phase 2) to test the effect of a polypill (containing 100 mg aspirin, 40mg simvastatin, and 2.5, 5 or 10 mg ramipril) compared to the three drugs given separately. Tested outcomes were treatment adherence, blood pressure (BP) and serum low density lipoprotein cholesterol (LDL-C), as well as safety and tolerability over a 9-month follow-up.

In phase 1, a 5-country cohort (Argentina, Brazil, Italy, Paraguay, and Spain) of 2118 patients was analyzed. Patients were randomized to either the polypill or the three drugs separately for phase 2. The primary end-point was treatment adherence measured at the final visit by the self-reported Morisky-Green questionnaire (MAQ) and pill count (patients had to meet both criteria for adherence at the in-person visit in order to be considered adherent).

In phase 1, overall CV medication adherence, defined as a MAQ score of ≥20, was 45.5%. In a multivariable regression model, the risk of being non-adherent (MAQ<20) was associated with younger age, depression, being on a complex medication regimen, poorer health insurance coverage, and a lower level of social support, with consistent findings across countries.



In phase 2, the polypill group showed improved adherence compared to the group receiving separate medications after 9 months follow-up: 50.8% vs 41% (p=0.019; intention-to-treat population) and 65.7% vs 55.7% (p=0.012; per protocol population) for the primary endpoint (attending the final visit with MAQ=20 and an 80-110% pill count () combined, to assess adherence. Adherence was also higher in the FDC group when measured by MAQ alone (68% vs. 59%, p=0.049). No treatment difference was found at follow-up in mean SBP (129.6 vs 128.6 mmHg), mean LDL-C levels (89.9 vs 91.7 mg/dL), serious adverse events (23 [6.6%] vs. 21 [6%]) or death (1, 0.2% in each group).

In conclusion, for secondary prevention following an AMI, younger age, being depressed and following a complex drug treatment are associated with lower medication adherence, while adherence is higher in patients with higher levels of insurance cover and social support. Compared with the three drugs given separately, the use of a polypill strategy met the primary endpoint for adherence—increased self-reported and directly measured medication—for secondary prevention following an AMI.

Polypill/CNIC-Ferrer

AETNA/CNIC/Mt. Sinai/ Ferrer Consortium to study the clinical and economic impact of non-adherence to cardiovascular medication in a US population.

In October 2013, Aetna partnered with the CNIC among others to carry out a series of retrospective analyses using the Aetna Database to study the effect on major adverse cardiovascular outcomes on a post MI population of more than 4000 patients. The results of this study were presented at the Registry Hotline at the European Society of Cardiology in Barcelona. A manuscript based on this study will be submitted for review and publication in January 2015.

The same study was carried out in a diabetic population. The results of this study have been submitted for a presentation at the next American College of Cardiology meeting in March 2015.

A third study with a similar outline has been carried out, but in an atherosclerotic vascular disease population (comprised of participants who have had MI, stroke or peripheral artery disease). The results of this study are being analyzed and a manuscript will be submitted in January 2015.

Selected Publications

Castellano JM, Sanz G, Peñalvo JL, Bansilal S, 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 SC Jr, Taubert K, Pocock S, Brotons C, Farkouh ME, Fuster V. **A polypill strategy to improve adherence: results from the FOCUS project.** *J Am Coll Cardiol* (2014) 64: 2071–82

Castellano JM, Sanz G, Fuster V. **Evolution of the polypill concept and ongoing clinical trials.** *Canad J Cardiol* (2014) 30: 520-6

Castellano JM, Narula J, Castillo J, Fuster V. **Promotion of Global Cardiovascular Health: strategies, challenges, and opportunities.** *Rev Esp Cardiol (Engl Ed)* (2014) 67:724-30

Castellano JM, Peñalvo J, Bansilal S, Fuster V. **Promotion of cardiovascular health in three stages of life: never too early, never too late.** *Rev Esp Cardiol (Engl Ed*) (2014) 67:731-7

Castellano JM, Sanz G, Garrido E, Bansilal S, Fuster V. **A polypill strategy to improve global secondary cardiovascular prevention: from concept to reality.** *J Am Coll Cardiol* (2014) 64:613-21

Sanz G, Castellano JM, Fuster V. **Polypill: Chimera or Reality?** *Rev Esp Cardiol (Engl Ed)* (2014) 67:689-92

METOCARD-CNIC trial

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 pre-clinical data, CNIC lead the METOCARD-CNIC trial, the first randomized trial testing the effect of i.v. ß-blockers on infarct size in patients undergoing primary angioplasty.

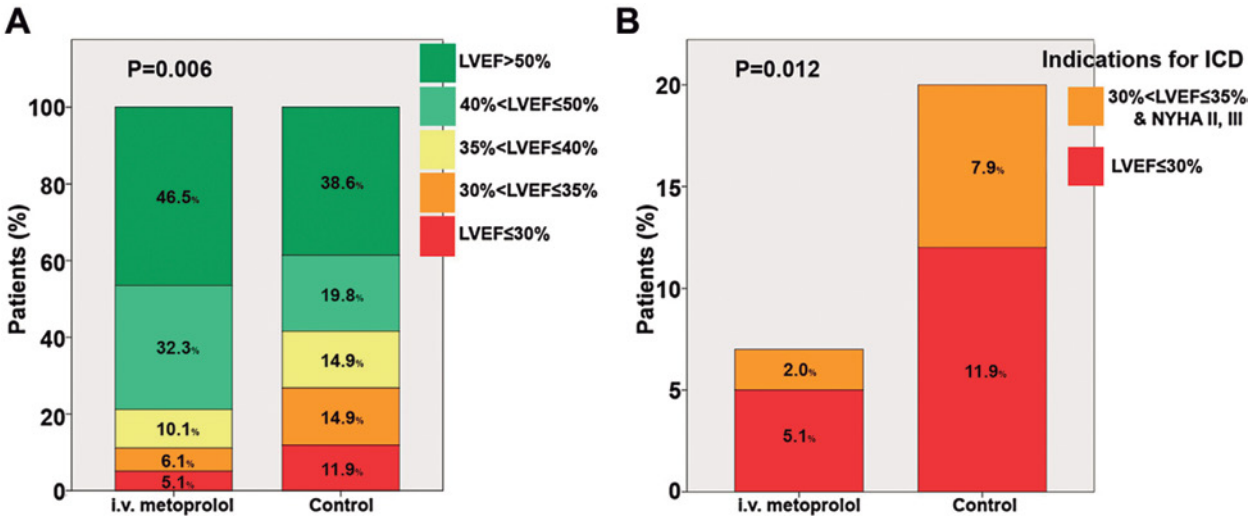
METOCARD-CNIC was a multicenter randomized clinical trial comparing the effect of early and delayed metoprolol initiation on infarct size and clinical events. The trial has already been completed, with a total of 270 patients recruited by the emergency medical services (55%) and participating hospitals (45%). A total of 220 patients underwent a magnetic resonance imaging (MRI) scan five days after infarction, and 202 patients underwent follow-up MRI at six months. All patients underwent one year clinical follow-up. Studies of patients recruited in Madrid were performed at the CNIC's human imaging facility, where the advanced imaging protocol is performed with a novel cutting-edge MRI system. MRI scan data were analyzed in a core laboratory at the CNIC.

The primary endpoint of the trial (infarct size as evaluated by MRI) was published in 2013 (Circulation 2013;128:1495-503), and the follow-up of the study was published in 2014 (J Am Coll Cardiol. 2014;63:2356-62). In summary, the administration of early metoprolol before reperfusion resulted in a very significant reduction of infarct size (see Figure). This effect was seen along with a significant increase in left ventricular ejection fraction. Follow-up MRI studies revealed that patients receiving metoprolol had a significant reduction in the incidence of severe heart dysfunction, along with a significant reduction in hospital readmission due to heart failure.

METOCARD-CNIC is the result of a multidisciplinary effort requiring close cooperation between investigators at the CNIC, hospitals across Spain, and, importantly, the emergency medical services. Hospitals participating in the METOCARD-CNIC trial are Hospital Clínico San Carlos, Puerta de Hierro, Hospital de la Princesa, Hospital 12 de Octubre and Hospital Quirón in Madrid, Hospital Meixoeiro in Vigo, Hospital Marqués de Valdecilla in Santander, and Hospital de León. Emergency medical services actively participating as co-investigators are SUMMA112, 061 Galicia, and SAMUR. The randomization center was located in the headquarters of SUMMA112 and was run 24/7 by trained full time staff.

CNIC is already working in the design of a multinational clinical trial, the MOVE ON! Trial. This trial will follow the same design of METOCARD-CNIC but will be powered to detect differences in clinical endpoints (mortality, heart failure, and arrhythmias). The MOVE ON! Trial will be conducted in several European countries.

Follow-up LVEF categories and indications for ICD according to treatment allocation



(A) Distribution of patients according to LVEF categories. B) Rate of formal indication (Class I recommendation in clinical guidelines) for an implantable cardioverter defibrillator. (from *J Am Coll Cardiol* 2014;63:2356-62: METOCARD-CNIC follow-up publication.)

METOCARD-CNIC trial



Members of the METOCARD-CNIC research group.

Selected Publications

Pizarro G, Fernández-Friera L, Fuster V, Fernández-Jiménez R, García-Ruiz JM, García-Álvarez A, Mateos A, Barreiro MV, Escalera N, Rodriguez MD, de Miguel A, García-Lunar I, Parra-Fuertes JJ, Sánchez-González J, Pardillos L, Nieto B, Jiménez A, Abejón R, Bastante T, Martínez de Vega V, Cabrera JA, López-Melgar B, Guzman G, García-Prieto J, Mirelis JG, Zamorano JL, Albarrán A, Goicolea J, Escaned J, Pocock S, Iñiguez A, Fernández-Ortiz A, Sánchez-Brunete V, Macaya C, Ibanez B. **Long-term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction).** *J Am Coll Cardiol* (2014) 63: 2356-62

Ibanez B, Macaya C, Sánchez-Brunete V, Pizarro G, Fernández-Friera L, Mateos A, Fernández-Ortiz A, García-Ruiz JM, García-Álvarez A, Iñiguez A, Jiménez-Borreguero J, López-Romero P, Fernández-Jiménez R, Goicolea J, Ruiz-Mateos B, Bastante T, Arias M, Iglesias-Vázquez JA, Rodriguez MD, Escalera N, Acebal C, Cabrera JA, Valenciano J, Pérez de Prado A, Fernández-Campos MJ, Casado I, García-Rubira JC, García-Prieto J, Sanz-Rosa D, Cuellas C, Hernández-Antolín R, Albarrán A, Fernández-Vázquez F, de la Torre-Hernández JM, Pocock S, Sanz G, Fuster V. **Effect of early metoprolol on infarct size in ST-segment–elevation myocardial infarction patients: The effect of metoprolol in cardioprotection during an acute myocardial infarction (METOCARD-CNIC) trial.** *Circulation* (2013) 128: 1495-503

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

The TAN SNIP study includes 4 projects whose unifying purpose is to develop a model for improved risk stratification based on the detection, quantification and characterization of subclinical atherosclerosis. The goal is for this improved risk stratification to enable novel targeted therapies and risk reduction strategies. This transatlantic network brings together expertise and resources, aligning (a) leaders from complementary fields (imaging, biomarkers, and population sciences), (b) existing patient cohorts, (c) state-of-the-art imaging resources and know-how, and (d) sophisticated biomarker platforms.

PURPOSE AND AIMS

Existing tools for characterizing atherosclerosis and determining the risk of its complications are inadequate. 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 treatments for high-risk populations with manifest disease are administered almost indiscriminately. This leads to high 'numbers-needed-to-treat' (NNT), unnecessary patient risk, wasted resources and unsustainable costs for health care providers.

AIM 1. In a relatively low-risk population (the PESA-CNIC cohort), we will study whether a personalized worksite-based lifestyle intervention, driven by imaging data (3D-ultrasound of carotid and ilio-femoral arteries, and coronary calcification) can engender behavioural changes, improved control of risk factors, and reduced progression of subclinical atherosclerotic plaque burden (SAPB).

Output: Non-pharmacological worksite lifestyle intervention validated against modification of conventional risk factors.

AIM 2. We will evaluate the predictive value of the Framingham risk score (FRS) and SAPB on the prevalence of microvascular/parenchymal brain changes (MPBC) and the incidence of cerebrovascular disease (CD) and cerebrovascular events (CVE) in asymptomatic individuals with a varying extent of CV risk factors and SAPB. This study will be carried out at the Icahn School of Medicine at Mount Sinai (ISMMS), New York. We will quantify SAPB by 3D-US of the carotid arteries (CA) and ilio-femoral arteries (IFA) in participants with no cardiovascular symptoms. MPBC will be detected through a combination of 1) MRI to assess microvascular perfusion and parenchymal changes in structural and functional brain connectivity and 2) 18F flumetamol/florbetapir-PET to quantify parenchymal amyloid deposition. CD will be assessed by standard neurophyschological tests.

Output: Understanding, achieved through cutting-edge imaging analysis, of the complex interaction between FRS, SAPB, MPBC and CD.

AIM 3. In an intermediate-risk population (the HRP-USA population) we will validate the added value of SAPB quantification (vascular 3-D ultrasound of carotid and ilio-femoral arteries) on top of classical risk factors (FRS) for predicting SAPB progression/regression and CVD events (CVDE).

Output: A cost-effective method to determine and monitor SAPB and determine its incremental value over FRS, and to increase understanding of the natural history of SABP over a 5-year period.

AIM 4. In the 3 complementary cohorts detailed above, we will validate recent proteomic and metabolomic discoveries related to major atherosclerotic CVDE and the metabolic risk factors for CVD identified by the Framingham Heart Study (FHS). This will allow the development of improved CVD risk assessment methods that incorporate cutting-edge 'omics' discoveries made by the FHS as well as state-of-the-art imaging results from the TAN SNIP network in conjunction with established CVD risk factors.

Outputs: Validation of FHS-derived novel biomarker panels ('omics') in 3 populations at graded cardiovascular risk. Novel algorithms that incorporate 'omics', SAPB, and established FRS criteria to improve personalized risk assessment.

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

AIM 1 (the PESA-CNIC cohort):

The study population for this part of the TAN SNIP study consists of participants in the PESA study: employees aged 40 to 54 years of the Banco de Santander Headquarters in Madrid (Spain). Two parallel randomized controlled trials (RCT) will be conducted within the PESA cohort population. One RCT will focus on a sample of employees with high imaging-defined CV risk, whereas the second RCT will be conducted on a sample with low imaging-defined CV risk. In both RCTs, the participants will be randomized to receive a comprehensive 3-year worksite lifestyle intervention or standard occupational health care. The worksite-based lifestyle intervention program will consist of three elements: (A) twelve 1-hour sessions of personalized lifestyle counseling ; (B) provision of a pedometer for self-monitoring of physical activity (Polar) ; and (C) use of a sit-to-stand workstation (optional). Data will be collected at baseline (concurrent with PESA Visit 1) and at follow-up at 1 year (T1), 2 years (T2), and 3 years (T3; concurrent with PESA Visit 3). Primary outcome measures are CVD risk assessment and MVPA. Secondary outcomes are physical activity, sedentary behavior, standing behavior, diet, smoking, vitality and quality of life and risk factor profiles, as well as specific changes in anthropometric measures, blood biomarkers, work-related outcomes (including work productivity and sickness absenteeism). A process evaluation and a cost-effectiveness study will be conducted during the intervention.

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 MVPA, compared to standard practice. We further predict that the level of compliance with the worksite-based lifestyle intervention will be higher in the high imaging-defined CV risk group than in the low imaging-defined CV risk group.

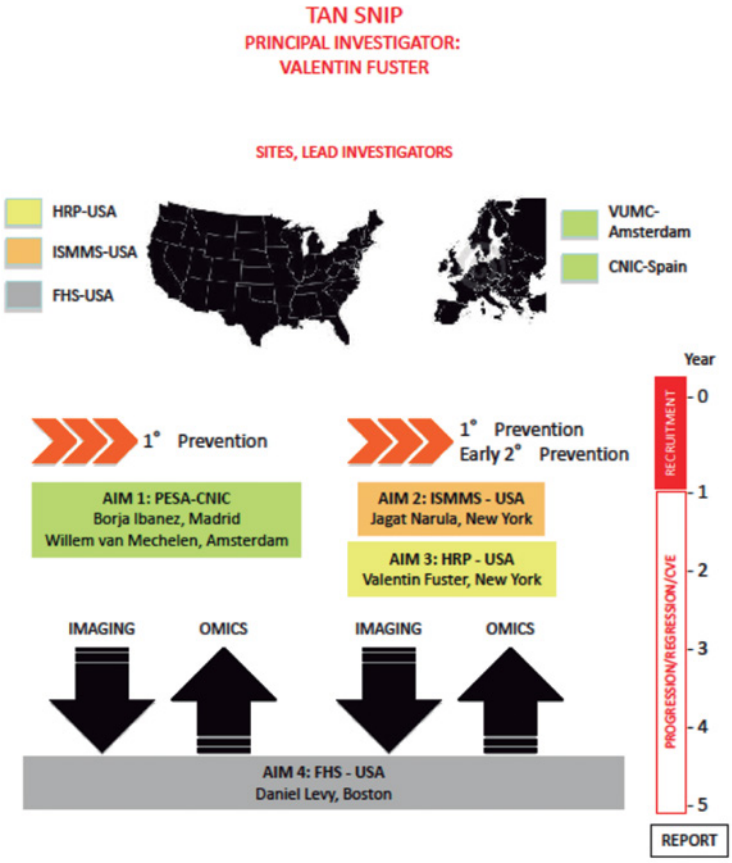


Figure A

TRANSLATIONAL PLATFORM

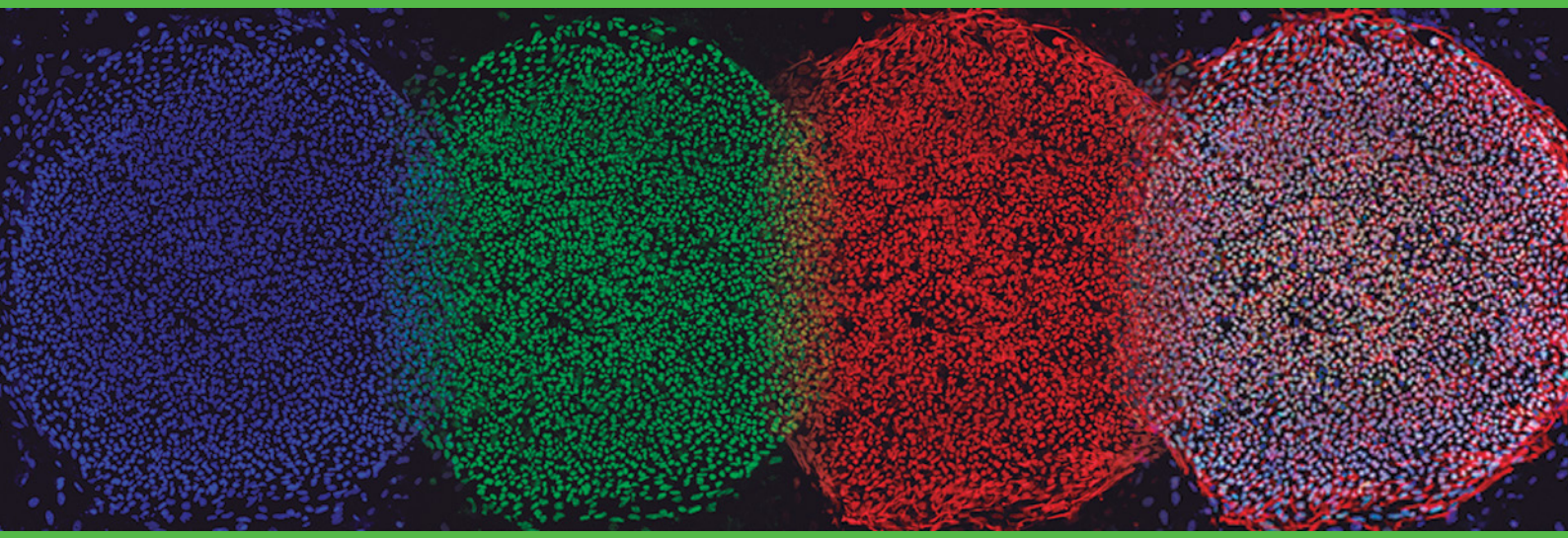


Technology Transfer & Translational Research Platform

The Technology Transfer & Translational Research Platform runs initiatives that foster translational research at the CNIC, in Spanish clinical facilities, and with international partners. The Platform also identifies, promotes, and co-develops CNIC research with potential for industrial application by facilitating the granting of patents and their subsequent development or licensing.

