ANNUAL REPORT 2016

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Centro Nacional de Investigaciones Oncológicas

EXCELENCIA SEVERO OCHOA 2012 - 2019

CONTENTS

“Commercialisation and return on investment in research remain priorities. CNIO’s royalty income in 2016 is double that of 2012. A total of 44 inventors, about 10% of the researchers at CNIO, have contributed and benefited from this achievement.”

MARIA A. BLASCO
Director

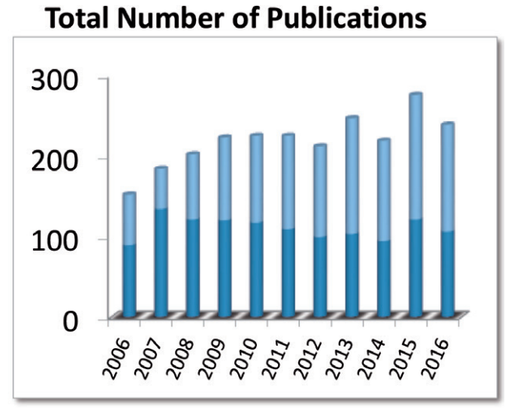
FOREWORD

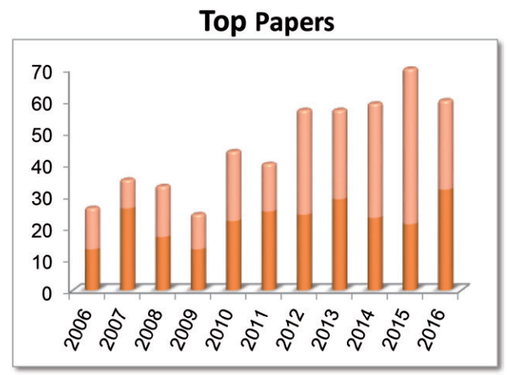
Maria A. Blasco Director

This year, once again, the data on CNIO's scientific performance illustrate that we are doing a superb job contributing towards scientific breakthroughs. During 2016, the CNIO authored a total of 239 papers, 28 of which were published in journals with impact factors (IF) ranging from 10 to 15, and 32 papers in journals with impact factors greater than 15. Comparison with previous years (2006-2016) indicates that CNIO has continued to increase the numbers of papers published in top journals with IF greater than 15.

With the aim of upholding the CNIO's strategy to remain an international Research Centre of Excellence and an influential institution in cancer research worldwide, 2016 saw the establishment of a CNIO Department of International Affairs. This new Department is headed by Carolina Pola who was the Editor of *Nature Medicine* for over seven years, as well as the former Director of Communications at Pharmamar, the most successful Spanish pharma company devoted to anti-tumour therapies. Since the creation of the Department of International Affairs, Carolina has redefined our international approach to embed the European concept of 'Responsible Research and Innovation' (RRI) in our institutional and research core. This new perspective, alongside several other efforts made by the Department, have resulted in several international collaborations that will bear fruit next year in the form of joint scientific symposiums to strengthen our research groups, as well as a plan to boost our recruitment of international talent. Our capacity to attract and retain scientific talent, providing new investigators with an interdisciplinary and multicultural environment, has become an integral part of our international agenda, which we will continue to nurture in the upcoming years.

I am also happy to mention that we continue to attract new and talented young group leaders to the CNIO. After the incorporation, in 2015, of two Junior Groups working in metastasis (led by Hector Peinado and Manuel Valiente) in the Molecular Oncology Programme, we welcomed another new Junior Group to the Programme in 2016: the Metabolism and Cell Signalling Group, led by Alejo Efeyan who joined the CNIO at the beginning of 2016





after a brilliant postdoc with David Sabatini at the Massachusetts Institute of Technology (MIT, Boston, USA).

During 2016, we also completed the selection process to incorporate two new Groups in the Structural Biology and Biocomputing Programme, with the aim of reinforcing lines of research in structural biology at the CNIO. The two new Junior Group Leaders who will start at CNIO in 2017 are: Ivan Plaza Menacho, currently Senior Research associate at the Structural Biology Laboratory at the Biozentrum, Univesity of Basel, Switzerland; and Rafael Fernández Leiro, from the MRC Laboratory of Molecular Biology, Cambridge, UK.