The activity of the Technology Transfer & Translational Research Platform is divided into the following areas: Technology Development Unit, Technology Transfer Office, Projects Office, Biobank, and Translational Research & Epidemiology.

TECHNICAL UNITS



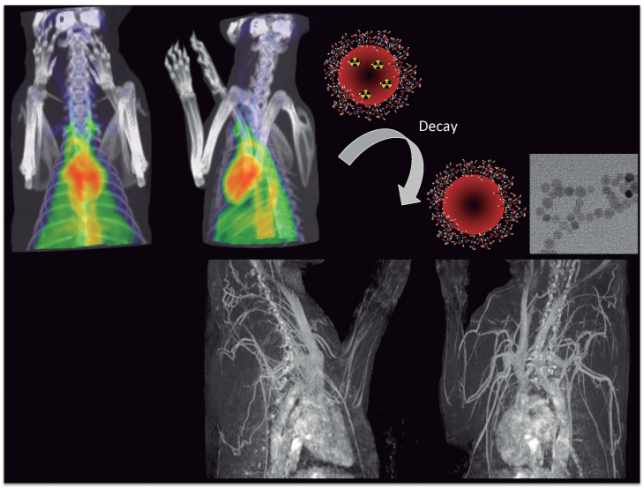
Advanced Imaging
Bioinformatics
Cellomics
Comparative Medicine
Genomics
Microscopy and Dynamic Imaging
Pluripotent Cell Technology
Proteomics
Transgenesis
Viral Vectors



Advanced Imaging

RESEARCH INTEREST

The Advanced Imaging Unit (AIU), established in early 2012, 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. 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 (bi and tri-dimensional luminescence and fluorescence). For its nanomedicine and radiochemistry program, the AIU has a dedicated nanotechnology and organic chemistry laboratory in which we develop new nanoparticles, molecular probes, and biofunctionalization techniques for the diagnosis and treatment of different cardiovascular diseases. Currently the unit produces multifunctional nanoparticles for all imaging techniques available at the CNIC—including iron oxide, liposomes, up-converting nanophosphors and gold nanoparticles—all of them functionalized with specifc cardiovascular biomarkers. The unit's radiochemistry laboratory is now fully operative for ^{68}Ga (and since early 2014) ^{89}Zr, providing the Center with specific PET radiotracers for cardiovascular nuclear imaging. The unit also has long experience in the study of disease by metabolic data analysis using magnetic resonance spectroscopy and mass spectrometry and statistical tools developed by the group. Our research in this area ranges from technical developments and chemistry advances to *in vitro* studies and tracking of biological processes *in vivo*.



Intravenous injection of dual (PET-MRI) single core nanoparticles. ^{68}Ga was inserted into the iron oxide core of nanoparticles, which were intravenously injected into a rabbit.



Head of Unit
Dr. Jesús Ruiz-Cabello Osuna

Postdoctoral Researchers:
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Jesús Mateo de Castro
Samuel España
Teresa Arias

Predoctoral Researchers:
Ana Victoria Lechuga
Hugo Groult
Juan Pellico
Riju Bhavesh
Ehsan Yazdanparast

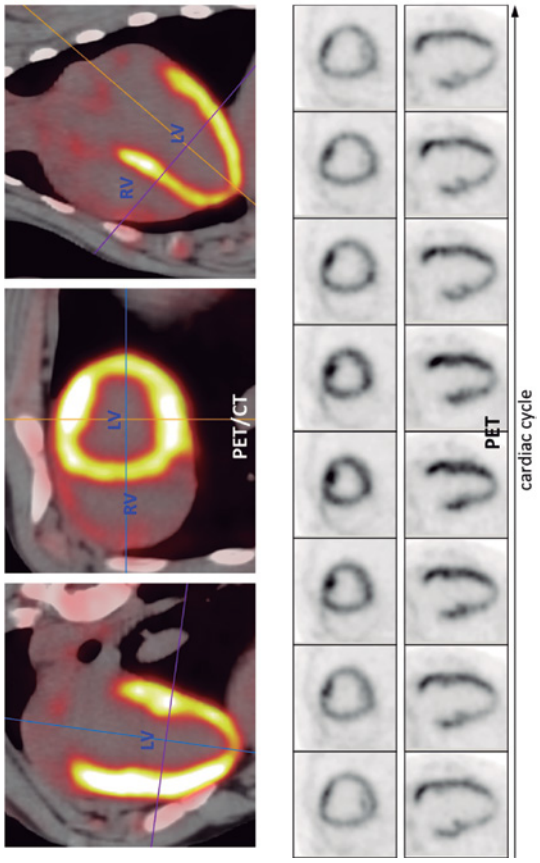
Technicians:
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Marina Benito
Coral Velasco

CardioImage Fellows:
Carlos Pérez Medina
Jose Manuel Pérez Sánchez

Res@CNIC Fellows:
Alberto Ullate de la Torre
Jorge Sanz Sánchez
Juan Carlos Gómez

Masters Students:
Adriana Mota
Mario Martín Veganzones

Visiting Scientists:
Ignacio Rodríguez
Palmira Villa
Marco Filice
Sandra Pérez Rial
Arnoldo de Jesús Santos Oviedo
José Gabriel Venegas



Study of cardiac metabolism using FDG-PET. Upper images show the long and short axis views of fused non-gated PET and CT images acquired in a pig animal model. Lower images show the short and long axis views of the ECG-gated PET images (8 bins per cardiac cycle).

MAJOR GRANTS

- **Ministerio de Sanidad y Consumo (CIBERES CB06/06/1090)**
- **European Commission FP7-PEOPLE-2010-ITN (Π-NET 264864) (NO CNIC)**
- **European Commission FP7-PEOPLE-2013-ITN (CardioNext PITN-GA-2013-608027)**
- **Fundació La Marató TV3 (70/C/2012) PI WP2, Borja Ibañez, Colaborador Jesus Ruiz Cabello**
- **Ministry de Economía y Competitividad. FIS RETICS (Terapia Celular: RD12/0019/0005) PI: Miguel Torres, Colaborador Jesus Ruiz Cabello**
- **Ministry de Economía y Competitividad. Modalidad Generación Conocimiento (MAT2013-47303-P) PI: Fernando Herranz**
- **Ministry de Economía y Competitividad. FIS (PI14/01427) PI: Jesús Mateo**
- **Madrid-MIT M+Visión (PRMIT2013) PI: Samuel España**
- **Madrid-MIT M+Visión (MIT14) PI: Teresa Arias**

Selected Publications

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. **Phosphatidylcholine-Coated Iron Oxide Nanomicelles for In Vivo Prolonged Circulation Time with an Antibiofouling Protein Corona**. *Chem Eur J* (2014) 20:16662-71

Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, Nácher M, Pitaval C, Radovanovic I, Fukui Y, McEver RP, Filippi MD, Lizasoain I, Ruiz-Cabello J, Zarbock A, Moro MA, Hidalgo A. **Neutrophils scan for activated platelets to initiate inflammation**. Science (2014) 346:1234-8

Pérez-Medina C, Abdel-Atti D, Zhang Y, Longo VA, Irwin CP, Binderup T, Ruiz-Cabello J, Fayad ZA, Lewis JS, Mulder WJ, Reiner T. **A modular labeling strategy for in vivo PET and near-infrared fluorescence imaging of nanoparticle tumor targeting**. *J Nucl Med* (2014) 55:1706-11

Mateo J, Izquierdo-Garcia D, Badimon JJ, Fayad ZA, Fuster V. **Noninvasive assessment of hypoxia in rabbit advanced atherosclerosis using ^{18}F-fluoromisonidazole positron emission tomographic imaging**. *Circ Cardiovasc Imaging* (2014) 7:312-20

España S, Marcinkowski R, Keereman V, Vandenberghe S, Van Holen R. **DigiPET: sub-millimeter spatial resolution small-animal PET imaging using thin monolithic scintillators**. *Phys Med Biol* (2014) 59:3405-20



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

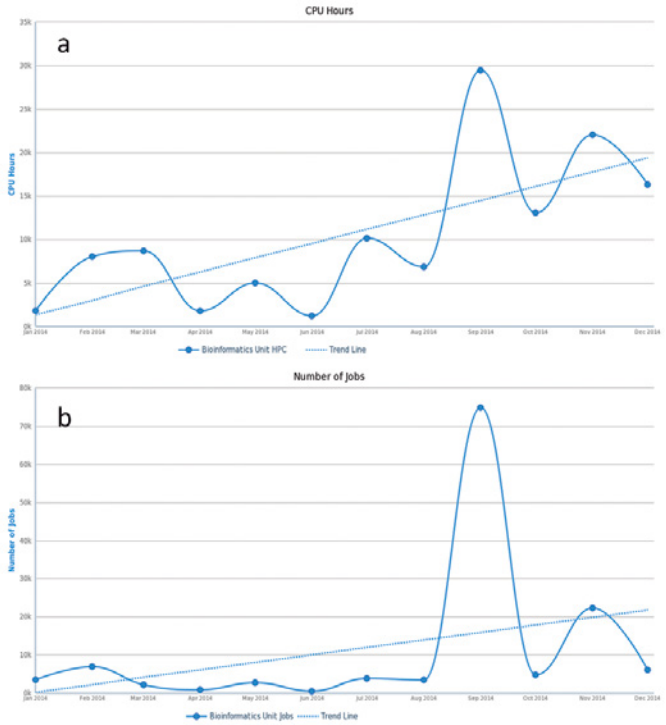
The CNIC Bioinformatics Unit was established in the last quarter of 2010. The main goal of the Unit is to establish a collaborative environment within which to contribute to CNIC research projects, thereby providing CNIC researchers with *ad-hoc*, state-of-the-art bioinformatic and computational biology solutions to support and enhance their research.

In the last year, the Bioinformatics Unit has established four new pipelines using state-of-the-art algorithms for the analysis and interpretation of high-throughput biological data: 4C-Seq; alternative splicing detection from RNA-Seq data; expression profiling plus GO Enrichment and GSEA from RNA-Seq data; and SNP detection with STAR-GATK from RNA-Seq data.

To accommodate increasing demand, the Unit's infrastructure grew in 2014 with the installation of a new 80 TB HPC storage EMCIsilon Solution™ and, starting at the beginning of 2015, installed HPC clusters are being upgraded from 246 threads and 700 GB of RAM to 1736 threads and 5TB of RAM. Another main focus during 2014 and into the New Year has been the implementation of the Bioinformatics Unit Galaxy Platform. This platform enables CNIC researchers to analyze their data using the pipelines developed by the Bioinformatics Unit through Galaxy, bringing them closer to their data.

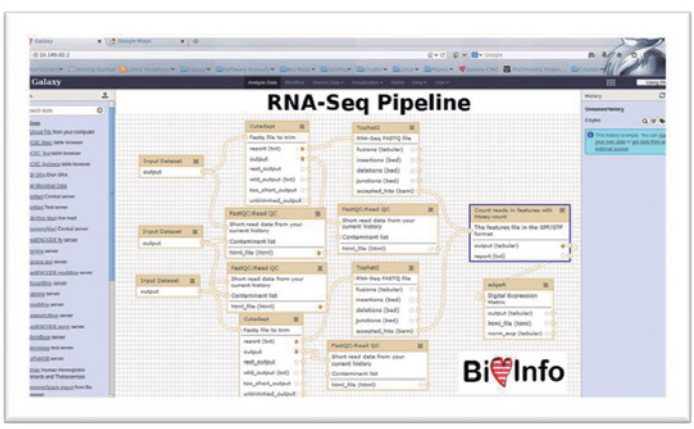
The Unit has also continued to provide customized advice and training to CNIC researchers in the analysis and interpretation of their experimental data. We also participated in the doctoral training at the Medical University of Bialystok in Poland.

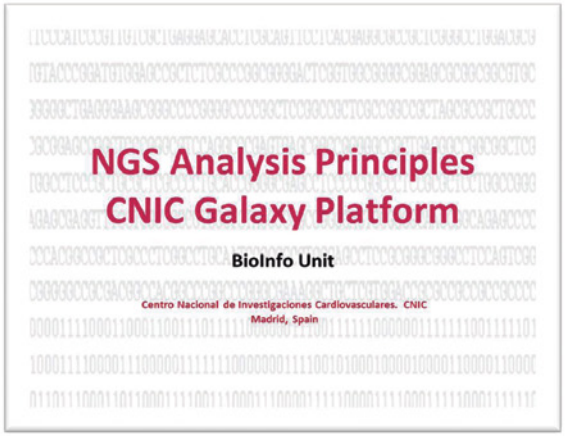
The Unit is closely involved in providing support and guidance to new PhD students working on bioinformatics projects at the CNIC, and co-directs 3 PhD projects.



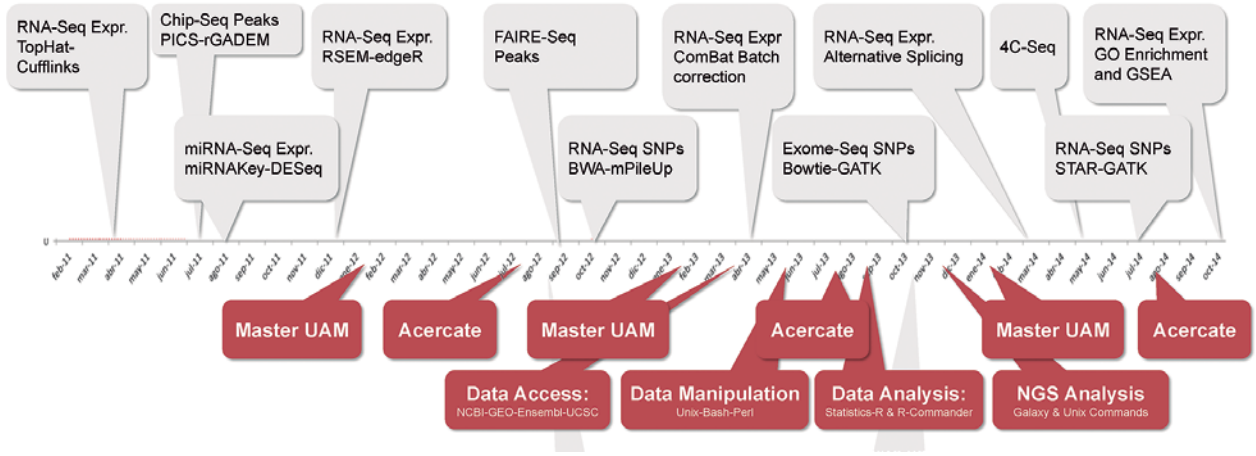
a. **Bioinformatics Unit HPC:** Summary of total CPU hours (number of CPU cores x wall time hours, excluding non-Bioinformatics Unit usage of the resource) from Jan 1 to Dec 1, 2014.

b. **Bioinformatics Unit Jobs:** Summarizes the total number of running Bioinformatics Unit jobs (excludes non-Bioinformatics Unit usage of the resource) from Jan 1 to Dec 1, 2014.





Bioinformatics Unit Galaxy Platform
Toward the end of 2014 the Unit began implementing its Analysis Pipelines into an in-house Galaxy Platform and will provide training sessions for researchers to involve them more closely in the analysis of their data.



Top: Bioinformatics Unit Analysis Pipelines under constant development
Bottom: The Bioinformatics Unit's training activities

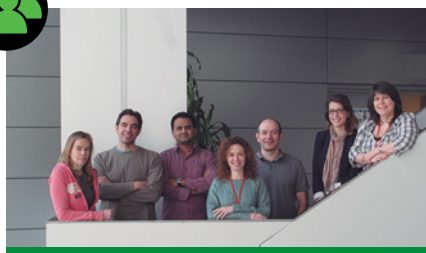
Selected Publications

Isern J, Garcia-Garcia A, Martin AM, Arranz L, Martin-Perez D, Torroja C, Sanchez-Cabo F, Mendez-Ferrer S. **The neural crest is a source of mesenchymal stem cells with specialized hematopoietic stem-cell-niche function.** *eLife* (2014) e03696

Gatto A, Torroja-Fungairino C, Mazzarotto F, Cook SA, Barton PJ, Sanchez-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



Cellomics



Head of Unit
María Montoya

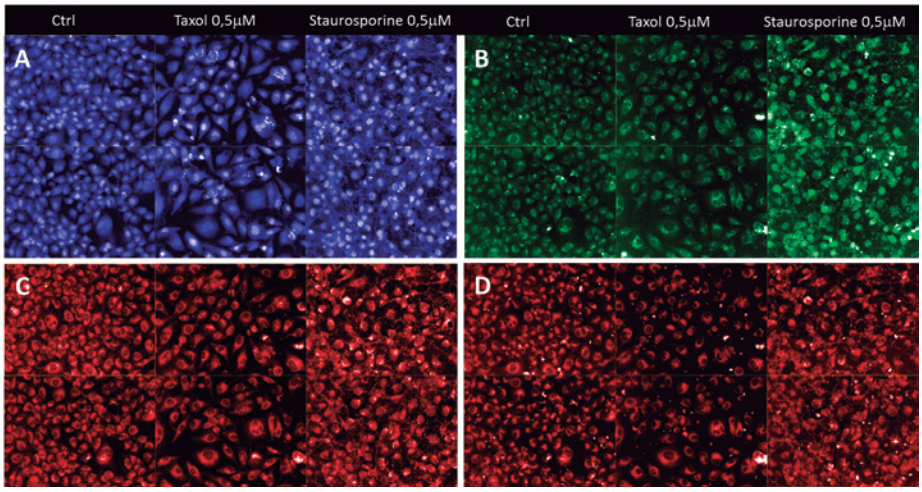
Support Scientists:
José Manuel Ligos
Laura Fernández
Gopal Karemore/Daniel Jiménez

Technicians
Raquel Nieto
Mariano Vitón
Irene Palacios Doiztua

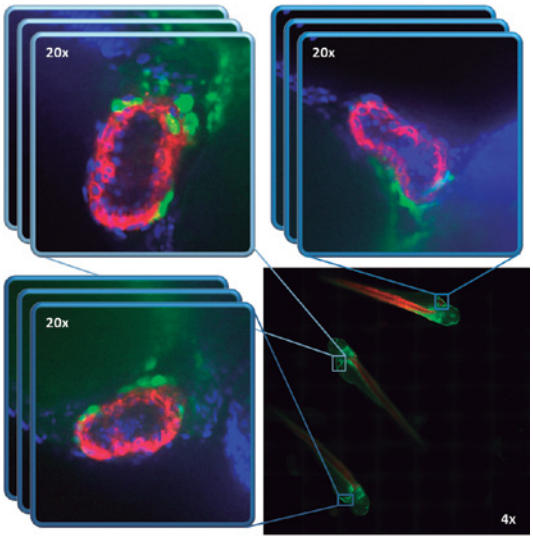
RESEARCH INTEREST

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. The Unit has expanded its sorting capabilities with the acquisition of an automated magnetic sorter (Automacs). Daniel Jiménez, a young mathematician, computer and biomedical engineer with PhD training in biomedical image processing and analysis from UPM Madrid and BWH Boston, joined Cellomics Unit in November, replacing Gopal Karemore.