In 2016, we also created a new service unit at the CNIO: the Biological Text Mining Unit, which is focused on the application and development of biomedical text mining technologies that are becoming a key tool for the efficient exploitation of information contained in unstructured data repositories. This Unit is fully funded through the '*Plan de Impulso de las Tecnologías del Lenguaje de la Agenda Digital* (PITL)', in the framework of an agreement ('encomienda') between the Secretary of State of Telecommunications of the Spanish Ministry of Energy, Tourism and Digital Agenda (MINETAD) and the CNIO. We also revised the organisation of the two Bioinformatics Units that were providing support and expertise to our investigators; namely, the Bioinformatics Unit of the Structural Biology and Biocomputing Programme, and the Translational Bioinformatics Unit of the Clinical Research Programme. Under a new single structure, the CNIO Bioinformatics Unit, headed by Fátima Al Shahrour, we have joined the efforts of both previous units in order to better serve the needs of the CNIO Research Groups and to better coordinate and manage bioinformatics projects.

The valorisation of the research results generated by CNIO's scientists, with the aim of turning them into high-potential diagnostic or therapeutic products and services, is one of our ways of creating added value for society and boosting public benefit through improving cancer patient outcomes, in particular. Óscar Fernández Capetillo, Vice-Director of Translational Research and Director of Innovation since end 2015, has been leading these key strategic areas for the CNIO, enhancing translational research at CNIO in collaboration with the CNIO Experimental Therapeutics Programme. In 2016, we leveraged on public-private partnerships in order to bring our research results closer to the patient. A project based on Dr. Djouder's findings that boosting levels with nicotinamide riboside (NR) prevented and abolished aggressive tumour formation, received nearly 1 Mio in funding from *MINECO*'s *Retos-Colaboración* Programme. The aim of the project is the development of a new NR-based therapy for use in hepatocellular carcinoma and other tumours.

We continue to track previously licensed programmes from our drug discovery projects led by the Experimental Therapeutics Programme; one focused on Pim kinase inhibitors and another one on ATR inhibitors, both are very close to reaching the clinical testing stage.

Commercialisation and return on investment in research remain priorities. Royalty income and milestone payments collected in 2016 raised more than 650 thousand euros. This includes revenues from patent licences as well as from commercialisation of research tools such as monoclonal antibodies. Following CNIO's policy of royalty revenue share, this income flows back to the CNIO's research activities as well as to the inventors themselves. A total of 44 inventors, about 10% of the researchers at CNIO, have contributed and benefited from this achievement.

Industrial partnerships remain key to achieve valorisation of scientific knowledge. Valorisation is not just about 'money', but also about the impact that can be created through successful alliances with industry. In such collaborations, both the CNIO and its industrial counterpart are committed to working towards a common goal and to jointly undertaking all the steps needed to conduct the research, as well as to identifying the best possible protection and commercialisation of the results. In 2016, new contracts with industry secured future revenues for collaborative research that amount to nearly 4 million euros, which represents about 10 % of CNIO's annual income.

The CNIO External Scientific Advisory Board (SAB), currently chaired by Mariann Bienz, is of utmost importance for guiding the strategic plans of the CNIO as well as for the review of our research groups. We would like to wholeheartedly thank our former SAB member Joan Massagué (Memorial Sloan Kettering Cancer Center, New York, USA) for his committed dedication to the CNIO SAB that lasted for over a decade (2003-2016). Joan Massagué, served as Chair of the CNIO SAB from 2011 to 2014, and as member for the remaining 9 years. During 2016, we welcomed two new members to our SAB, namely Stephen Frye, Director of the Centre for Integrative Chemical Biology and Drug Discovery at the University of North Carolina Eshelman School of Pharmacy in Chapel Hill (USA); and Ada E. Yonath, Nobel Prize winner in Chemistry (2009) and Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly at the Weizmann Institute of Science in Rehovot (Israel).

I would like to take this opportunity to thank all those who helped the CNIO by sponsoring our students, postdoctoral programmes and the stays of several researchers. I hereby extend my gratitude to the *Banco Santander* Foundation for funding postdoctoral stays at the CNIO and the *IE Business School* course, the *La Caixa* Foundation for fostering international PhD fellowships, the *Seve Ballesteros* Foundation that supports the Seve-Ballesteros Foundation-CNIO Brain Tumour Group, and the *Jesus Serra* Foundation for supporting the Visiting Scientists Programme and the Dean's Office. During 2016, we hosted Patrick Sung, Professor of Molecular Biophysics and Biochemistry and of

Therapeutic Radiology, Yale University School of Medicine in New Haven (USA), who started his Sabbatical at CNIO in 2015 and split his stay at the Centre in two short periods.