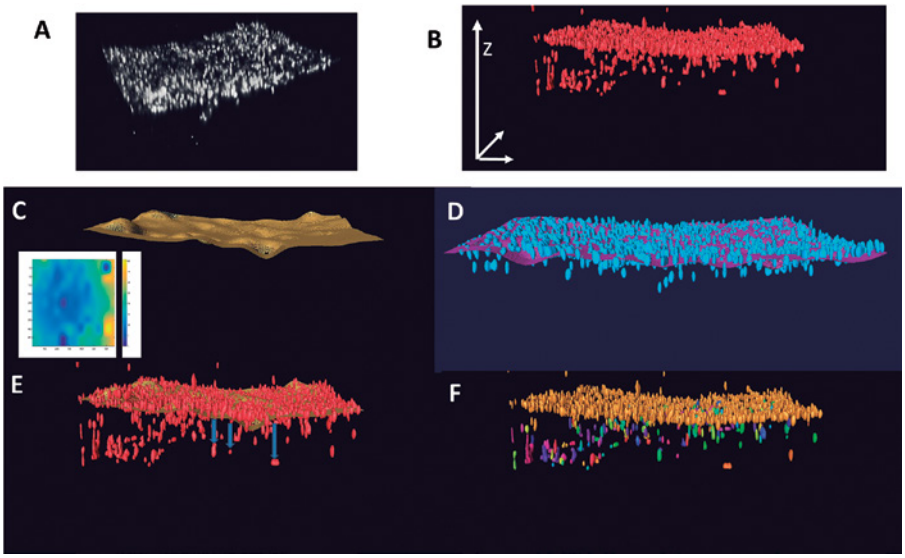
In 2014 we successfully completed a drug repurposing screen to identify FDA-approved drugs that regulate Cav1 expression in pancreatic cancer associated fibroblasts, in partnership with the Integrin Signaling group at the CNIC and the Clinical Research and Experimental Therapeutics Programs at the CNIO. A secondary/validation screen is currently in progress. The Unit has also developed an automated multiparametric assay for toxicity assessment (Fig. 1) that has been used for in vitro toxicity testing of nanoparticles in partnership with the Advanced Imaging Unit. Our Unit has also programmed a script for automatic scanning of high resolution Z stacks of zebrafish embryo hearts using the Opera imaging device. This work formed part of the development of an HCS assay in collaboration with the Development of the Epicardium and its Role during Regeneration group at the CNIC (Fig.2). The Unit has also developed customized image analysis tools for a variety of purposes, for example quantification of tumor cell invasion in 3D matrices (Fig 3), detection of candida-infected cells and proliferating cells from immunofluorescence tissue images, and quantification of second harmonic microscopy images.



***In vitro* toxicity assay**. EA.hy926 cells were treated with 0.5 mM taxol or staurosporine or left untreated (control). A) DAPI stain. B) Staining with a fluorescent substrate for activated caspases 3 and 7. C) Mitotracker. D) Cell Rox. The figure shows four images per field obtained from duplicate wells using the Opera automated imaging device.



Prescan-rescan script for automatic acquisition of high resolution 3D images from zebrafish embryos. Software was developed to allow the Opera System to automatically detect heart locations on 2D low resolution (4x) images and acquire 3D high resolution (20x) images from multiple zebrafish embryos on multiwell plates. Images show staining with fluorescent markers of epicardium (green) and myocardium (red) and nuclear DAPI (blue) as overlaid images obtained at the magnifications indicated



Analysis of tumor cell invasion through 3D matrices. Tumor cells were loaded onto 3D collagen matrices and allowed to invade for 5 days, then fixed and stained with DAPI. A) DAPI staining raw image. B) Nuclear segmentation. C) Top layer estimation from spatial sampling and cubic interpolation of the maximum Z coordinate values of the nuclei centroids. D) Top layer and overlaid cell nuclei segmentation. E) Sub-pixel cell-by-cell Z penetration calculation (distance to top layer in microns). F) Classification of cells as non invaders (brown) and invaders (colors).

Selected Publications

Azegrouz H, Karemore G, Torres A, Alaíz CM, Gonzalez AM, Nevado P, Pellinen T, del Pozo MA, Dorronsoro JR, Montoya MC. **Cell-based imaging- and biology- metrics enhances HCS assay robustness.** *J Biomol Screen* (2013) 1810: 1270-83

Fernandez-de-Manuel L, Wollny G, Kybic J, Jimenez-Carretero D, Tellado JM, Ramon E, Desco M, Santos A, Pascau J, Ledesma-Carbayo MJ. **Organ-focused mutual information for nonrigid multimodal registration of liver CT and Gd–EOB–DTPA-enhanced MRI.** *Med Image Anal* (2014) 18:22-35

Ding H, Fernandez-de-Manuel L, Schär M, Schuleri KH, Halperin H, He L, Muz Zviman M, Beinart R, Herzka DA. **Three-dimensional whole-heart T2 mapping at 3T.** *Magn Reson Med* doi: 10.1002/mrm.25458 19 SEP 2014 [Epub ahead of print]

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* (2014) 19:187-202

Maška M, Ulman V, Svoboda D, Matula P, Matula P, Ederra C, Urbiola A, España T, Venkatesan S, Balak DM, Karas P, Bolcková T, Streitová M, Carthel C, Coraluppi S, Harder N, Rohr K, Magnusson KE, Jaldén J, Blau HM, Dzyubachyk O, Křížek P, Hagen GM, Pastor-Escuredo D, Jimenez-Carretero D, Ledesma-Carbayo MJ, Muñoz-Barrutia A, Meijering E, Kozubek M, Ortiz-de-Solorzano C. **A benchmark for comparison of cell tracking algorithms.** *Bioinformatics*. (2014) 30:1609-17

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 source of non-representative data, or have a major impact of the 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 scanner 3001 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.

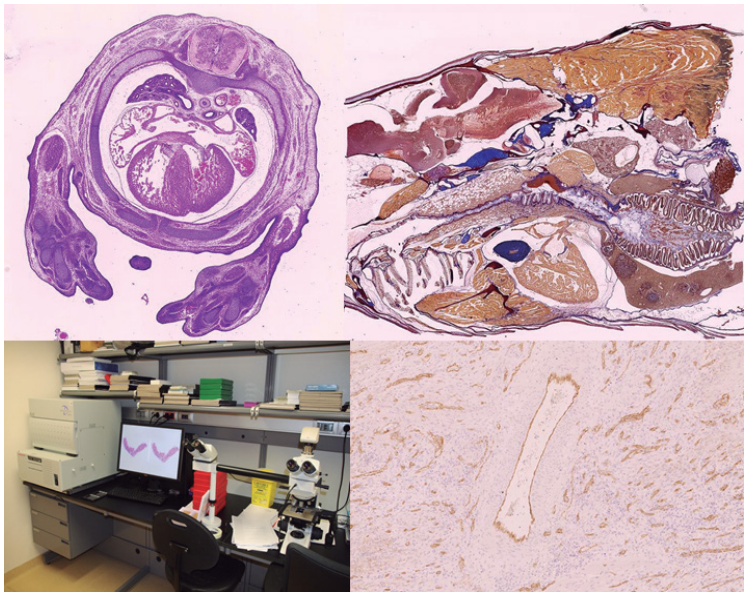
- *Veterinary Medicine and Experimental Surgery Core* (VMESC). The VMESC provides highly specialized expertise in the surveillance and monitoring of animal health status, disease follow-up, development of surgical animals models with enphasis on minimally invasive procedures, life support, and setting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

- *Quality Control Core* (QCC). The QCC follows the recommendation the last FELASA report (Laboratory Animals 2014, Vol. 48(3): 178-192).

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



Coagulation analyzer



1. Thoracic section of a 16.5 day mouse embryo, stained with H&E. **2**. Section of a zebrafish whole-body stained with Acid Fuchsin Orange G. **3**. Hamamatsu scanner for histological sample digitalization. **4**. Immunostaining of the CD31 marker to highlight the newly formed vessels in the granulation tissue of a pig myocardial infarct.



Metabolic Cages System



Genomics



Head of Unit
Ana Dopazo

Support Scientists:
Sergio Callejas
Alberto Benguría

Technician:
Rebeca Álvarez

RESEARCH INTEREST

During the last 4 years, the Genomics Unit has focused on second generation sequencing (NGS) technologies for genome analysis using an Illumina Genome Analyzer IIx sequencer. In 2014 the Unit expanded its sequencing capacity by acquiring a new Illumina HiSeq 2500 sequencer. In addition to its larger production power, the HiSeq 2500 delivers a lower cost per sample across most of the applications.

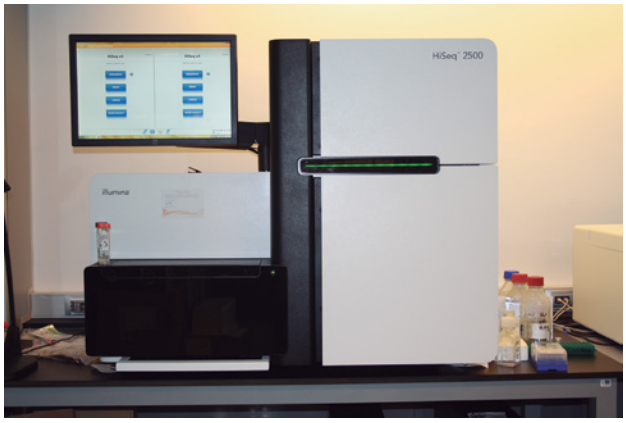
The Unit provides these cutting-edge genomic technologies to the scientific community at the CNIC and beyond, offering a wide variety of NGS applications (RNA Seq, Low input RNA Seq, small RNA-Seq, ChIP Seq, PCR Seq, Exome Sequencing, targeted resequencing, etc). On each sequencing project the Unit's tasks include project consultation, sample quality check, sample library preparation, and data generation. At least 3 of the CNIC top scientific publications in 2014 include NGS experiments performed in the Genomics Unit.

The team is also very much committed to continuing to improve methods for low-input RNA Seq applications, in which RNA Seq can be performed from tiny amounts of starting biological material or even directly from cells.

We are also automating the newly incorporated NGS library preparation protocols by using an open liquid handling platform. Automation of this step avoids the bottleneck created by the high sample number typically included in sequencing runs, and also reduces the risk of human error during this step.

On request, the Unit continues to offer microarray analysis services using the Agilent microarray platform. 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 genomic services, the Unit performs its own research.



Liquid handling platform used for the automation of Illumina-NGS steps

MAJOR GRANTS

- Ministerio de Economía y Competitividad. FIS (PI10/01124)

Selected Publications

Callejas S, Alvarez R, Benguria A and Dopazo A. **AG-NGS: A powerful and user-friendly computing application for the semi-automated preparation of next-generation sequencing libraries using open liquid handling platforms.** *Biotechniques* (2014) 56: 28-35

Hill R, Kalathur R, Callejas S, Colaco L, Brandao R, Serelde B, Cebria A, Blanco-Aparicio C, Pastor J, Futschik M, Dopazo A and 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
Veronica Labrador Cantarero

Visiting Scientists:
Antonio Trullo
Andrea Orsi
Mª Eugenia Pérez-Ojeda Rodríguez

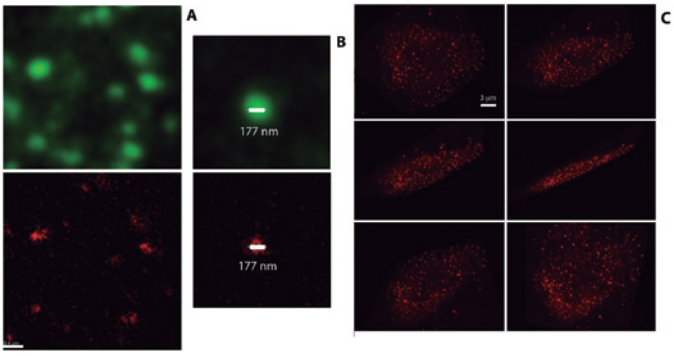
Visiting Student:
Irene Hernández Lozano

RESEARCH INTEREST

2014 saw a major technological development with the installation of a nanoscopy plaftform and the acquisition of a single plane illumination microscopy (SPIM) system. The nanoscopy platform includes an innovative gated STED-3X microscope and a system for single molecule localization by ground state depletion (dSTORM). The platform allows a comprehensive approach to super-resolution imaging using a variety of fluorescent probes, ranging from spectral variants of the fluorescent protein to standard chemical dyes. The customized SPIM system joins the state-of-art confocal and multiphoton microscopes already in use, completing a large portfolio of imaging modalities capable of imaging individual biomolecules in cells as well as sub-micron compartments in living model organisms. To maximize the performance of this portfolio, we have also dedicated considerable effort to developing sofware packages and protocols suitable for handling and analyzing large 3D-image files.

The considerable technological expansion of the Unit has laid the groundwork for new collaborative research projects with internal and external partners that have been granted funding by national and international funding agencies. For example, in a project in partnership with the Institute of General Organic Chemistry (CSIC) and the San Raffaelele Foundation in Milan, the Unit is designing novel fluorophores for increased perfomance in super-resolution microscoscopy to reveal the sub-structure of IRE1 proteins rearranged into composite domains in the ER membrane as sensor of cellular stress.

The Unit continues its intensive individual theoretical and practical training activities and workshops, and contributes to the CNIC-JOVEN training plan through the ACERCATE, CICERONE and the Master Programs.



IRE1 domains in the endoplasmic reticulum of HeLa cells during the unfolded protein response. (A) Confocal image (top) and STED image (bottom). (B) single domain zoom in confocal (top) and STED (bottom) resolution. (C) 3D-STED gallery of IRE1 domains in a whole cell.

MAJOR GRANTS

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

Selected Publications

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**. *Bioconjugate Chem* (accepted)

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

Trullo A, Corti V, Arza E, Caiolfa VR, Zamai M. **Application limits and data correction in number of molecules and brightness analysis.** *Microsc Res Tech* (2013) 76: 1135-46

Rocha-Perugini, V., M. Zamai, J.M. Gonzalez-Granado, O. Barreiro, E. Tejera, M. Yanez-Mo, V.R. Caiolfa, and F. Sanchez-Madrid. **CD81 controls sustained T cell activation signaling and defines the maturation stages of cognate immunological synapses.** *Mol Cell Biol* (2013) 33: 3644-58



Pluripotent Cell Technology



Head of Unit
Giovanna Giovinazzo

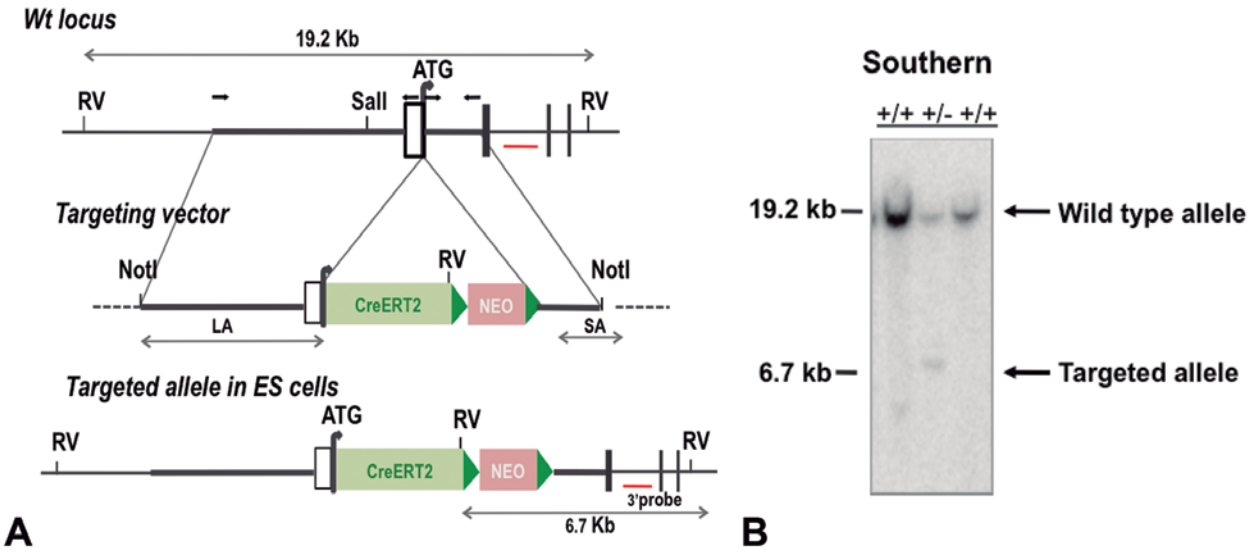
Support Scientists:
Francisco Gutiérrez
Elisa Santos

Technician:
María Ángeles Sanguino

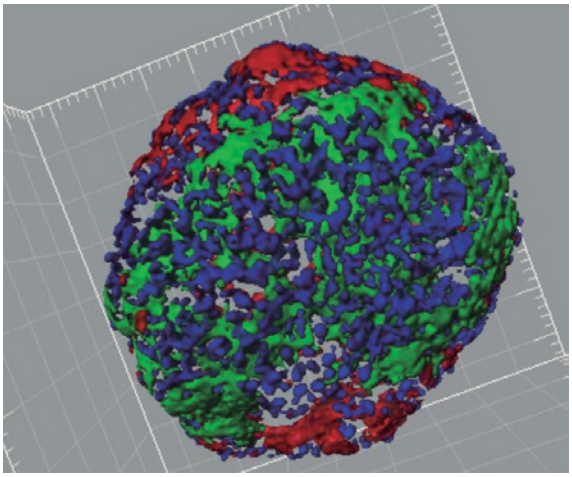
RESEARCH INTEREST

The Pluripotent Stem Cell Technology Unit provides knowledge, training, and state-of-the-art technological support with the culture and manipulation of mouse and human pluripotent stem cells. A suitable working environment for work with stem cells is provided by tow supervised culture rooms, one devoted to human stem cells and the other to mouse embryonic stem cells (mESCs). In 2014, our staff continued to facilitate gene-targeting experiments by supply genetically modified mESCs under tight quality-control, an essential requirement for germline transmission and the generation of mutant mice. Taking charge of all key steps in the gene targeting protocol (electroporation of the targeting vector, screening, karyotyping, culture and the preparation of cells for blastocyst injection) the Unit contributed to the generation of six new mouse mutant lines in 2014. The Unit also applied its wide expertise in genetic modification to the generation of mouse stem cells modified using the CRISPR/cas system. The Unit supplies knockout stem cell lines for the development of research projects. On request, we can also assist CNIC researchers in fine-tuning differentiation protocols for a specific lineage.

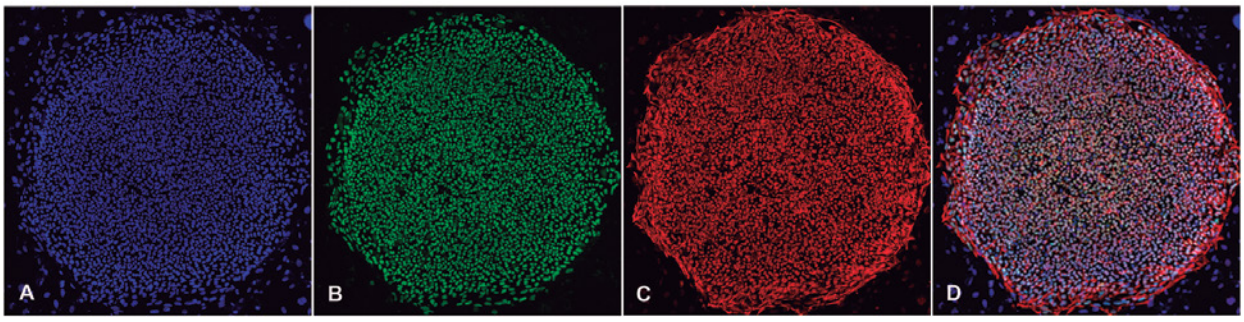
Human induced pluripotent stem cells (hiPSC) are an extraordinarily valuable source of cells for basic and translational research, including drug development and disease modeling. The PCT Unit also dedicates effort to the transgene-free reprogramming of somatic cells obtained from cell banks. Several hiPSC lines have already been derived, characterized and banked. We also establish differentiation programs to specific lineages and provide the latest cutting-edge technology for genome editing.