I also wish to thank the *Banc Sabadell* Foundation for sponsoring a series of Distinguished Seminars at the CNIO given by 'outside-the-box speakers' who provided novel perspectives that contribute to the CNIO's transdisciplinary environment. During 2016, we had the privilege to listen to: Romain Quidant, ICREA Research Professor at the Institute of Photonic Sciences (ICFO), Barcelona, Spain; Andrés Moya, Professor at the University of Valencia, Spain; Francisco Juan Martínez Mojica, Professor at the University of Alicante, Spain; and Francisco J. Ayala, an evolutionary biologist and philosopher at the University of California, Irvine, USA. The *Banc Sabadell* Foundation also sponsored one of the WISE seminars, given by Edurne Pasaban, Mountaineer, Tolosa, Spain.

Furthermore, I would like to highlight the work that is being carried out at the CNIO Women and Science (WISE) Office. During 2016, we had the pleasure of listening to María Concepción Ferreras, Head of You Tube Partnerships in Southern Europe and Russia; Margery Resnick, Professor at the Massachusetts Institute of Technology in Cambridge (USA), and President of the International Institute in Madrid; Pilar Garrido, Oncologist at the *Instituto Ramón y Cajal de Investigación Sanitaria* (IRYCIS), *Ramón y Cajal* University Hospital, Madrid; Edurne Pasaban, Mountaineer, Tolosa, Spain; María Teresa Fernández de la Vega, President of Women for Africa Foundation (*Fundación Mujeres por África*), and former First Deputy Prime Minister of Spain; Tánia Balló, documentalist and film director, Barcelona, Spain; Ángeles González-Sinde, scriptwriter, film director and former Spanish Minister of Culture; and Christina Rosenvinge, Spanish singer-songwriter, actress and producer. Together with Belén Yuste and Sonnia L. Rivas Caballero, from *Rocaviva Eventos*, Madrid, we also organised and hosted the CNIO Exhibition 'Marie Skłodowska-Curie: a Polish girl in Paris'. Guadalupe Martín Martín, Medical Physicist at the *Hospital Universitario de Fuenlabrada*, gave us the opening talk about the role of Marie's research breaking barriers for women in science at the time and starting the field of radiotherapy.

During 2016, we were also very excited to incorporate a journalist into our Communications Office; Cristina de Martos previously worked as a journalist for the health section of *El Mundo* (the second most read newspaper in Spain) for eight years. In 2016, the CNIO appeared in the printed and digital press more than 2,600 times, thus helping to bring the CNIO to the forefront of public awareness and interweaving it into our country's culture. Throughout the year, the featured stories received nearly 83,000 hits (EurekAlert! news service) from around the world and were taken up by prestigious international media such as the BBC, The Guardian, The Scientist or Scientific American. One of the most widely commented articles in 2016 was the one authored by the researchers Manuel Serrano and Lluc Mosteiro on cell reprogramming; it was published in November in the prestigious *Science* journal. Their discovery was covered by important media outlets such as radio and television, and even made it to the first page of the daily, *El Mundo*.

The 'CNIO Friends' initiative, devoted to raising funds for cancer research at the CNIO, celebrated its first two years of existence at the end of 2016. At that time, the initiative had about 800 Friends who showed their unwavering commitment at all times. Thanks to them, we put in place the first three cancer research grants in 2016. The first two grants sponsored the researchers Paulina Gómez, from the Genetic and Molecular Epidemiology Group, and Vera Pancaldi, from the Structural Computational Biology Group. The third grant came from an agreement with the *Juegaterapia* Foundation (http://juegaterapia.org/) that is devoted to helping children affected by cancer. Thanks to this collaboration, the CNIO will hire a researcher in 2017 to investigate neuroblastoma and central nervous system tumours, two of the most common tumours in children.

Businesses also joined our CNIO Friends community. In this context, the CNIO signed a collaboration agreement with *ASISA Vida* in relation to a new company product that includes specific coverage for gynaecological cancer. The CLH Group also participated in the sponsorship of the CNIO through an agreement aimed to support research and training of CNIO's scientific personnel.

Last but not least, I would like to thank all the CNIO volunteers who make it possible for us to move our mission forward, and of course, to the entire CNIO Friends community. Combining society's efforts with the endeavours of the research community can make a significant difference for the future of cancer.

Finally, I would like to thank all of those who have once again collaborated on the elaboration of this Annual Report, with especial thanks to Sonia Cerdá who is responsible for this CNIO publication, as well as to our collaborators: the visual artist Amparo Garrido and the underbau graphic design team.