Generation of mouse reporter line. A) Diagram showing the 5' wild-type (wt) region of the gene involved in homologous recombination, the targeting vector and the screening strategy designed to detect recombination events. B) Detection of targeted clones by Southern blot.



iMARIS reconstruction of the direct differentiation of an embryoid body toward the cardiac lineage. Expression of fluorescent EGFP (green) is under the control of the cardiac TroponinT promoter.



Immunocytochemistry detection of pluripotent cell markers in reprogrammed cells derived from human dermal fibroblasts. A) DAPI. B) Oct4. C) Nanog. D) Merged view.

Selected Publications

Rosello-Diez A, Arques CG, Delgado I, Giovinazzo G and 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 and Torres M. **Two new Targeted alleles for the comprehensive analysis of Meis1 functions in the mouse** Genesis (2014) 52: 967-75

Claveria C, Giovinazzo G, Sierra R, Torres M. **Myc-driven endogenous cell competition in the early mammalian embryo**. *Nature* (2013) 500: 39-44



Proteomics



Head of Unit
Juan Antonio López

Support Scientists:
Enrique Calvo
Emilio Camafeita
Iakes Ezkurdia

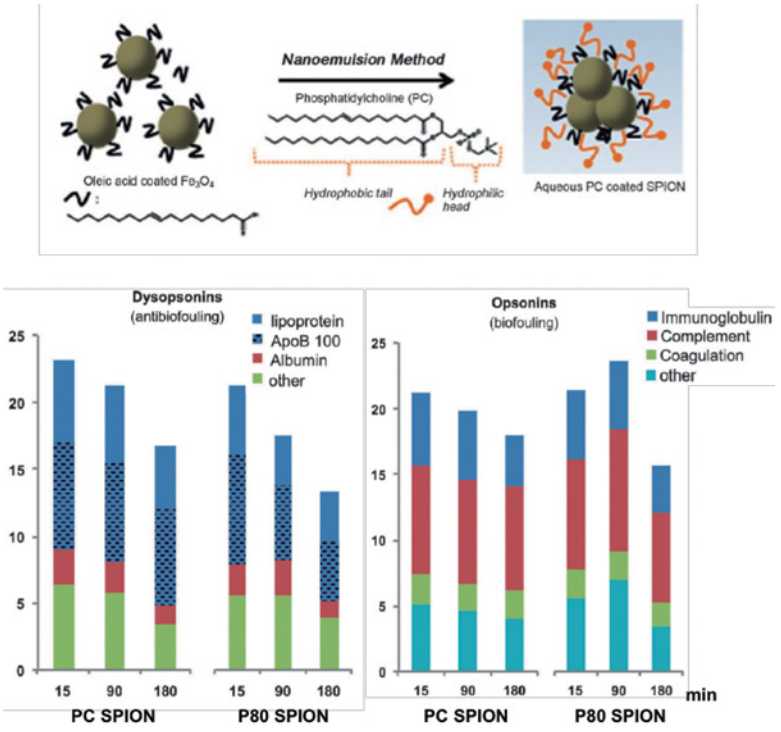
RESEARCH INTEREST

The Proteomics Unit is dedicated to technological innovation and the development of new applications of interest to the research community. The Unit has been working on improvements to the separation and quantitative analysis of protein expression by shotgun proteomics using high-throughput technologies based on nanoHPLC coupled to mass spectrometry. The Proteomics Unit houses several nano-HPLC systems coupled to state-of-art mass spectrometers for ultra-deep proteome analysis. During 2014 substantial progress was made in quantitative proteomics approaches, mainly using stable isobaric labeling (iTRAQ and TMT). Particular improvements were made in the development of the chromatographic conditions for peptide separation, optimization of fragmentation parameters, statistical analysis of quantitative data, and systems biology interpretation of results using programs developed in house.

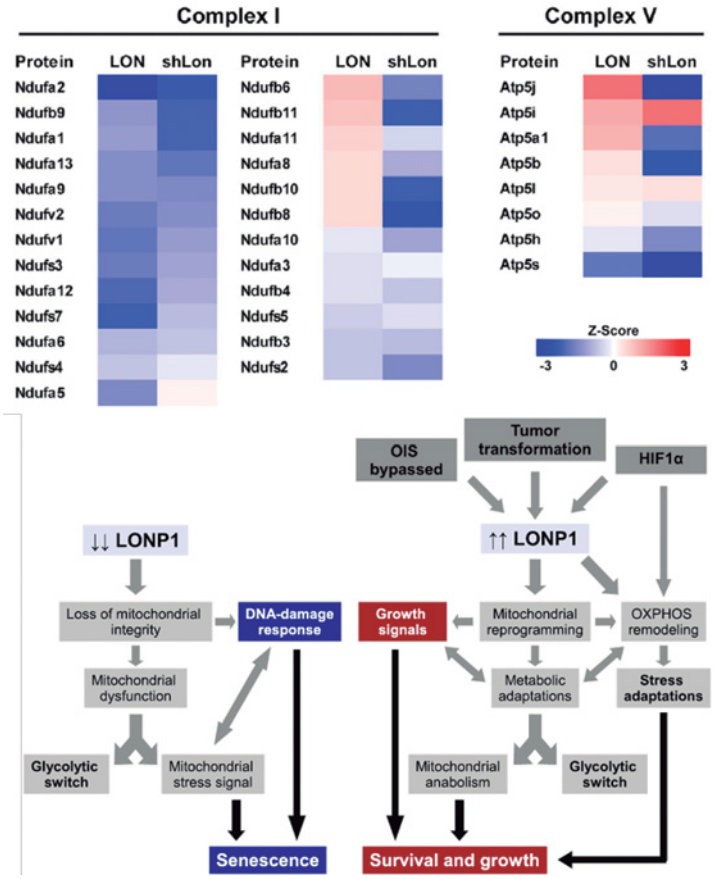
These approaches are being extended to the quantitative analysis of oxidative post-translational modifications, an area of paramount interest in the cardiovascular field, and for biomarker discovery in the clinical setting to the analysis of dozens of plasma samples, using depletion protocols of the most-abundant proteins.

One of the most interesting developments is a data-independent scanning acquisition mode that mixes targeted and shotgun approaches, based on signal-independent fragmentation. This novel approach is being explored in selected subproteomes to increase the coverage and number of identified peptides, allowing us to conduct post-acquisition targeted in-silico analysis of selected peptide sequences.

This robust analytical platform, together with our recognized experience in the field, enables us to manage large research projects that require qualitative and quantitative proteomic approaches to measure differential protein expression, characterize chemical and posttranslational modifications, and map protein-protein interactions in different biological systems.



Phosphatidylcholine-coated iron oxide nanomicelles for in vivo prolonged circulation time with an antibiofouling protein corona. *(Top)* Production of phosphatidylcholine-coated superparamagnetic iron oxide nanoparticles (PC SPION) nanomicelles. *(Bottom)* Relative % weight of proteins with known antibiofouling properties (dysopsonins) and biofouling properties (opsonins) classified by their biological function in the coronas of micellar PC and P80 SPION incubated in vitro in rat serum. Modified from Groult *et al*. 2014.



LONP1 controls tumor bioenergetics by remodeling OXPHOS subunits. *(Top)* Heatmap showing protein abundance changes in LON and shLon cells in Complex I and Complex V subunits of enriched mitochondrial preparations, obtained with high-throughput iTRAQ quantitative proteomics. The relative abundance changes are expressed using the Z score in relation to each control. *(Bottom)* Model summarizing the functional relevance of Lon protease in reprogramming mitochondrial activity in cancer. Modified from Quiros *et al*. 2014.

Selected Publications

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

Ezkurdia I, Juan D, Rodriguez JM, Frankish A, Diekhans M, Harrow J, Vazquez J, Valencia A, Tress ML. **Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes**. Hum Mol Genet (2014) 23: 5866-78

Groult H, Ruiz-Cabello J, Lechuga-Vieco AV, Mateo J, Benito M, Bilbao I, Martínez-Alcázar MP, Lopez JA, Vázquez J, Herranz F. **Phosphatidylcholine-coated iron oxide nanomicelles for in vivo prolonged circulation time with an antibiofouling protein corona**. *Chem Eur J* (2014) 20:16662-71

Ezkurdia I, Vázquez J, Valencia A, and Tress M. **Analyzing the First Drafts of the Human Proteome**. *J Proteome Res* (2014) 13: 3854–55

Camafeita E, Lamas JR, Calvo E, Tornero-Esteban P, Lopez JA, Fernández-Gutiérrez B. **Selected reaction monitoring assays in mesenchymal stem cells from osteoarthritis patients**. *Clin Proteomics* (2014) 11: 33



Transgenesis



Head of Unit
Luis-Miguel Criado Rodríguez

Support Scientists:
José Mª Fernández Toro
Juan de Dios Hourcade Bueno

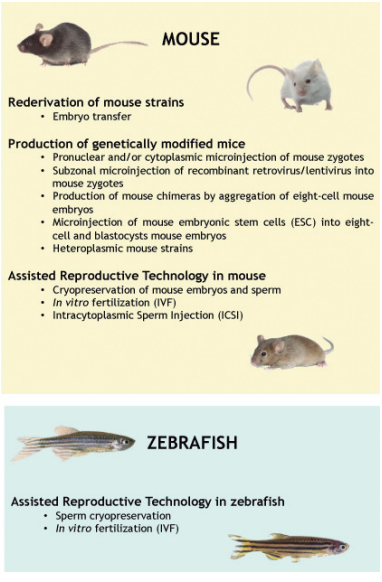
RESEARCH INTEREST

The Unit's work with mice *(Mus musculus)* is divided into three areas: rederivation of mouse strains, production of genetically modified mice, and assisted reproductive techniques (ART). Rederivation to establish colonies in the SPF zone of the animal facility is done by embryo transfer. Genetically modified mouse strains (transgenic mice) are produced using well established standard techniques, mainly microinjection of DNA and/or RNA into the zygote pronuclei or cytoplasm, or microinjection of embryonic stem cells (ESC) into 8-cell or blastocyst-stage mouse embryos. For assisted reproduction, valuable mouse strains are cryopreserved by freezing sperm and embryos, and other ARTs used include in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

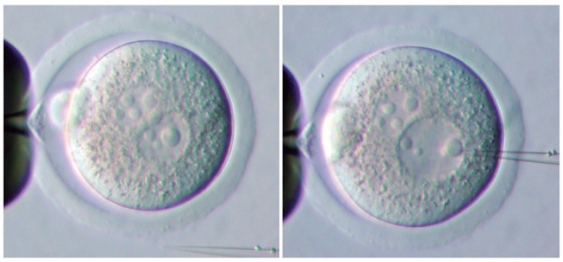
Another important vertebrate model organism used in scientific research is the zebrafish *(Danio rerio)*. The Unit cryopreserves sperm from this species and performs in vitro fertilization using fresh and frozen sperm.

In 2014, following the general trend toward production of transgenic animals by gene edition using engineered nucleases, the unit succeeded in the production of genetically modified mice using zinc finger nucleases (ZFN) and also the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system.

The Unit collaborates with several CNIC groups on specific aspects of their research programs, and Unit members participate in the CNIC's training plans by providing theoretical and practical sessions.



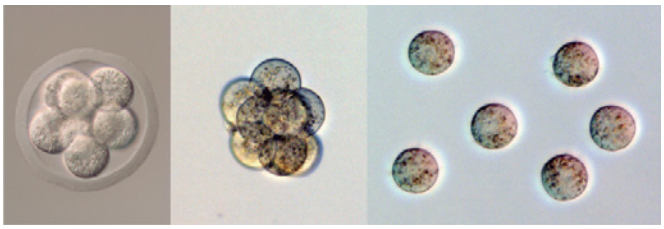
Transgenesis Unit activities



Pronuclear microinjection with DNA solution for the production of transgenic mice. Left: Pronuclear microinjection of a mouse zygote (B6CBAF2 strain) anchored with a holding needle; the injection needle containing the DNA solution is visible al the bottom of the image. Right: expanded pronucleus after microinjection



Production of a heteroplasmic mouse strain. One NZB cytoplast—the cytoplasm portion without nuclear genetic material—has been introduced by micromanipulation into the perivitelline space of a C57BL/6JOlaHsd zygote with two pronuclei. After an electrofusion process, cytoplast and zygote will fuse to produce a heteroplasmic zygote bearing C57BL/6JOlaHsd genomic DNA (gDNA) and a mix of mitochondrial DNAs (mtDNAs) from C57BL/6JOlaHsd and NZB origins.



Blastomere disaggregation of an 8-cell mouse embryo: genetic analysis of individualized blastomeres. A single 8-cell B6CBAF2 mouse embryo (left) is chemically treated to remove its surrounding zona pellucida (middle), and is then disaggregated to separate the eight blatomeres (right). The zona pellucida is a glycoprotein layer surrounding the cytoplasmic membrane of preimplantation mammalian embryos.



Viral Vectors



Head of Unit
Juan C. Ramírez

Support Scientists:
Raúl Torres

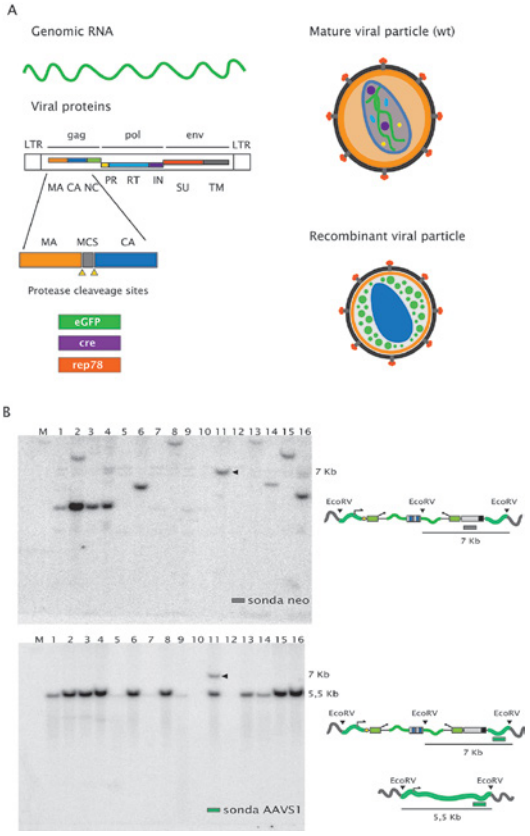
Technicians:
Aida García

Visiting Scientist:
Catarina Reis
(CNIO)

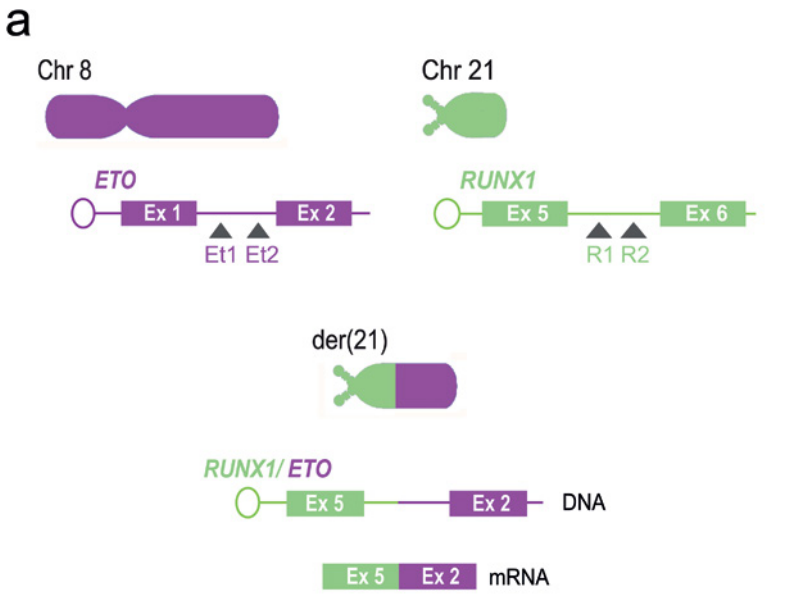
RESEARCH INTEREST

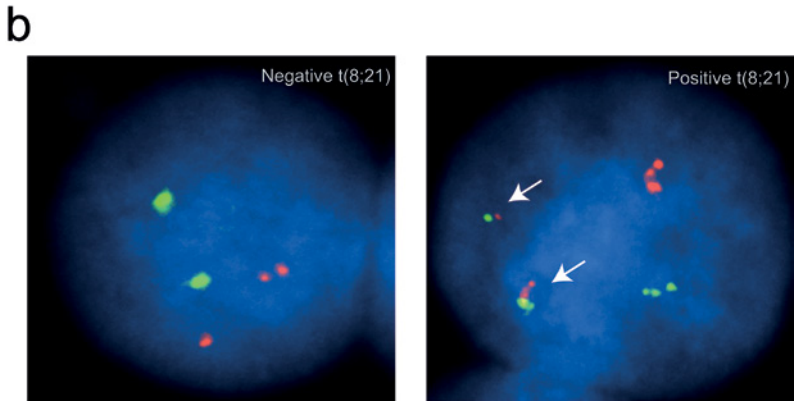
The Viral Vector Unit continues to provide the non-clinical grade recombinant viral vectors (lentivirus and AAV) to CNIC researchers and to external collaborators in Spain, accommodating most investigators' needs regarding virally mediated gene transfer.

We have implemented specific programs aimed at developing novel approaches to gene editing and novel applications of currently available tools. First, we have developed a simple system based on integrase-deficient lentivirus (IDLV) vectors to direct the insertion of genes into the safe AAVS1 locus, mediated by the HUH-site-specific recombinase Rep78 of AAV. By packaging Rep78 in lentiviral particles together with a construct encoding the minimal recognition site for the protein to promote site-specific recombination, we have obtained integration frequencies of up to 10% at the AAVS1 safe harbor locus in human cells, with no evidence of off-target insertions. Remarkably, this hit-and-run method limits the cytotoxic effects derived from long exposure to the protein, both in the producer and the target cells. Second, we have used the popular RGEN technology (RNA-guided endonuclease), also known as the CRISPR/Cas9 system, to demonstrate that it is possible to generate cells undergoing human-cancer-specific chromosomal translocations at high efficiency. We have generated chromosomal translocations identical to those observed in Ewing's Sarcoma (ES) and Acute Myeloid Leukemia (AML), either in a well establish cell line (HEK293) or in primary cells; human mesenchymal stem cells (hMSC) in the case of ES and hematopoietic stem cells (HSC) in the case of AML. This work was conducted in close partnership with members of the Molecular Cytogenetics group at the CNIO.



Viral Vector Figure 1. Site-Specific integration by Rep78 in AAVS1 locus.
A. Strategy for generating lentivirus containing the recombinase Rep78 and control ORFs (eGFP, cre). The heterologous ORFs were cloned between the matrix (MA) and capsid (CA) ORFs of the gag-gene in the plasmid.
B. Southern blot analysis of G418R HEK293 clones showing single copy insertion in the AAVS1 locus in clone #11 (arrowhead). The diagrams indicate the expected sizes of the neo (internal probe, gray bar) and AAVS1 probes (external probe, green bar).