ORGANISATION OF RESEARCH

MARIA A. BLASCO DIRECTOR

ALFONSO VALENCIA VICE-DIRECTOR OF BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

Manuel Serrano Programme Director

Manuel Serrano
Tumour Suppression Group

Mariano Barbacid
Experimental Oncology Group

Maria A. Blasco
Telomeres and Telomerase Group

Marcos Malumbres
Cell Division and Cancer Group

Óscar Fernández-Capetillo
Genomic Instability Group

Ana Losada
Chromosome Dynamics Group

Juan Méndez
DNA Replication Group

María S. Soengas
Melanoma Group

Héctor Peinado
Microenvironment and Metastasis Junior Group

Manuel Valiente
Brain Metastasis Junior Group

Alejo Efeyan
Metabolism and Cell Signalling Junior Group

CNIO CANCER CELL BIOLOGY PROGRAMME

Erwin F. Wagner Programme Director

Erwin F. Wagner
Genes, Development and Disease Group

Francisco X. Real
Epithelial Carcinogenesis Group

Mirna Pérez-Moreno
Epithelial Cell Biology Junior Group

Nabil Djouder
Growth Factors, Nutrients and Cancer Junior Group

Massimo Squatrito
Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

Alfonso Valencia Programme Director

Alfonso Valencia
Structural Computational Biology Group

Daniel Lietha
Cell Signalling and Adhesion Junior Group

Santiago Ramón-Maiques
Structural Bases of Genome Integrity Junior Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Fátima Al-Shahrour
David G. Pisano (until May)
Bioinformatics Unit

Salvador J. Capella Gutierrez (since June)
National Bioinformatics Institute Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Crystallography and Protein Engineering Unit

ÓSCAR FERNÁNDEZ-CAPETILLO VICE-DIRECTOR OF TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez Programme Director

Javier Benítez
Human Genetics Group

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Miguel Urioste
Familial Cancer Clinical Unit

Juan C. Cigudosa
Molecular Cytogenetics and Genome Editing Unit

Anna González-Neira
Human Genotyping-*CEGEN* Unit

CLINICAL RESEARCH PROGRAMME

Manuel Hidalgo (until December) Programme Director

Manuel Hidalgo (until December)
Gastrointestinal Cancer Clinical Research Unit

Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

David Olmos
Prostate Cancer Junior Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

Joaquín Martínez-López
H12O-CNIO Haematological Malignancies Clinical Research Unit

Luis Paz-Ares
H12O-CNIO Lung Cancer Clinical Research Unit

BIOBANK

Manuel M. Morente Director

ÓSCAR FERNÁNDEZ-CAPETILLO DIRECTOR OF INNOVATION

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Genomics Core Unit

Sagrario Ortega
Transgenic Mice Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Lola Martínez
Flow Cytometry Core Unit

Diego Megías
Confocal Microscopy Core Unit

Javier Muñoz
Proteomics Core Unit

Alba De Martino
Histopathology Core Unit

Isabel Blanco
Animal Facility (Vivotecnia Management & Services)

EXPERIMENTAL THERAPEUTICS PROGRAMME

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Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling Therapies Section

María José Barrero
CNIO-Lilly Epigenetics Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz Director

Vice-Direction of Basic Research

ALFONSO VALENCIA
Vice-Director of Basic Research

“My main activity, as Vice-Director for Basic Research, is to work together with CNIO’s Basic Research Groups in order to enhance scientific excellence and foster collaboration.”

There have been several encouraging developments this year pertaining to the organisational aspects within the CNIO’s Basic Research domain: 1 Junior Group Leader, Alejo Efeyan, joined the CNIO to conduct a new line of research in the area of metabolism and cell signalling in cancer; the two previous Units that provided support in Bioinformatics and Translational Bioinformatics were reorganised and merged into one single Bioinformatics Unit headed by Fátima Al-shahrour (it has already started operations in collaboration with a number of CNIO Groups); the inclusion of CNIO’s protein production facility (previously integrated in the Proteomics Unit) in the Crystallography Unit, now called the Crystallography and Protein Engineering Unit; and the recruitment of Salvador J. Capella to lead the National Bioinformatics Institute (INB-ISCIII) Unit.

This constructive trend will be further strengthened in the coming year thanks to the creation of the Biological Text Mining Unit in the area of Computational Biology, headed by Martin Krallinger (this new Unit will become operational at the beginning of 2017); and the new recruitments in strategic areas of biochemistry-structural biology and electron microscopy - protein complexes. Furthermore, the increase of collaborations with internal and external groups, as well as the consolidation of the projects developed with the Experimental Therapeutics Programme, will also lead to a further bolstering of our overall scientific output.

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It is my pleasure to introduce the highlights of the Molecular Oncology Programme in 2016. First of all, my enthusiastic and warm welcome to Alejo Efeyan, who joined the CNIO early this year to lead the Metabolism & Cell Signalling Junior Group. Alejo is a brilliant young scientist who trained as a postdoctoral fellow with David Sabatini, at the Massachusetts Institute of Technology, Cambridge. Cancer cells are metabolically hyperactive and an exciting discovery in recent years has been the realisation that cancer cells have mutations in the pathways that detect nutrient availability. Understanding how the nutrient sensing mechanisms contribute to cancer is the main goal of the Metabolism & Cell Signalling Junior Group. Alejo's outstanding career and his original project have been awarded with a prestigious and generous grant from the European Research Council. His Group is now settled and fully operative. We are all very proud of having him here with us and we wish him all the best!

It is also very gratifying that the two other Junior Groups that joined the CNIO during 2015 have continued to successfully consolidate their teams and their projects throughout 2016. The Brain Metastasis Junior Group, led by Manuel Valiente, now has promising candidate small compounds that inhibit metastasis initiation in brain slices. Likewise, the Microenvironment & Metastasis Junior Group, led by Héctor Peinado is making impressive progress towards detecting how the vesicles shed by tumours (known as exosomes) are distributed throughout the organism, modifying it, and thereby making it more receptive for metastatic seeding.

In the following pages, you will read about several surprising discoveries that expand our understanding of cancer and that might pinpoint new therapeutic strategies in the near future. For example, a protein that regulates mRNA stability, CEBP4, and contributes to cancer progression (Melanoma Group, *Nat. Commun.* 2016); or telomere-derived transcripts that play a key role in chromosomal integrity through the stabilisation of telomeres (Telomeres & Telomerase Group, *Nat. Commun.* 2016).

"Congratulations to Marcos Malumbres and Óscar Fernández-Capetillo for being elected as members of the European Molecular Biology Organization (EMBO)."

Other discoveries concern basic mechanisms that are altered in cancer. For example, it has been found that the infliction of damage to tissues triggers cell plasticity in the surrounding cells and that cytokine IL-6 is a key factor in this process (Tumour Suppression Group, *Science* 2016). You will also read about a rather unique DNA polymerase named PrimPol (DNA Replication Group); about how cohesins regulate transcription, an unsuspected role for these proteins traditionally involved in sister chromatid cohesion (Chromosome Dynamics Group); and about a kinase named MASTL that is upregulated in cancer and its inhibition blocks the proliferation of some cancer cells (Cell Division & Cancer Group).

The identification of preclinical anti-cancer treatments is the ultimate goal of the Molecular Oncology Programme. In this regard, we are proud of two relevant contributions: a novel therapeutic approach for lung adenocarcinoma based on two inhibitory molecules that are effective even against aggressive cancers lacking p53 (Experimental Oncology Group, *Nat. Med.* 2016); and the identification of compounds that block DNA repair with therapeutic efficacy against acute myeloid leukaemia (Genomic Instability Group, *Sci. Signal.* 2016).

TUMOUR SUPPRESSION GROUP

Manuel Serrano
Group Leader

Staff Scientists
Susana Llanos, Bárbara Martínez, Daniel Muñoz (until August), Cristina Pantoja

Post-Doctoral Fellows
Timothy Cash, Cian J. Lynch, Gianluca Varetti

Graduate Students
Noelia Alcázar, Raquel Bernad, Selim Chaib, Dafni Chondronasiou, Elena López-Guadamillas (until September), Lluc Mosteiro, Miguel Rovira

Technician
Maribel Muñoz (TS)*

**Titulado Superior* (Advanced Degree)

Student in practice
Isabel Calvo

OVERVIEW

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes, but they can become defective over time. The affected cells thus become partially unprotected and, combined with additional mutations in other genes, can give rise to cancer. Understanding how tumour suppressor genes work may help us to design drugs that block cancer. Tumour suppressor genes are now known to control many aspects of cell biology and organismal physiology, such as cellular pluripotency, cell senescence, and metabolism. We aim to achieve an integrated understanding of cancer protection.

"We have found that damaged cells secrete factors that promote reparative activities in the surrounding cells, including loss of differentiation and plasticity. This could be beneficial to repair the damaged tissues, but it could also favour the expansion of dormant cancer cells."

Our goals are to:

- → Understand the mechanisms of tumour suppression and identify new tumour suppressor regulators.
- → Study the interplay between tumour suppression and ageing.
- → Analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage.
- → Characterise cellular senescence as a tumour suppression mechanism.
- → Investigate cellular pluripotency and the involvement of tumour suppressors in the process of reprogramming to induced pluripotent stem (iPS) cells.
- → Explore the role of cell plasticity in cancer, tissue regeneration, and ageing.
- → Search for new frontiers in cell plasticity.