Viral Vector Figure 2. Strategy for engineering chromosomal translocations using CRISPR/ Cas9.
A. Translocation strategy. Double-strand breaks are introduced by the sgRNAs (arrowheads) mapping to introns in ETO (purple) and RUNX1 (green). **B**. FISH analysis of chromosome 8 (red) and 21 (green), verifying reciprocal chromosomal translocation in CD34+ human hematopoietic stem cells

MAJOR GRANTS

- Ministerio de Economía y Competitividad (PI11/02041)

Selected Publications

Torres R, Rodriguez-Perales S, Ramirez JC. **The use of innovative tools to reproduce human cancer translocations: lessons from the CRISPR/C as system.** *Curr Biotechnol* doi: 10.2174/2211550103666141023221106

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

Rojas JM, Moreno H, Garcia A, Ramirez JC, Sevilla N, Martin V. **Two replication-defective adenoviral vaccine vectors for the induction of immune responses to PPRV.** *Vaccine* (2014) 32: 393-400

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

APPENDIX



Publications
Training Programs and Courses
Seminars, Events and Awards
Strategic Alliances
Funding
Patent Portfolio
Staff Figures

There were 210 CNIC publications in 2014, 186 of them in JCR-listed journals with an Impact Factor (IF). Of the total publications, 68% were produced through collaboration with foreign institutions, 26% with national institutions, and 6% were authored solely by CNIC researchers.

A CNIC scientist was a main author on 59% of the publications. The average IF for all the articles was 7.386.

Articles with a CNIC Main Author

Articles with an IF

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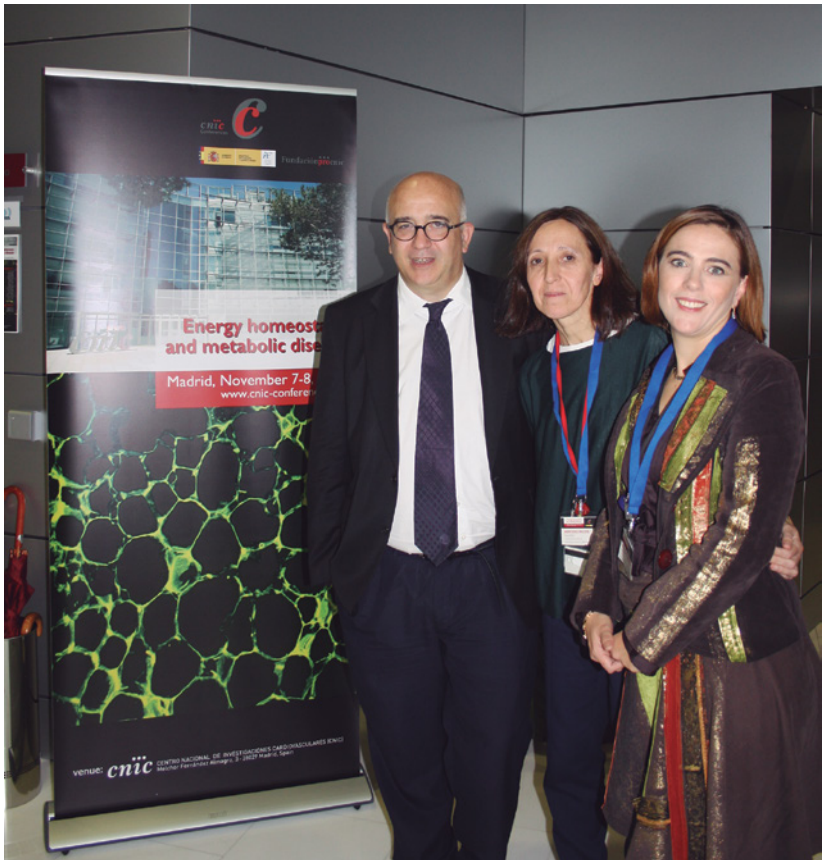
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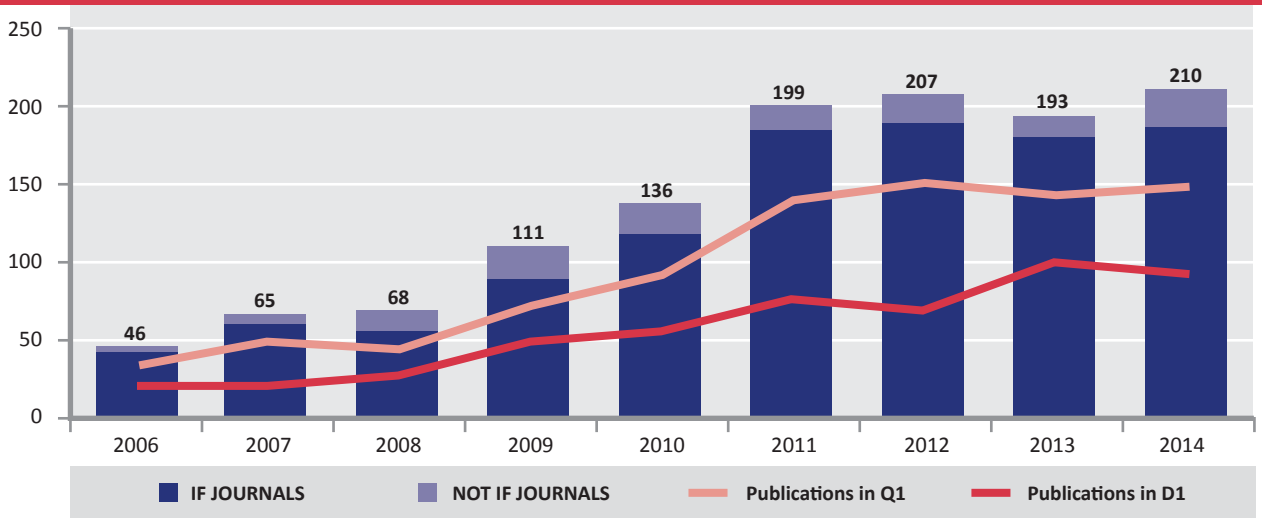
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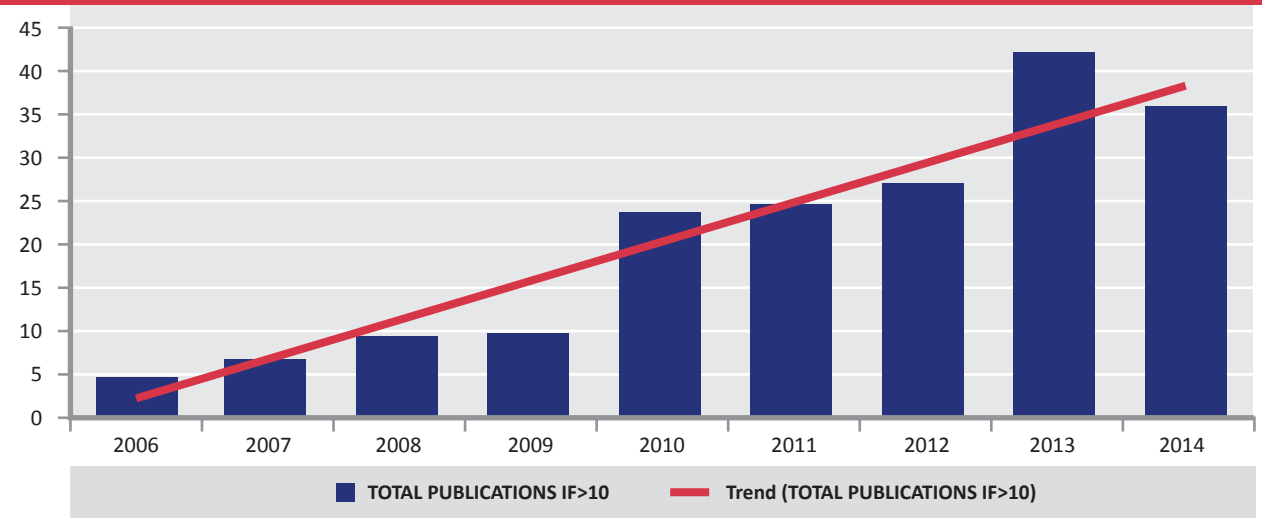
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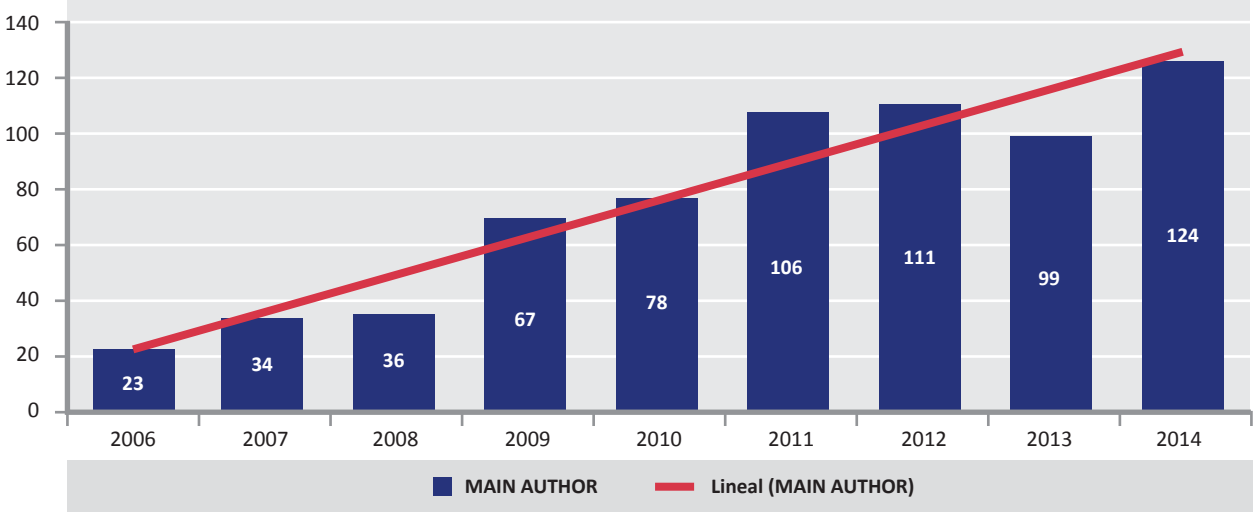
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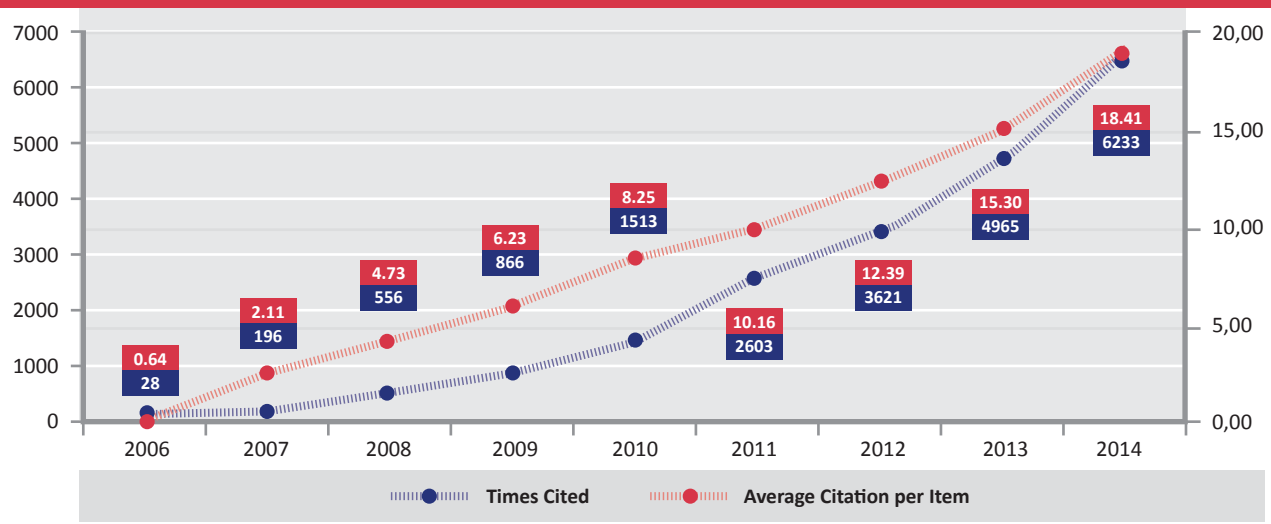
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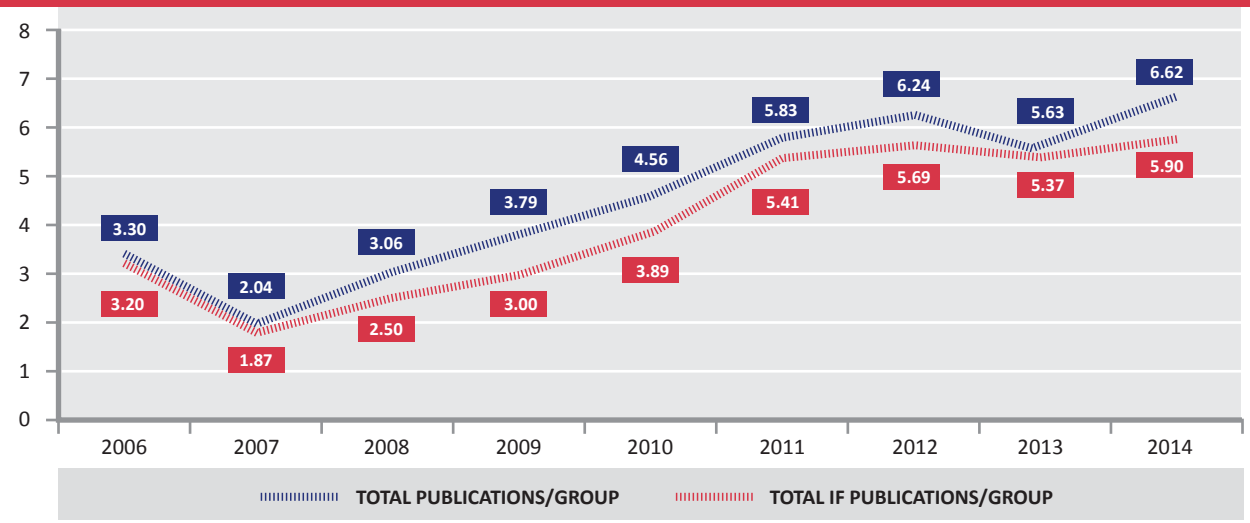
PUBLICATIONS CNIC MAIN AUTHOR



TOTAL AND AVERAGE CITES (until December 2014)



PUBLICATIONS/GROUP



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 2014

Name	Secondary School	Autonomous Region
Becerra Rodríguez, María de los Ángeles	IES Medina Azahara	Andalucía
Cuella Martín, Isabel	Colegio San Juan Bosco	Castilla y León
Gallego Cortés, Ana	Escolapios Santander	Cantabria
Lobato Martínez, Ester	Colegio San Agustín	Comunidad Valenciana
Martínez Paz, Patricia	IES Plurilingüe San Rosendo	Galicia
Quirós Aguirre, Carmen	IES Sancti Petri	Andalucía
Vega Barreto, Víctor	Atlantic School Garoé	Islas Canarias
Vila García, Joaquín	IES Rosalía de Castro	Galicia

CICERONE Program

The CICERONE Program is open to advanced undergraduate students studying towards 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 2014*

Candidate	Degree	University
Alfonso, Jose Manuel	Biotecnología	Universidad Politécnica de Valencia
Aparicio, José Juan	Biotecnología	Universidad Pablo de Olavide
Azagra, Ignasi	Farmacia	Universidad de Barcelona
Bermejo, David	Ingeniería Biomédica	Universidad Politécnica de Madrid
Brines, Miriam	Biotecnología	Universidad Politécnica de Valencia
Climent, Guillem	Biotecnología	Universidad Politécnica de Valencia
Duart, Leticia	Farmacia	Universidad de Barcelona
Fernández, Irene	Bioquímica	Universidad Autónoma de Madrid
Ferrández, Luis José	Biología	Universidad de Alicante
Fulgoni, Giulio	Biotecnología	Università degli studi di Parma
García, Irene	Biología	Universidad Autónoma de Madrid
García, Lorena Belén	Bioquímica	Universidad Autónoma de Madrid
García, María	Bioquímica	Univ. de Sevilla y Univ. de Málaga
González, Alicia	Medicina	Universidad de Castilla La Mancha
Herrera, Leticia	Biología Sanitaria	Universidad de Alcalá de Henares
Jauset, Cristina	Biomédicas	Universitat de Lleida
Jiménez, Natalia	Bioquímica	Universidad Autónoma de Madrid
Lavado, Jesús	Biotecnología	Universidad Pablo de Olavide
López, Carlos	Biología	Emory University
Martí, Carlos	Biotecnología	Universidad Politécnica de Valencia
Montañés, Pablo	Biotecnología	Universidad de Zaragoza
Palacios, José Luis	Biología	Universidad de Granada
Pérez Howell, Oliver	Biotecnología	Univ. Miguel Hernández de Elche
Policarpo, Guillermo	Medicina	Universitat de Valencia
Rivera, Mario	Medicina	Universidad de Granada
Sahún, Álvaro	Bioquímica y Biología Molecular	Universitat Rovira i Virgili
Salguero, Alejandro	Biotecnología	Universidad Pablo de Olavide
Saz, Paula	Bioquímica	Universidad de Zaragoza
Sendín, Mercedes	Medicina	Universidad de Sevilla
Serrano, Álvaro	Bioquímica	Universidad de Castilla la Mancha
Suárez, Javier	Biología Sanitaria	Universidad de Alcalá
Suay, Carmen	Bioquímica y Ciencias Biomédicas	Universidad de Valencia
Torregrosa, Rebeca	Biología	Universidad de Murcia
Vicente, Alba	Farmacia	Universidad de Salamanca
Victorino, Jesús	Biotecnología	Universidad Pablo de Olavide
Yleina, Cendón	Biología	Universidad Rey Juan Carlos

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 collaborated 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 2014

Name	Degree - University	MSc Degree
Castañs, Celia	Univ. Complutense de Madrid	Biomedicina Molecular (Autónoma de Madrid)
Martí, Carlos	Univ. Politécnica de Valencia	Bioinformática (Autónoma de Barcelona)
Fernández, Macarena	Univ. Pablo Olavide (Sevilla)	Biomedicina Molecular (Autónoma de Madrid)
Palacios, José Luis	Univ. de Granada	Biomedicina Molecular (Autónoma de Madrid)
Pérez, Oliver	Univ. Miguel Hernández (Elche)	Biomedicina Molecular (Autónoma de Madrid)
Sahún, Álvaro	Univ. Rovira i Virgili (Tarragona)	Biomedicina Molecular (Autónoma de Madrid)
Saz, Paula	Univ. de Zaragoza	Biomedicina Molecular (Autónoma de Madrid)
Suárez, Javier	Univ. Alcalá de Henares (Madrid)	Dianas Terapéuticas en Señalización Celular: Investigación y Desarrollo (Alcalá de Henares)
Torregrosa, Rebeca	Univ. de Murcia	Biomedicina Molecular (Autónoma de Madrid)
Valverde, José	Univ. de Murcia	Biomedicina Molecular (Autónoma de Madrid)
Victorino, Jesús	Univ. Pablo Olavide (Sevilla)	Biomedicina Molecular (Autónoma de Madrid)

PREDOCTORAL (PhD) Program

The PREDOCTORAL Program provides a unified framework for all researchers at the CNIC who are working towards 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 2014