RESEARCH HIGHLIGHTS

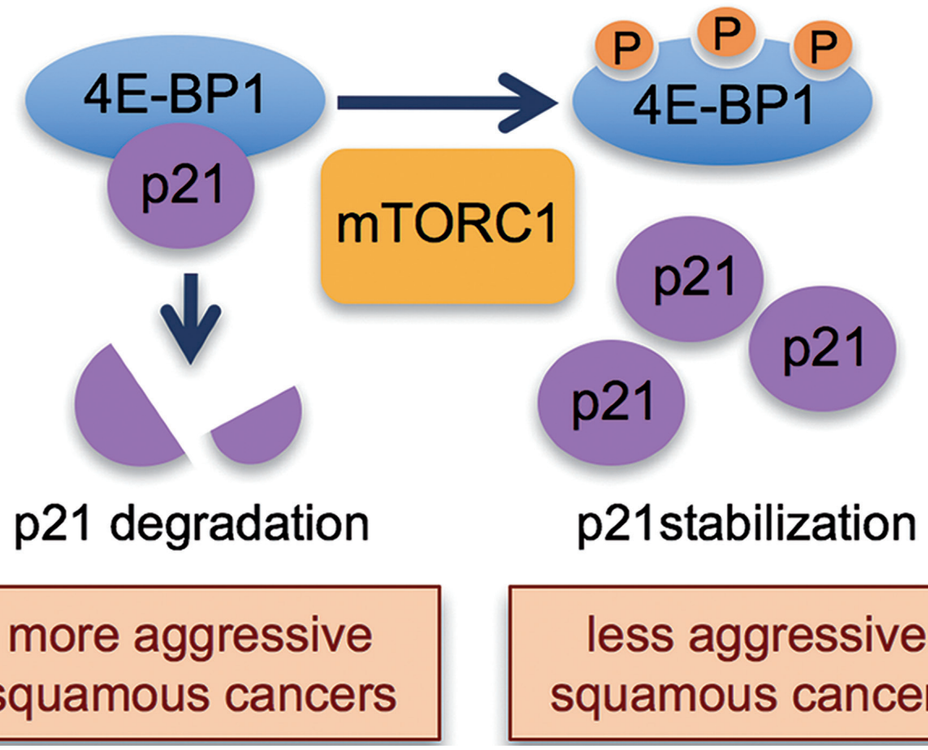

Figure 1 Model of mTOR-mediated regulation of p21. Non-phosphorylated 4E-BP1 binds to p21 and promotes p21 degradation, and this is associated to more aggressive cancers. 4E-BP1 phosphorylation by mTOR disrupts the 4E-BP1/p21 complex ensuing stable p21 accumulation, and this is associated to less aggressive cancers.

New tumour markers for the prognosis of head and neck cancer

Head and neck cancers include a heterogeneous group of tumours located in the oral cavity, pharynx and larynx. The survival rate of patients with this pathology has hardly improved over the last decade. Stratification of patients has been limited, until now, to a clinical classification and not a molecular one. Analysis of patients' biopsies showed that about half of them possess high levels of the p21 protein as well as mTOR activation. We have unravelled the molecular mechanism by which p21 levels are linked to the activity of mTOR (FIGURE 1). When the mTOR protein is inactive, it dictates the degradation of p21, and, conversely, when mTOR is active, p21 becomes stable. The presence of both markers, active mTOR and high levels of p21, predict a less aggressive evolution of the disease. This may help in choosing from amongst different therapeutic options for these patients.

Antioxidant defences delay ageing and age-related diseases

Accumulation of cell damage plays an important role in ageing. There is no clear answer about which types of cellular damage

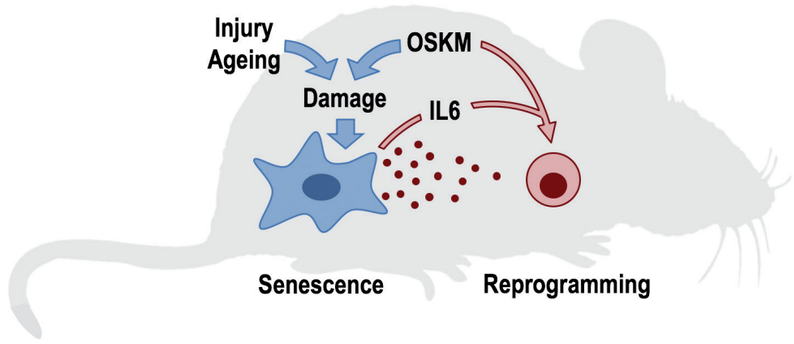

Figure 2 Tissue damage and ageing favour *in vivo* reprogramming. Expression of OSKM *in vivo* induces the reprogramming of a small population of cells, as well as damage and senescence in many other cells. Senescent cells release factors that promote the reprogramming of neighbouring cells, and IL6 is a critical mediator. Tissue injury and ageing also favour *in vivo* reprogramming through the accumulation of senescent cells.