Name	Thesis Title	University	CNIC Department	Thesis Advisor(s)
Alameda, Daniel	Transcriptional regulation of inflammation in macrophages by retinoid x receptor	UAM	Cardiovascular Development and Repair	Mercedes Ricote Pacheco
Blanco, Noelia	SHIP-1 phosphatase associates to dectin-1 hemitam to modulate functional responses to candida albicans in dendritic cells	UAM	Vascular Biology and Inflammation	David Sancho Madrid y Carlos del Fresno Sánchez
Casanova, María	Characterization of a population of aged neutrophils and its effect on hematopoietic niches.	UAM	Atherothrombosis, Imaging and Epidemiology	Andrés Hidalgo
Cedenilla, Marta	Role of macrophages in cardiac homeostasis and repair following myocardial infarction	UAM	Cardiovascular Development and Repair	Mercedes Ricote Pacheco
Escolano, Amelia	Calcineurin in macrophages: polarization and modulation in the inflammatory response	UAM	Vascular Biology and Inflammation	Juan Miguel Redondo Moya
Latorre, Ana	Oxphos system: functional integration of two genomes	UAM	Cardiovascular Development and Repair	José Antonio Enríquez Domínguez
Martín, Laura	Adipose stem cells characterization from obese subjects	UAM	Cardiovascular Development and Repair	Beatriz González Gálvez
Méndez, Nerea	Functions of calcineurin and Rcan1 in the pathological vascular wall remodelling	UAM	Vascular Biology and Inflammation	Juan Miguel Redondo Moya y Miguel Campanero García
Peralta, Marina	Dissecting the role of heartbeat-driven pericardiac fluid forces on epicardium morphogenesis through in vivo imaging in the zebrafish	UAM	Cardiovascular Development and Repair	Nadia Mercader
Rayón, Teresa	Understanding the first lineage choice in the embryo: regulation of CDX2 in the blastocyst through the notch and hippo pathways	UAM	Cardiovascular Development and Repair	Miguel Manzanares Fourcade
Tomé, María	Role of microRNAs in the therapeutic activity of human mesenchymal stem cells	UAM	Cardiovascular Development and Repair	Manuel Ángel González de la Peña y Antonio Bernad Miana
Uribe, Verónica	Analysis of the role of ARID3D in cardiac development	UAM	Cardiovascular Development and Repair	Juan José Sanz Ezquerro
Valiente, Iñigo	Role of the polycomb gene bmi1 in the biology of resident cardiac stem cells in mammals	UAM	Cardiovascular Development and Repair	Antonio Bernad Miana

> Graduate students studying for their PhD theses at the CNIC during 2014

Name	Funding Agency	University	CNIC Department	Joined previously through Training Program
Adrover Montemayor, Jose Mª	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad Complutense de Madrid	Atherothrombosis, Imaging and Epidemiology	No
Albacete Albacete, Lucas	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2010
Alonso Herranz, Laura	La Caixa Foundation Fellowship	Universidad Complutense de Madrid	Cardiovascular Development and Repair	CICERONE Program 2012/BMM9 2013-2014/MASTER Program 2013, La Caixa CNIC 2014
Álvarez Prado, Ángel Francisco	CNIC's contract	Autónoma de Madrid	Vascular Biology and Inflammation	No
Aix Sacido, Esther	FPU (Spanish Ministry of Education)	Autónoma de Madrid	Cardiovascular Development and Repair	BMM9 2009-2010 / MASTER Program 2009
Bartolomé Izquierdo, Nahikari	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad del País Vasco	Vascular Biology and Inflammation	No
Bednareck, Dorota	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Bernal, Aurora	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2010 / MASTER Program 2010
Bernardo Vasco, Edgar	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Bustos Morán, Eugenio	La Caixa Foundation Fellowship	Autónoma de Madrid	Vascular Biology and Inflammation	No
Clemente Toribio, Cristina	FPU (Spanish Ministry of Education)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Cruz Uréndez, Francisco Miguel	FPU (Spanish Ministry of Education)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2010 / MASTER Program 2010
Chiodo, Yuri	FPI (Spanish Ministry of Economy and Competitiveness)	U. degli Studi di Modena e Reggio Emilia	Vascular Biology and Inflammation	No
D'Amato, Gaetano	Marie Curie Initial Training Network (NotchIT)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Díaz Díaz, Covadonga	FPU (Spanish Ministry of Education)	Universidad de Oviedo	Cardiovascular Development and Repair	No
Enamorado, Neris Michele	La Caixa Foundation Fellowship	Universidad de la Habana	Vascular Biology and Inflammation	BMM9 2012-2013/ MASTER Program 2012, La Caixa-CNIC 2013

> *Graduate students studying for their PhD theses at the CNIC during 2014*

Name	Funding Agency	University	CNIC Department	Joined previously through Training Program
Fanjul Hevia, Víctor	La Caixa Foundation Fellowship	Universidad de Oviedo	Atherothrombosis, Imaging and Epidemiology	CICERONE Program 2012-2013, MASTER Program 2013
Fernández Jiménez, Rodrigo	Río Hortega (ISCIII)	Universidad de Zaragoza	Atherothrombosis, Imaging and Epidemiology	No
García García, Andrés	La Caixa Foundation Fellowship	Universidad de Málaga	Cardiovascular Development and Repair	BMM9 2012-2013, La Caixa-CNIC 2013
Gatto, Alberto	Eurpean International Training Network (FP7)	Autónoma de Madrid	Cardiovascular Development and Repair	No
García-Prieto Cuesta, Jaime	CNIC' contract	Autónoma de Madrid	Atherothrombosis, Imaging and Epidemiology	No
Gómez Salinero, Jesús Mª	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	PRACTICALS Program 2009-10 / MASTER Program 2010
Gómez Velázquez, Melisa	CNIC' contract	Autónoma de Madrid	Cardiovascular Development and Repair	MASTER Program 2009/ Cardiovascular Postgraduate Program 2009-2010
González Hernández, Sara	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad de Sevilla	Cardiovascular Development and Repair	No
González Sainz de Aja, Julio	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	No
González Terán, Bárbara	European Research Council	Autónoma de Madrid	Vascular Biology and Inflammation	No
González Valdés, Ileana Beatriz	Reseach National Project (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Grivas, Dimitris	Research European Agency - Cardionet	Autónoma de Madrid	Cardiovascular Development and Repair	No
Guarás Rubio, Adela Mª	FPI (Spanish Ministry of Economy and Competitiveness)	Zaragoza	Cardiovascular Development and Repair	No
Gutiérrez Vázquez, Cristina	CAM (Madrid Autonomous Region)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2007 / Cardiovascular Postgraduate Program 2008-2009
Hamczyk, Magda	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Atherothrombosis, Imaging and Epidemiology	CICERONE Program 2010
Hidalgo Gavilán, Isabel	CNIC's contract	Autónoma de Madrid	Cardiovascular Development and Repair	No

> Graduate students studying for their PhD theses at the CNIC during 2014

Name	Funding Agency	University	CNIC Department	Joined previously through Training Program
Izquierdo Hernández, Helena	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2008 and 2009 / PRACTICALS Program 2009-10
Lechuga Vieco, Ana Victoria	FPI (Spanish Ministry of Economy and Competitiveness)	Pablo Olavide (Sevilla)	Cardiovascular Development and Repair	CICERONE Program 2011-2012-2013, BMM9 2012-2013
Lioux, Ghislaine	Research European Agency - Cardionet	Université Paris	Cardiovascular Development and Repair	No
Lozano Vidal, Noelia	FPU (Spanish Ministry of Education)	Autónoma de Madrid	Vascular Biology and Inflammation	Cardiovascular Postgraduate Program 2009-2010 / MASTER Program 2009
Manieri, Elisa	La Caixa Foundation Fellowship	Autónoma de Madrid	Vascular Biology and Inflammation	No
Martín Alonso, Mara	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2008 / Cardiovascular Postgraduate Program 2009-2010
Martín García, Elena	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	BMM9 2012-2013
Martínez López, María	FPU (Spanish Ministry of Education)	Universidad de Granada	Vascular Biology and Inflammation	CICERONE Program 2010, MASTER Program 2010
Molina Sánchez, Pedro	FPU (Spanish Ministry of Education)	Universidad de Valencia	Atherothrombosis, Imaging and Epidemiology	No
Montes Menéndez, Iván	La Caixa Foundation Fellowship	Universidad de Oviedo	Cardiovascular Development and Repair	No
Moreno Vicente, Roberto	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	CICERONE Program 2011
Munch, Juliane	Notch IT, Marie Curie	Autónoma de Madrid	Cardiovascular Development and Repair	Cardiovascular Postgraduate Program 2009-2010
Muñoz Martín, Noelia	La Caixa Foundation Fellowship	Universidad de Salamanca	Cardiovascula Development and Repair	MASTER Program 2013, La Caixa-CNIC 2014
Nieto Arellano, Rocío	FPU (Spanish Ministry of Education)	Universidad de Valencia	Cardiovascular Development and Repair	CICERONE Program 2012
Núñez Andrade, Norman	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Núñez Buiza, Ángel	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad Complutense de Madrid	Cardiovascular Development and Repair	No
Oller Pedrosa, Jorge	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	No

> *Graduate students studying for their PhD theses at the CNIC during 2014*

Name	Funding Agency	University	CNIC Department	Joined previously through Training Program
Pérez García, Atrantxa	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Vascular Biology and Inflammation	No
Pérez Menchero, Sergio	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Petra, Eleni	CNIC's contract	Imperial College (London)	Cardiovascular Development and Repair	No
Puentes Querol, Samuel	La Caixa Foundation Fellowship	Universidad Complutense de Madrid	Cardiovascular Development and Repair	La Caixa-CNIC 2014
Pun Hidalgo, Andrés	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad Complutense de Madrid	Atherothrombosis, Imaging and Epidemiology	No
Roche Molina, Marta	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad de Sevilla	Cardiovascular Development and Repair	No
Rouco Varela, Raquel	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	CICERONE Program 2012-2013, MASTER Program 2013, BMM9 2012-2013
Sánchez Ferrer, Carlota	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad Complutense de Madrid	Cardiovascular Development and Repair	No
Sánchez Iranzo, Héctor	FPU (Spanish Ministry of Education)	Universidad de Valencia	Cardiovascular Development and Repair	MASTER Program 2011
Travisano, Stanislao Igor	FPI (Spanish Ministry of Economy and Competitiveness)	Autónoma de Madrid	Cardiovascular Development and Repair	No
Uzhova, Irina	FPI (Spanish Ministry of Economy and Competitiveness)	Karolinska Institute	Atherothrombosis, Imaging and Epidemiology	No
Villa del Campo, Cristina	FPI (Spanish Ministry of Economy and Competitiveness	Autónoma de Madrid	Cardiovascular Development) and Repair	CICERONE Program 2007-09 / Cardiovascular Postgraduate Program 2009-2010 / MASTER Program 2009
Villahoz Lázaro, Silvia	FPI (Spanish Ministry of Economy and Competitiveness)	Universidad de León	Cardiovascular Development and Repair	CICERONE Program 2011-2012

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 edition of the Severo Ochoa Award. In 2014 the la Caixa Foundation provided support for four 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, 2014*

Name	CNIC Supervisor	CNIC Department
Alonso Herranz, Laura	Mercedes Ricote	Cardiovascular Development and Repair
García León, Daniel	David Filgueiras	Atherothrombosis, Imaging and Epidemiology
Muñoz Martín, Noelia	Miguel Torres	Cardiovascular Development and Repair
Ortiz Sánchez, Paula	Enrique Lara	Cardiovascular Development and Repair



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 collaboration with the CNIC.

RES@CNIC was launched in 2012. Students selected for the third call will join the CNIC during January and February 2015.

> Selected Candidates for the third call

Candidate	Hospital	CNIC Supervisor
Asla Ormaza, Cristina	H. U. de Basurto (Bilbao)	Jesús J. Borreguero
Cruz Utrilla, Alejandro	H. Clínico San Carlos (Madrid)	David Filgueiras
Fernández Vega, Ana	H. Clínico San Carlos (Madrid)	José Luis de la Pompa
García Arribas, Daniel	H. Clínico San Carlos (Madrid)	Pilar Martín
García Rubio, Julio C.	H. de Cabueñes (Gijón)	Borja Ibáñez
Marco del Castillo, Álvaro	H.U. Ramón y Cajal (Madrid)	Martín Laclaustra
Martínez Vives, Pablo	H. Clínico San Carlos (Madrid)	Vicente Andrés
Mendoza Cuartero, Paula	H. U. de Basurto (Bilbao)	Jesús J. Borreguero
Monteagudo Ruiz, Juan M.	H.U. Ramón y Cajal (Madrid)	Enrique Lara
Pardo Sanz, Ana	H.U. Ramón y Cajal (Madrid)	Guadalupe Sabio
Pérez Nogales, Eliú	C. H. Insular (Las Palmas de Gran Canaria)	Miguel Torres
Sobrino Balandrón, Adolfo	H.G.U. Gregorio Marañón (Madrid)	Jesús Ruiz-Cabello
Valandrón Sucasas, Isabel	H. Son Espases (Palma de Mallorca)	Borja Ibáñez
Varela Barca, Laura	H.U. Ramón y Cajal (Madrid)	José Antonio Enríquez



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 2014*

Name	Hospital	CNIC Department
Ayaon Albarrán, Ali	Hospital Clínico San Carlos, Madrid	Atherothrombosis, Imaging and Epidemiology
Marina Breysse, Manuel	Hospital General Universitario de Ciudad Real	Atherothrombosis, Imaging and Epidemiology



FICNIC Program

The CNIC has partnered with the Fundación Jesús Serra (FJS) and the *Fundación Interhospitalaria para Investigación Cardiovascular* (FIC) to create this new 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.

> *Fellowships in 2014*

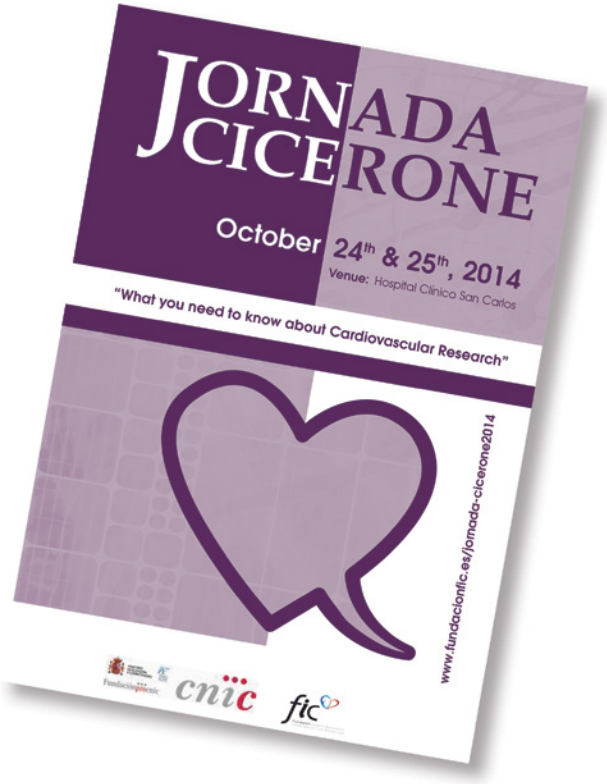
Name	Hospital	CNIC Supervisor
Fernández Jiménez, Rodrigo	Hospital Clínico San Carlos, Madrid	Borja Ibáñez

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

Fundación Interhospitalaria Investigación Cardiovascular

This group of lectures 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 editions of the Jornada CICERONE have been run in collaboration with the Fundación Interhospitalaria para la Investigación Cardiovascular and takes place in the Hospital Clínico San Carlos, Madrid.

Dates: *October 24-25, 2014*
Attendees: *98*



CARDIOVASCULAR PATHOPHYSIOLOGY Course: "From symptoms to genes"

SOCIEDAD ESPAÑOLA DE Cardiología

The course in CARDIOVASCULAR PATHOPHYSIOLOGY 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: *November 21-22, 2014*
Venue: *CNIC Lecture Hall*
Attendees: *101*



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 21-22, 2013*
Attendees: *190*



Research Professionals

CNIC International Postdoctoral Program

The CNIC International Postdoctoral Program (CNIC IPP) is aimed at supporting transnational mobility of postdoctoral researchers and to broaden and deepen their individual competence, particularly in terms of 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.

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

> Fellowships in 2014

Name	CNIC Department
Fernández Alvira, Juan Miguel	Atherothrombosis, Imaging and Epidemiology
Saraswati, Sarita	Cardiovascular Development and Repair
Sánchez Álvarez, Miguel	Vascular Biology and Inflammation

CNIC International Incoming Fellowships for Young Group Leaders

The CNIC IFF program aims to increase the mobility within Europe of experienced researchers in the cardiovascular research area. The program has been designed to support transnational mobility of researchers and to broaden and deepen their individual competencies, particularly in terms of acquisition of complementary skills needed to attain or strengthen a senior independent position in biomedical research.

The CNIC IIF is supported by the CNIC and the European Commission through the COFUND Programme, within the Marie Curie Actions theme in FP7. The EC contributes 40% of the total cost of the program.

> Fellowships in 2014

Name	CNIC Department
Fog Bentzon, Jacob	Atherothrombosis, Imaging and Epidemiology



January

13 **Ajay Chawla**
Cardiovascular Research Institute / University of California
San Francisco, USA

24 **Marco Tripodi**
Roma University
Italy

27 **Mike Murphy**
MRC Mitochondrial Biology Unit, Medical Research Council
Cambridge, UK

February

7 **Mariona Graupera**
Institut d'Investigació Biomèdica de Bellvitge (IDIBELL)
Barcelona, Spain

10 **Christer Betsholtz**
Uppsala University / Karolinska Institutet
Stockholm, Sweden

24 **Emmanouil Dermitzakis**
University of Geneva Medical School
Switzerland

March

7 **Francisco Real**
Centro Nacional de Investigaciones Oncológicas (CNIO)
Madrid, Spain

10 **Brian Hendrich**
Wellcome Trust-MRC Stem Cell Institute, Department of Biochemistry, University of Cambridge
UK

20 **Lluis Morey**
Centre for Genomic Regulation (CRG)
Barcelona, Spain

24 **Joseph Vita**
Journal of the American Heart Association
Boston, USA

31 **Ari Helenius**
ETH Zürich, Institute of Biochemistry
Switzerland

April

10 **Andrés Santos**
E.T.S.I. Telecomunicaciones, Universidad Politécnica de Madrid
Spain

11 **Richard Flavell**
Yale School of Medicine
New Haven, USA

21 **Daniel Levy**
National Heart, Lung and Blood Institute, National Institutes of Health
Bethesda, USA

24 **Manuel Irimia**
The Donnelly Centre, University of Toronto
Canada

25 **Xosé R. Bustelo**
Cancer Research Center, CSIC-University of Salamanca
Spain

May

5 **Gerd Heusch**
Direktor des Institutes fuer Pathophysiologie, Universitaetsklinikum Essen
Germany

8 **2014 Weinstein Cardiovascular Conference**

16 **Florent Ginhoux**
Singapore Immunology Network (SIgN), Agency for Science,
Technology and Research (A*STAR)

19 **Josef Penninger**
Institute of Molecular Biotechnolgoy (IMBA)
Vienna, Austria

June

2 **Gwendalyn J. Randolph**
Washington University School of Medicine
St. Louis, USA

9 **Stefan Schulte-Merker**
Hubrecht Institute
Utrecht, The Netherlands

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

30 **Manuel Serrano**
Centro Nacional de Investigaciones Cardiovasculares (CNIO)
Madrid, Spain

July

3 **Jacob Fog Bentzon**
Aarhus University
Denmark

4 **Inflammation MACS® Day – CNIC**

9 **Katrien De Bock**
KU Leuven
Belgium

September

4 **José Jalife**
University of Michigan Medical School
USA

22 **Michael A. Gimbrone**
Harvard Medical School / Center for Excellence in Vascular Biology,
Brigham & Women's Hospital
Boston, Massachusetts, USA

26 **La Noche de los investigadores**
Bioinformática: unir investigación cardiovascular básica y aplicada es el futuro

October

1 **John E. Dick**
Princess Margaret Cancer Centre, University Health Network / University of Toronto/ Ontario Institute for Cancer Research
Canada

3 **Multicolor panel design for flow cytometry Workshop**

6 **Luis Serrano**
Centre for Genomic Regulation (CRG)
Barcelona, Spain

16 **Fifth Madrid Zebrafish Club Meeting**

23 **Jennifer Nichols**
Wellcome Trust Centre for Stem Cell Research, University of Cambridge
UK

24 **Jornada Cicerone "What you need to know about Cardiovascular Research"**

27 **Miriam Merad**
Mount Sinai Hospital
New York, USA

November

7 **IV CNIC Conference "Energy homeostasis and metabolic disease"**

11 **Semana de la Ciencia "Ven a CNIC: Visita interactiva a sus departamentos para conocer la investigación cardiovascular"**

13 **TRANSCARDIO14 "Primer Encuentro Español de Ciencia Traslacional en Cardiología"**

17 **Goran k Hansson**
Karolinska Institutet
Stockholm, Sweden

21 **VIII Curso de Fisiopatología Cardiovascular ""Del síntoma a los genes"**

26 **Nicholas Robert Forsyth**
Institute for Science and Technology in Medicine, Keele University
UK

27 **Salim Seyfried**
Max-Delbrück-Center for Molecular Medicine
Berlin, Germany

December

1 **CNIC Phd Day - 2014 Conference**
Towards Leadership Development in Science



Awards

Cardiovascular Development and Repair Department

Award: Premio Fundación Princesa de Girona Investigación Científica
Awarded to: ***Rui Benedito***

Award: Premio Nacional de Fin de Carrera de Educación Universitaria.
Awarded to: ***Héctor Sánchez***

Award: Segundo premio del Área de Ciencias Biológicas y Biomédicas en el Certamen Arquímedes.
Awarded to: ***Laura Alonso***

Vascular Biology and Inflammation Department

Award: Estrella de la Comunidad de Madrid en el Día Internacional de la Mujer.
Awarded to: ***Guadalupe Sabio***

Epidemiology, Atherothrombosis and Imaging Department

Award: Included in the 25 outstanding figures of the last 25 years. Readers of Spanish national newspaper El Mundo.
Awarded to: ***Valentin Fuster, MD, PhD***

Award: Appointment Editor-in-Chief, American College of Cardiology. American College of Cardiology
Awarded to: ***Valentin Fuster, MD, PhD***

Award: 2014 Frontiers in Science Award. American Association of Clinical Endocrinologist
Awarded to: ***Valentin Fuster, MD, PhD***

Award: Title of Marquis for his "outstanding and unceasing research efforts and his educational outreach work". King Juan Carlos I of Spain
Awarded to: ***Valentin Fuster, MD, PhD***

Award: Opening keynote lecturer of six International meetings. Chest World Congress and The One Century Celebration of the Mayo Clinic Cardiovascular Dept.
Awarded to: ***Valentin Fuster, MD, PhD***

Award: "XII Premio Nacional de Investigación CMC - Barclays" al trabajo "Effect of early metoprolol on infarct size in ST-segment elevation myocardial infarction patients undergoing primary PCI: the METOCARD-CNIC trial". Colegio Oficial de Médicos de Córdoba – Barclays.
Awarded to: ***Borja Ibañez et al., Circulation. 2013;128:1495-1503***

Award: Young Researcher Award. The European Society of Cardiology Working Group on Pulmonary Circulation and Right Ventricular Function.
Awarded to: ***Ana García Álvarez***

Award: Award to the 2nd best communication. International Symposium NEUTROPHIL 2014 (Montreal, Canadá).
Awarded to: ***María Casanova Acebes***

Award: Mención de Alumno Distinguido por la Universidad de Extremadura. Universidad de Extremadura.
Awarded to: ***Federico Sierra Rodríguez de la Rubi***

Award: Premios Jóvenes Jaén 14ª Edición en la Categoría de Universidad. Instituto Andaluz de la Juventud (IAJ) de la Consejería de Igualdad, Salud y Políticas Sociales. Junta de Andalucía.
Awarded to: ***Ana Victoria Lechuga Vieco***

STRATEGIC ALLIANCES: The CNIC consolidates and expands its alliances to investigate, train, innovate and transfer

The central aim of biomedical research is to translate knowledge generated in basic research laboratories into improved and innovative clinical practice, and reciprocally to stimulate research into questions raised in healthcare centers. Excellence in this area requires an integrated network based on close contacts with a wide range of institutions in different sectors.

In the last year, the CNIC has signed 37 interinstitutional agreements to create or consolidate partnerships.

In the education sector, the CNIC has expanded its academic network by signing agreements with universities in Spain (Francisco de Vitoria de Madrid, San Pablo CEU de Madrid, Barcelona, Castilla-La Mancha, and Extremadura) and abroad (Universita degli Studi di Napoli, Italy). These agreements mostly establish student exchange programs and short visits for practical work in the CNIC's laboratories.

To strengthen the CNIC PhD Program, the Center has also consolidated its parthership with the *Fundación La Caixa*, receiving a major injection of funding for new PhD fellowships within the "La Caixa-Severo Ochoa International PhD Program".

The Center has also reinforced its relationships with the Fundación Interhospitalaria de Investigación Cardiovascular, establishing a new training program called the "FICNIC Program", aimed at young cardiologists.

Links with the clinical sector have been consolidated through the signing of new agreements with Spanish hospitals such as *Hospital Clínico San Carlos* (Madrid), *Hospital Puerta de Hierro* (Madrid), and *Hospital Clínico de Barcelona*.

The CNIC's international projection is greatly strengthened by a new agreement signed with the Icahn School of Medicine (Mount Sinai, New York) and the private sector (AstraZeneca) to create a translantic network for the study of cardiovascular prognosis and prevention by the use of non-invasive imaging tools (TAN SNIP Project).

Finally, visibility of CNIC's scientific activities has been reinforced through the establishment of new collaborations with media organizations such as *Radio Televisión Española* (RTVE) and the *Fundación para el Conocimiento Madrimasd* (Comunidad de Madrid).



Public-Private Partnership

In spite of the enormous advances in diagnosis and treatment witnessed over the last 20 years, cardiovascular diseases continue to be the main cause of death in the developed world. The costs generated in economic, social and human terms are immense. In response to this reality, the Spanish Government, through the Instituto de Salud Carlos III (Carlos III Health Institute), created the CNIC to bring together the best of Spanish cardiovascular research and provide it with a modern infrastructure and ample funding to carry out world-leading biomedical research.

To achieve the funding necessary for its ambitious plan, the Spanish government appealed to the sense of social obligation of some of the major players in Spanish civil society, inviting the largest businesses in the country to make an active and long-term commitment to this project. The outcome was an agreement, signed in December 2005, between the Spanish government and a group of some of the most important Spanish businesses. Through this agreement these companies pledged to fund the CNIC up to 2012. This commitment was later extended until 2020.

Shortly after the agreement was signed, on January 24, 2006, the group of companies was formally constituted as the ProCNIC Foundation, signaling the most significant act of business sponsorship in recent years in terms of the amount of funding provided, its social significance, the group of companies involved, and the anticipated outcomes.

Since the signing of this agreement, the CNIC's funding has been based on a public-private partnership of a broad, socially-committed nature. In this innovative PPP, state funding is complemented by financing through the ProCNIC Foundation (**http://www.fundacionprocnic.es**).

On November 5 2013, **Fundación Mapfre**—the non profit organization set up by Spanish insurance giant MAPFRE—became the fourteenth partner in the Pro-CNIC Foundation, through an agreement signed by Pro-CNIC president Luis de Carlos and MAPFRE President Antonio Huertas, in the presence of Dr. Fuster. The other thirteen Pro-CNIC members are **Acciona**, **BBVA**, **Endesa**, **Fundación Abertis**, **Fundación Mutua Madrileña**, **Fundación Botín**, **Fundación Ramón Areces**, **Fundación Repsol**, **Gas Natural Fenosa**, **Grupo Prisa**, **Inditex**, **la Caixa**, and **Telefónica**. These full members are joined by ProCNIC International Collaborator **Mitsubishi**. This unique PPP allows the CNIC to fund special programs for the discovery and training of young investigators, to award extramural grants aimed at integrating basic and clinical research to answer specific questions, to acquire specialized research equipment that would otherwise be difficult to fund, and to run programs to incentivize and retain valuable investigators.

But the ProCNIC Foundation does more than provide the CNIC with money; it also contributes its accumulated managerial and business expertise. Representatives of the ProCNIC Foundation sit on the CNIC's Board of Trustees and actively participate in the management, planning and decision taking related to the Center. In this way, some of the most important organizations in the private sector in Spain have committed themselves to a direct involvement in biomedical research and the fight against cardiovascular diseases.

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.

Private Funding

Fundaciónprocnic

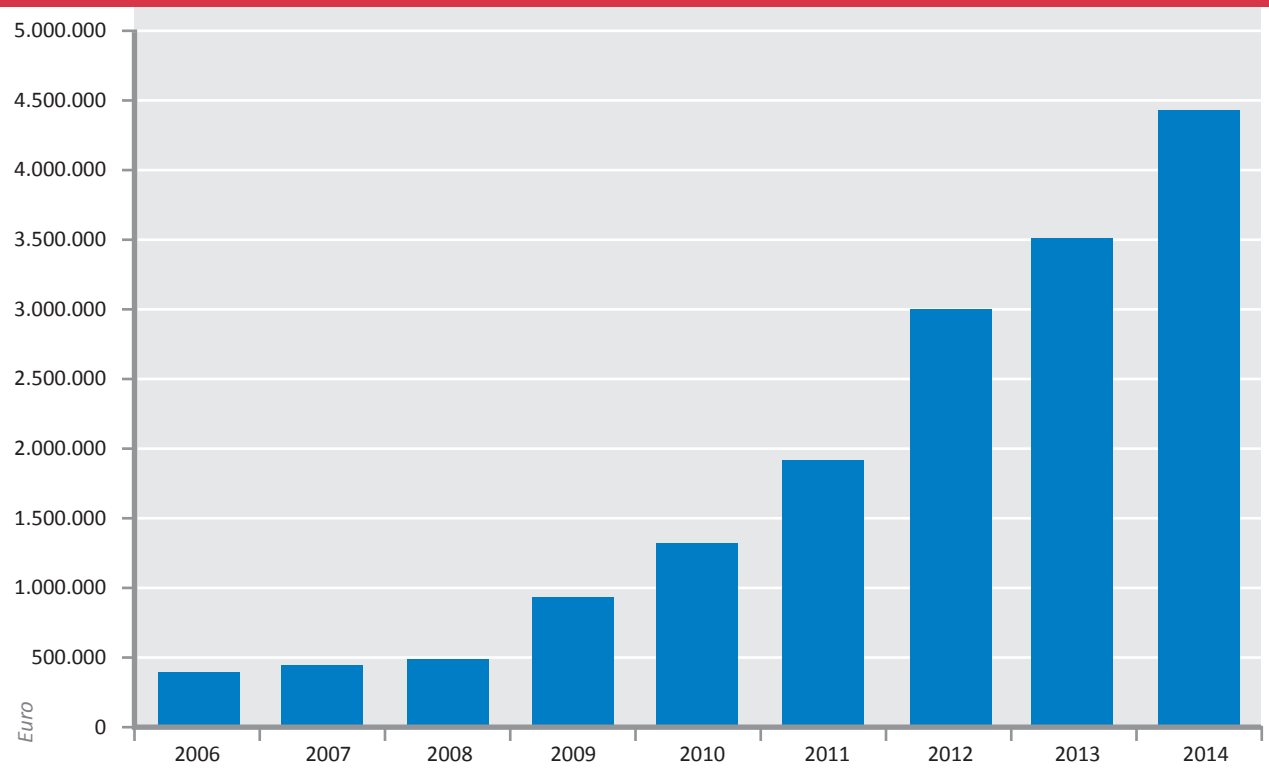
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FUNDACIÓN MUTUAMADRILEÑA FUNDACION RAMON ARECES Fundación REPSOL gasNatural fenosa INDITEX

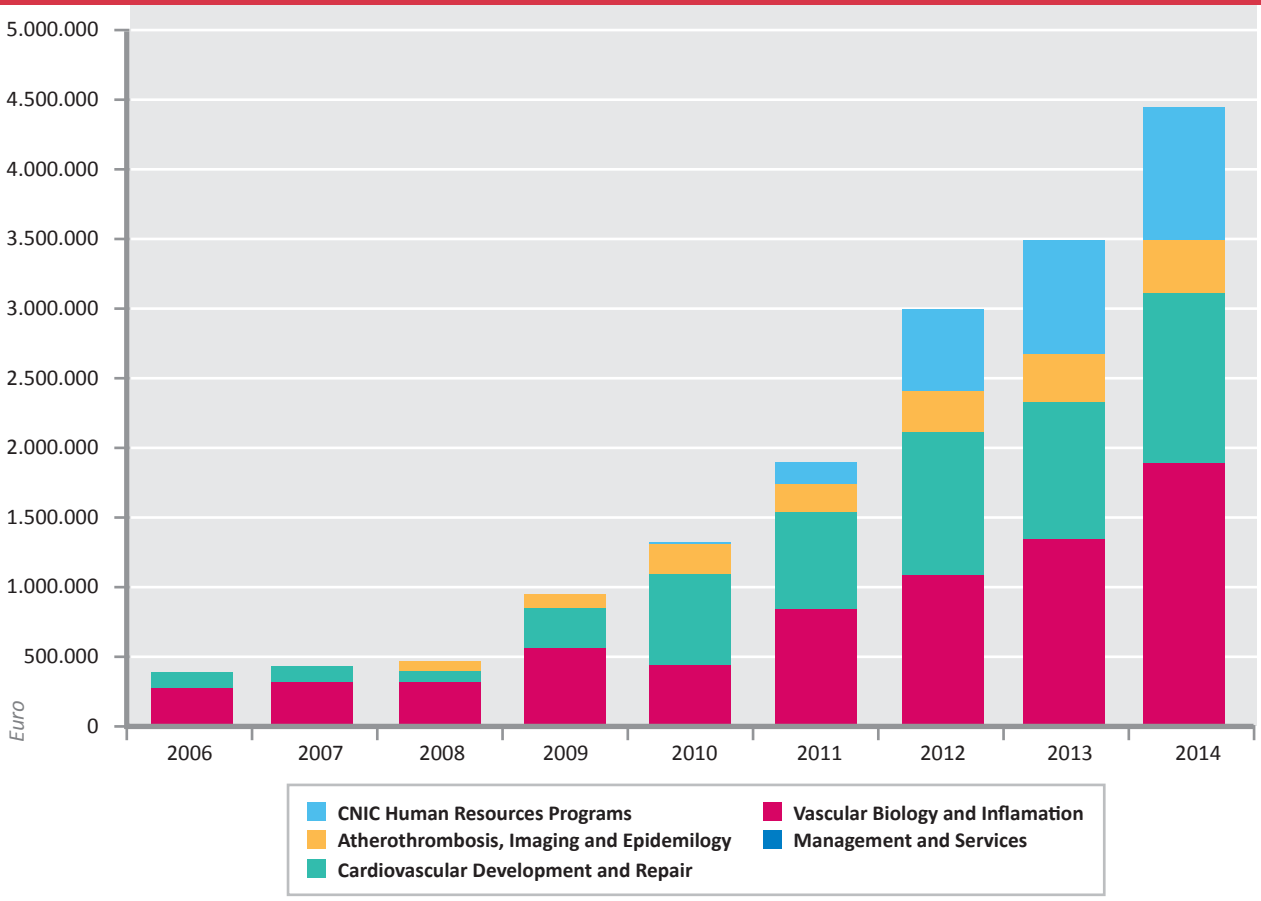
"la Caixa" Santander PRISA Telefónica ProCNIC International Collaborator Mitsubishi Corporation