are more relevant for ageing. Although the accumulation of oxidative damage with ageing is undisputed, the large majority of attempts to prove that oxidative damage is relevant for ageing have failed. All these attempts, however, have manipulated only one component of the complex network of antioxidant defences. In contrast to these previous attempts, we have approached this issue by increasing the levels of NADPH, a simple co-factor required for almost all antioxidant reactions and whose levels are known to determine the global antioxidant capacity of cells. To achieve this, we generated transgenic mice with an increased expression throughout their bodies of glucose-6-phosphate dehydrogenase (G6PD), one of the most important enzymes for the production of NADPH. We found that G6PD transgenic mice have overall higher levels of NADPH and, consequently, a better protection against oxidative damages. Importantly, these mice are not predisposed to cancer and, indeed, have a modest increase in longevity. These observations point to a novel strategy to delay ageing-related diseases, including cancer.

Senescent cells provide critical signals for cellular reprogramming

The mechanisms involved in the reprogramming of differentiated cells inside a living organism remain to be elucidated. Senescence is a cellular response to damage characterised by an abundant production of cytokines and other secreted factors that, together with the recruitment of inflammatory cells, results in tissue remodelling. We have shown that *in vivo* expression of the reprogramming factors OCT4, SOX2, KLF4 and cMYC (OSKM) triggers two divergent cellular outcomes: most cells undergo senescence, while other cells undergo reprogramming, both occurring in close physical association. OSKM-induced senescence requires the tumour suppressor locus *Ink4a/Arf*, which, via the production of the cytokine IL6, creates an optimal tissue environment for *in vivo* reprogramming. We concluded that tissue injury or ageing, through cellular senescence and cytokine IL6, favour *in vivo* reprogramming by OSKM (FIGURE 2). These findings could be relevant for tissue repair and open new strategies to manipulate reprogramming *in vivo*. ■

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EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Staff Scientists
Matthias Drosten, Carmen Guerra, Monica A. Musteanu, David Santamaría (until August)

Post-Doctoral Fellows
Chiara Ambrogio (until April), Raquel García, Harrys K.C. Jacob

Graduate Students
M. Teresa Blasco, Laura De Esteban, Magdolna Djurec, Fernando Fernández (since October), Jing Li (since December), Laura Martín (since September), Marina Salmón (since October), Manuel Sanclemente, Lucía Simón (until December)

Visiting Graduate Student
Lavinia Cabras (*Università di Cagliari,* Italy) (since September)

Technicians
M. Carmen González (TS)*, Silvia Jiménez (since August), Marta San Román. Raquel Villar

**Titulado Superior* (Advanced Degree)

External Associates
Alfredo Carrato (*Hospital Ramón y Cajal*, Madrid), Bruno Sainz (*Universidad Autónoma de Madrid*), Juan Velasco (Lilly Spain)

OVERVIEW

KRAS oncogenes have been implicated in about one fifth of all human cancers including lung and pancreatic adenocarcinomas, 2 of the tumour types with the worst prognosis. Unfortunately, identification of suitable therapies to treat these tumours remains elusive and patients are still treated with cytotoxic compounds approved over 2 decades ago. The recent discovery that these tumours display intra-tumour heterogeneity adds another layer of complexity that needs to be addressed. Hence, our laboratory has decided to search for novel therapeutic targets that may contribute to the early stages of lung tumour development, hoping that these targets will be present in all tumour cells – including cancer initiating cells and cancer stem cells – and not only in limited populations of evolving clones. In addition, we have continued our quest to validate known targets (mainly those of the MAPK and PI3K pathways) using genetically engineered mouse tumour models with the ultimate goal of establishing rational combination therapies that may provide significant therapeutic benefits in the clinic.

Significance

- We have shown that human lung tumours respond efficiently to combinations of DDR1 and NOTCH inhibitors in PDX models.
- We have provided a mechanistic explanation for the exclusive presence of K-*RAS* or *EGFR* mutations in human lung adenocarcinomas.
- We have demonstrated that the different incidence of H-*RAS* and K-*RAS* oncogenes in human tumours is due to the signalling intensity of their respective oncoproteins.