Competitive Funding

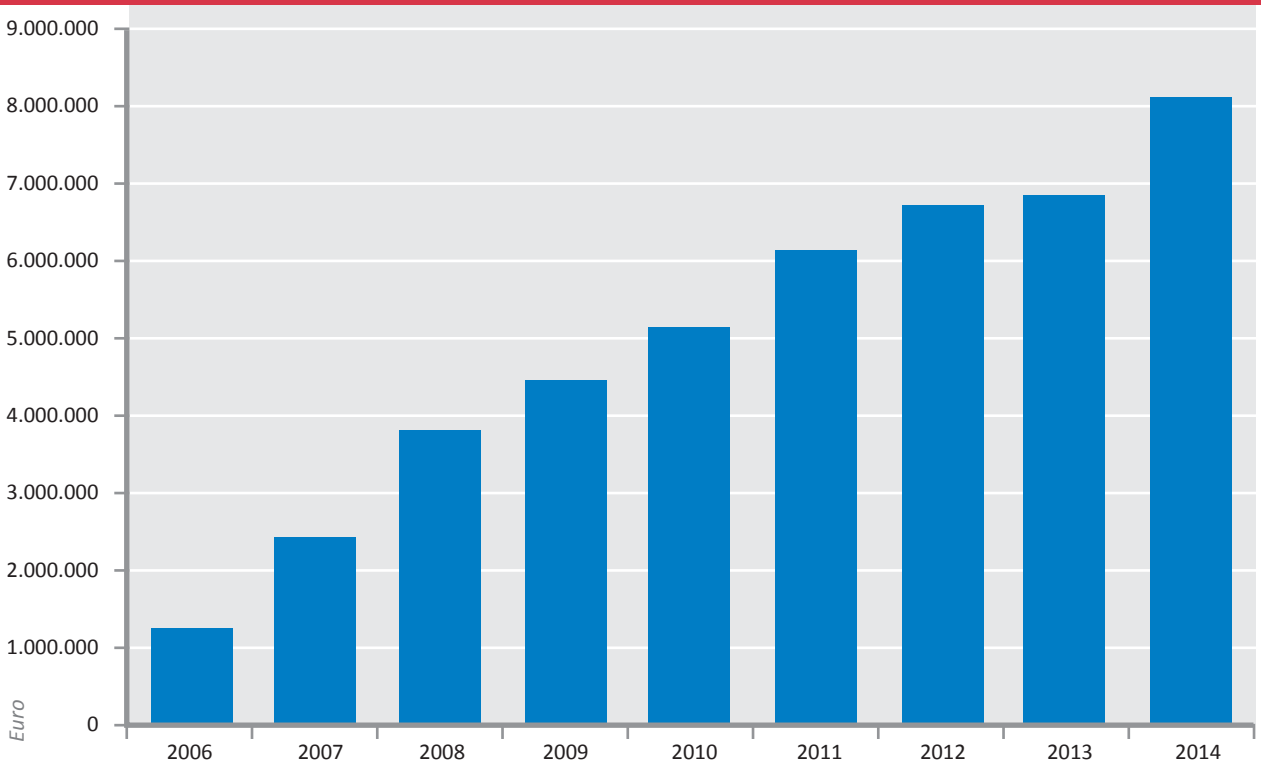
International Competitive Funding



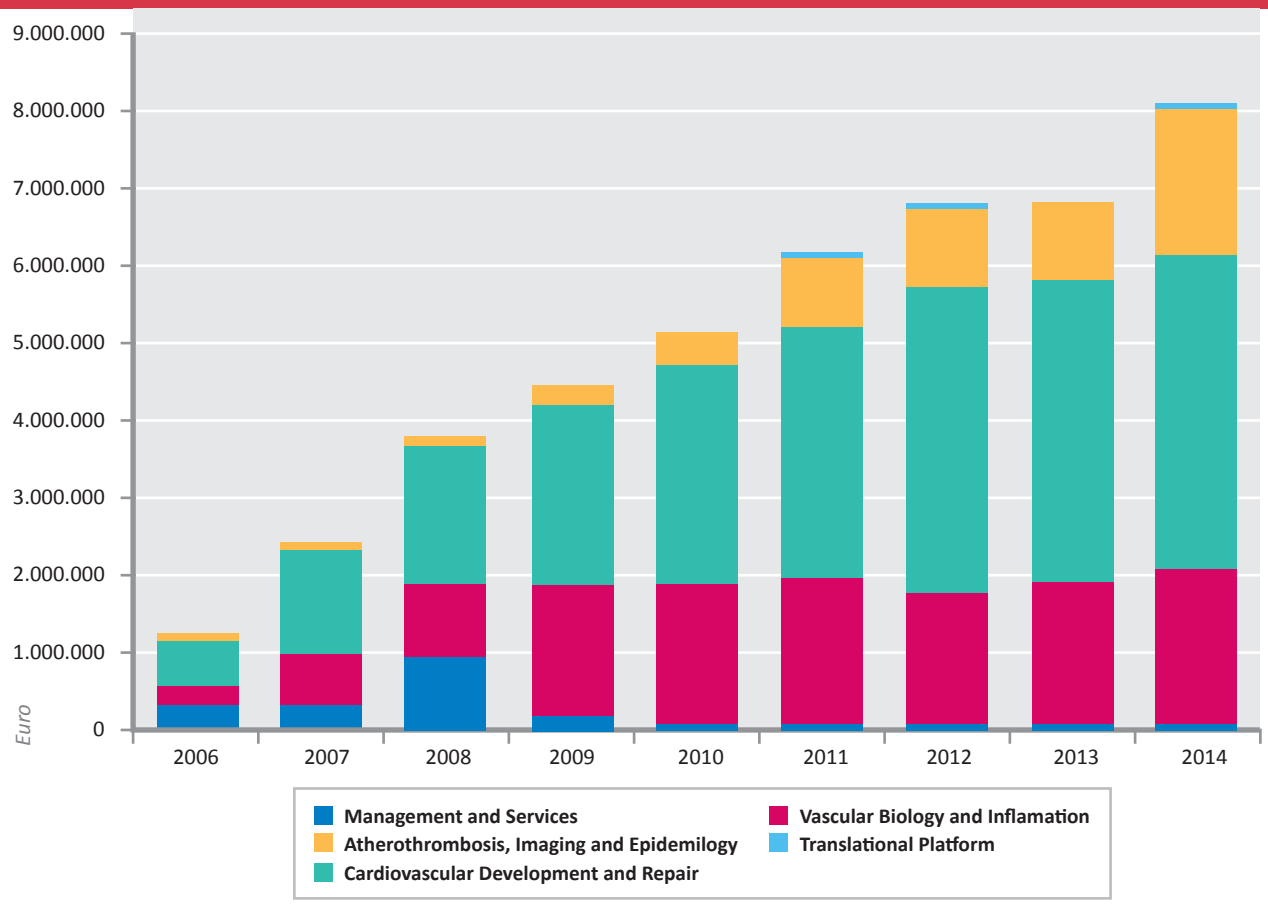
International Competitive Funding by department



National Competitive Funding



National Competitive Funding by department



Since 2004 the CNIC has attracted more than €25 million from international competitive sources, and around €50 million from national funds.

With just 33 research groups, the CNIC participated in 29 projects funded under the European Commission's Seventh Framework Programme (FP7). This included two key CNIC-coordinated projects funded under the Cooperation FP7-Health Programme:

- ***CARE-MI. PI:*** *Antonio Bernad Miana. Funding: €1.5 million*
- ***FOCUS. PIs:*** *Ginés Sanz y V. Fuster. Funding: €0.5 million*

Twenty projects were awarded to CNIC groups under the People (Marie Curie) FP7 Programme. These include two coordinated ITN projects (one of them in the Innovative Doctorate Training category, the first such project coordinated by a Spanish institution), and two COFUND projects (which bring a total funding of €4 million).

- ***ITN-IDT-2013.*** *CardioNext. PI: AG Arroyo. Funding: €2.7 million*
- ***ITN-2011.*** *CardioNet. PIs: Enrique Lara-Pezzi, Miguel Torres, José Luis de la Pompa. Funding: €1 million*

The scientific competitiveness of the CNIC research groups is highlighted by their participation in European Research Council (ERC) funded projects. The ERC funds scientific projects that enable Europe's brightest minds to tackle research challenges, and the CNIC contributes to the achievement of this goal through 5 ERC projects awarded under FP7 and one recently awarded under H2020 (under negotiation).

- ***ERC Advanced Grant.*** *GENTRIS: Mechanisms of MTOC guidance and Genetic Transfer at the Immune Synapse: novel modes of Immunomodulation. PI: Francisco Sánchez Madrid. Funding: €1.5 million*

- ***ERC Starting Grant.*** *CLR Sensing Necrosis - Immune functions of myeloid Syk-coupled C-type lectin receptors sensing necrosis. PI: David Sancho. Funding: €1.3 million*

- ***ERC Starting Grant.*** *OBECAN: Role of obesity in the development of hepatocellular carcinoma. PI: Guadalupe Sabio Buzo. Funding: €1.5 million*

- ***ERC Starting Grant.*** *BCLYM: Mechanisms of mature B cell lymphomagenesis. PI: Almudena R. Ramiro. Funding: €1.6 million*

- ***ERC Starting Grant.*** *ZebraHeart: Novel insights into cardiac regeneration through studies in the zebrafish. PI: Nadia Mercader Huber. Funding: €1.5 million*

CNIC patent portfolio 2014

Twenty two inventions are currently being filed, ten of them in cooperation with other institutions. Of these, four are licensed. The following table lists those patents available for licensing:

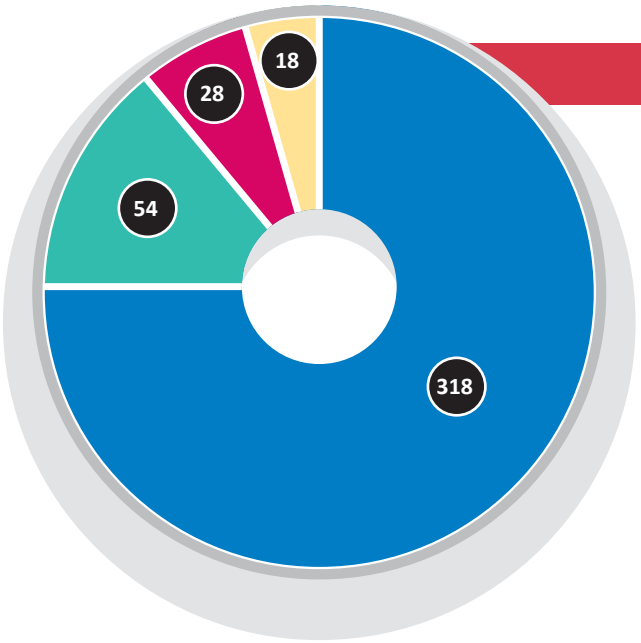
TITTLE	INVENTORS	APPLICANTS	PATENT APPLICATIONS
Method and system for generating MR images of a moving object in its environment	Javier Sanchez Gonzalez, Nils Dennis Nothnagel, Borja Ibáñez Cabeza, Rodrigo Fernández Jiménez, Valentín Fuster Carulla	CNIC, Philips	EP
Method of predicting or prognosticating neurological performance in patients which 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, IdiPAZ, UPV	EP
Antibodies and fragments thereof capable of binding the P-selectin glycoprotein ligand-1 (PSGL-1) for the treatment of thrombo-inflammatory disorders	Vinatha Sreeramkumar, Andrés Hidalgo Alonso	CNIC	EP
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
Bimodal fluorophore-labeled liposomes and associated methods and systems	Carlos Pérez-Medina, Thomas Reiner, Jason S. Lewis, Wilem J.M. Mulder, Zahi A. Fayad	CNIC, MSK, MOUNT SINAI, CIBER	US
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
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
Stable episomes based on non-integrative lentiviral vectors	Juan Carlos Ramírez Martínez, Raúl Torres Ruiz, Aida García Torralba	CNIC	EP
Modulador selectivo del receptor de estrógenos para el tratamiento de una enfermedad mieloproliferativa	Simón Méndez Ferrer, Lorena Arranz Salas, Abel Sánchez-Aguilera Peño	CNIC	ES
Terapia neuroregeneradora/ neurocompensatoria para el tratamiento de las neoplasias mieloproliferativas	Simón Méndez Ferrer, Lorena Arranz Salas, Joan Isern Marín	CNIC	ES
LxVP-mediated calcineurin inhibition in macrophages	Juan Miguel Redondo, Amelia Escolano	CNIC	EP, PCT
Secuencias nucleotídicas motivo que dirigen la localización de los ácidos nucleicos	Francisco Sánchez Madrid, María Mittelbrum Herrero, Cristina Gutiérrez Vázquez, Fátima Sánchez Cabo y Carolina Villarroya Beltri	UAM, CNIC	ES, PCT

CNIC patent portfolio 2014

TITTLE	INVENTORS	APPLICANTS	PATENT APPLICATIONS
Uso de agonistas selectivos de receptores beta-3 adrenérgicos para el tratamiento de hipertensión pulmonar	Borja Ibañez Cabeza, Valentín Fuster Carulla y Ana García-Álvarez	H. Clinic, CNIC	ES, PCT
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 y Jesús Gómez Salinero	EMBL, CNIC	EP, PCT
Nanopartículas recubiertas de gelatina	Fernando Herranz Rabanal, Jesús Ruíz-Cabello Osuna, Beatriz Salinas Rodriguez	UCM, CNIC	ES, PCT
Caveolin-1 in tumor-associated fibroblasts as biomarker for tumor progression	Miguel Ángel del Pozo, Jacky Goetz	CNIC	EP
Uso de células mesenquimales Nestina positivas para el mantenimiento de la hematopoyesis" /PCT: Células multipotenciales Nestina positivas	Simón Méndez Ferrer, Álvaro Urbano Ispizua	H. Clinic, Fund. Progreso y Salud, CNIC	EP, US
Selective peptides that inhibit the biological activity of calcineurin	Juan Miguel Redondo, Antonio Rodriguez, Sara Martínez	CNIC	EP, US

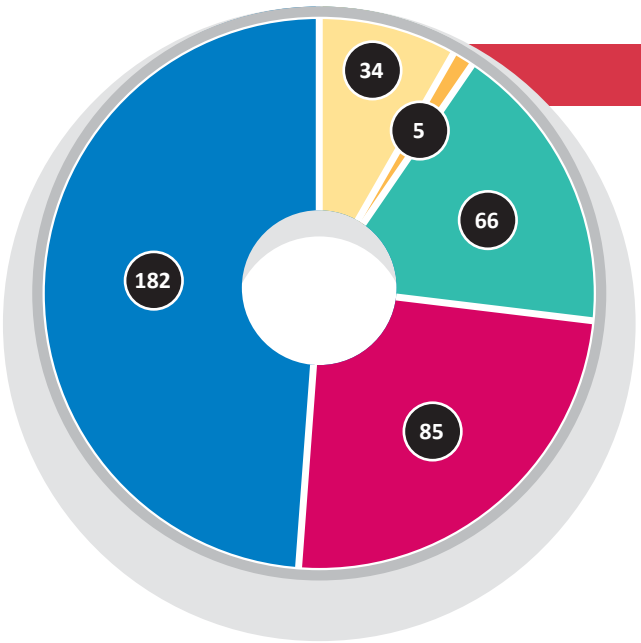
Patent Applications:
ES - Spain
PCT - International
EP - Europe
US - USA





CNIC staff 2014 (418)

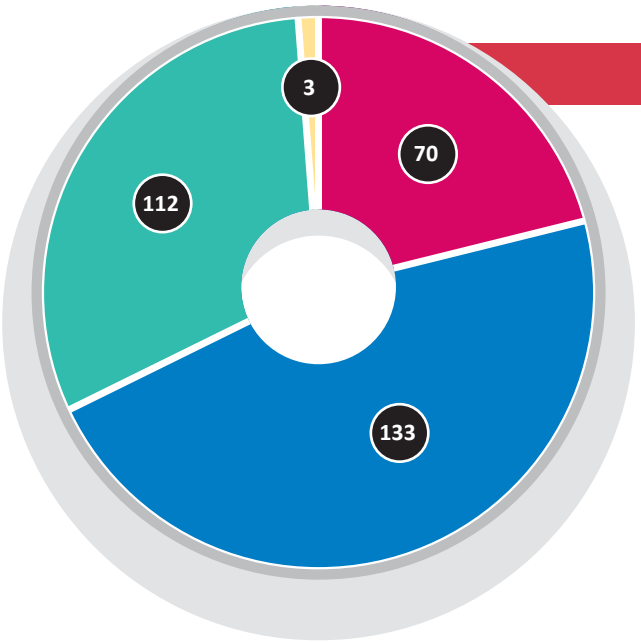
- Scientific Departments
- Technical Units
- Administration
- Scientific Services



CNIC research staff 2014 (372)

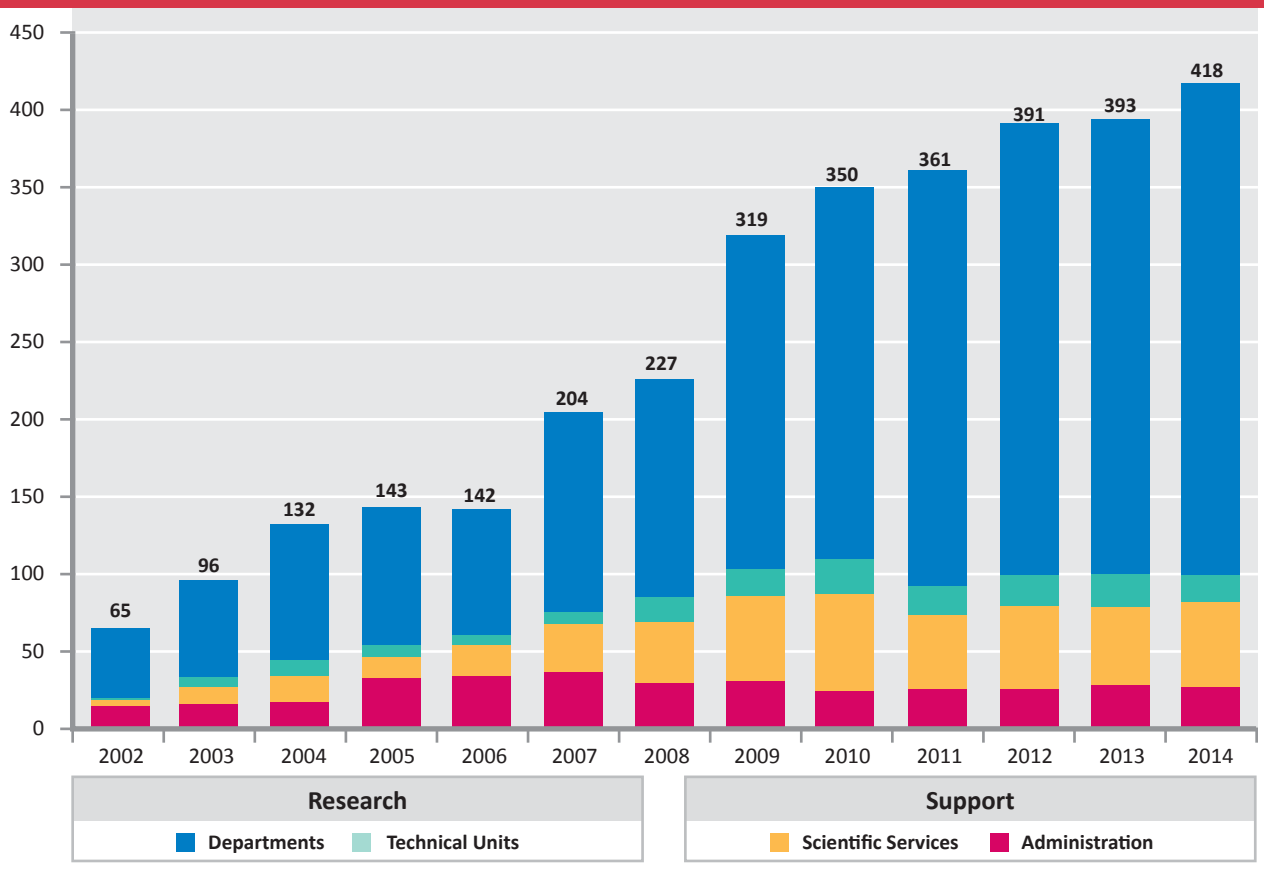
- Head of Laboratory/Unit
- Research Scientists
- Postdoctoral Researchers
- Predoctoral Researchers
- Technicians

Staff by department 2014 (318)

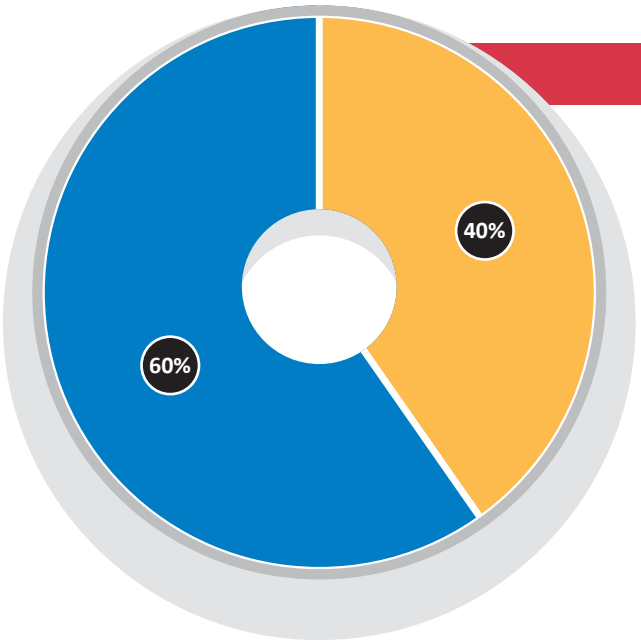


- Imaging, atherotrombosis and epidemiology
- Vascular Biology and Inflammation
- Cardiovascular Development and Repair
- Translational Platform

Gradual growth current status

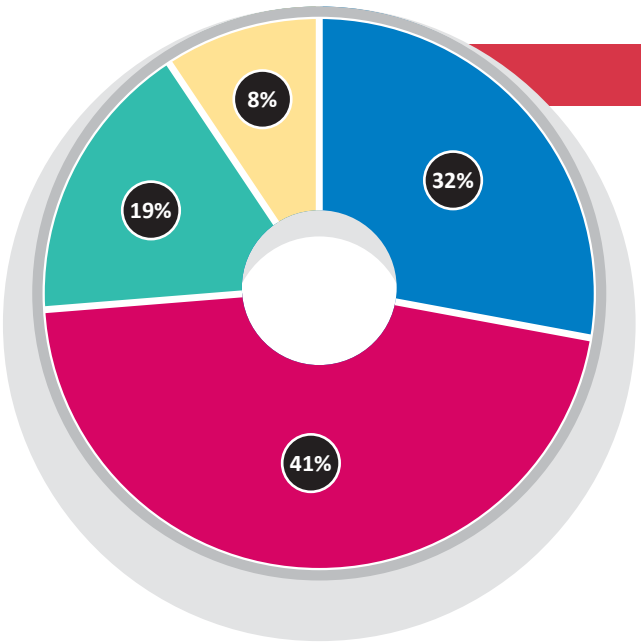


Gender Distribution 2014



- Men
- Women

Age distribution 2014



- < 30
- 30-39
- 40-50
- > 50



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