RESEARCH HIGHLIGHTS

Identification of novel therapeutic targets for the treatment of K-Ras driven lung adenocarcinoma

The recent discovery that lung tumours display significant levels of clonal heterogeneity (Govindan, *Science*, 2014) implies that effective therapies must target early oncogenic events/alterations present in all tumour cells and not only in clonal variants that appear during tumour development. To provide potential solutions to this key issue we decided to search for novel therapeutic targets present in the earliest stages of lung tumour development, expecting that such targets will be present in the entire tumour population including the putative cancer initiating/stem cells. Among the most highly expressed druggable genes we identified *Ddr*1, a locus that encodes a tyrosine protein kinase receptor. As reported early this year (Ambrogio *et al.*, *Nat Med*, 2016), genetic and pharmacological inhibition of Ddr1 prevented progression of K-Ras driven p53 wild type, but not p53 mutant tumours. Yet concomitant inhibition of Ddr1 and Notch, a downstream mediator of Ddr1 activity, led to a significant anti-tumour effect even in aggressive K-Ras^{G12V}; p53 mutant adenocarcinomas. More importantly, this treatment induced regression of K-RAS;p53 mutant patient-derived lung ortho-xenografts (PDX) with a therapeutic efficacy superior to standard chemotherapy. Identification of additional targets present in these early K-Ras mutant driven lung cells should expand the therapeutic opportunities to treat K-RAS mutant tumours in the clinic, thus by-passing the challenges derived from the development of intra-tumour heterogeneity.

Lack of selective advantage for lung cells expressing K-*RAS* and *EGFR* oncogenes

Activating mutations in *KRAS* and *EGFR*, the 2 most frequent oncogenic drivers in human lung adenocarcinoma, occur in a mutually exclusive manner suggesting functional redundancy and implying lack of positive selection. By means of a mouse model engineered to induce expression of mutant *EGFR*^{L858R} in advanced tumours driven by a resident *Kras*^{G12V} oncogene, we show that, instead, their co-expression is detrimental for the progression of lung adenocarcinoma. *In vivo* expression of *EGFR*^{L858R} in *Kras*^{G12V}-driven tumours triggers an immediate response with hallmarks of replicative stress resulting in apoptosis. Yet, a fraction of tumour cells survive, but enter a transient cytostatic state incompatible with tumour development that is fully reversible upon discontinuation of *EGFR*^{L858R} expression. Ultimately, continuous co-expression of both mutants results in the attenuation of the overall oncogenic signalling to levels compatible with cell proliferation and tumour growth. In sum, our results indicate that the mutual exclusivity of *KRAS* and *EGFR* activating mutations occurs as a combination of cellular toxicity and signal adjustment that results in the lack of selective advantage for those cells expressing both oncogenes.

Whereas the wild type H-Ras and K-Ras proteins are bioequivalent, their oncogenic isoforms H-Ras^{G12V} and K-Ras^{G12V} induce different tumour spectra

We have provided genetic evidence demonstrating that the H-Ras and K-Ras proteins are fully bioequivalent in mice. Previous studies have shown that replacement of the K-*Ras* alleles by H-Ras coding sequences resulted in viable mice (Potenza *et al.*, *EMBO Rep,* 2005). Yet, these mice displayed cardiovascular defects. Now, we have shown that these defects were due to the presence of the 4 H-*Ras* expressing alleles in these mice. Ablation of the 2 endogenous H-*Ras* alleles, hence generating mice that only express the H-Ras protein from the 2 targeted K-*Ras* alleles, is absolutely normal.

These results appear to be at variance with the well-established observation that H-*RAS* and K-*RAS* oncogenes are involved in different human tumour types. To determine whether the oncogenic versions of the H-Ras and K-Ras proteins are also bioequivalent, we knocked-in H-*Ras*^{G12V} oncogene sequences into the K-*Ras* locus. Germline expression of H-Ras^{G12V} or K-Ras^{G12V} from the K-*Ras* locus resulted in equal embryonic lethality. However, their expression in adult mice led to different tumour phenotypes. Whereas H-Ras^{G12V} elicited papillomas and haematopoietic tumours, K-Ras^{G12V} induced lung tumours and gastric lesions. The reason why H-Ras^{G12V} expression failed to cause lung tumours is due to the induction of a senescence-like state due to excessive MAP kinase signalling. Likewise, H-Ras^{G12V} but not K-Ras^{G12V} induced oncogene-induced senescence in mouse embryonic fibroblasts (MEFs). Label-free quantitative analysis revealed that minor differences in H-Ras^{G12V} expression levels led to drastically different biological outputs, suggesting that subtle differences in MAP kinase signalling influence the differential tumour spectra induced by RAS oncoproteins. ■

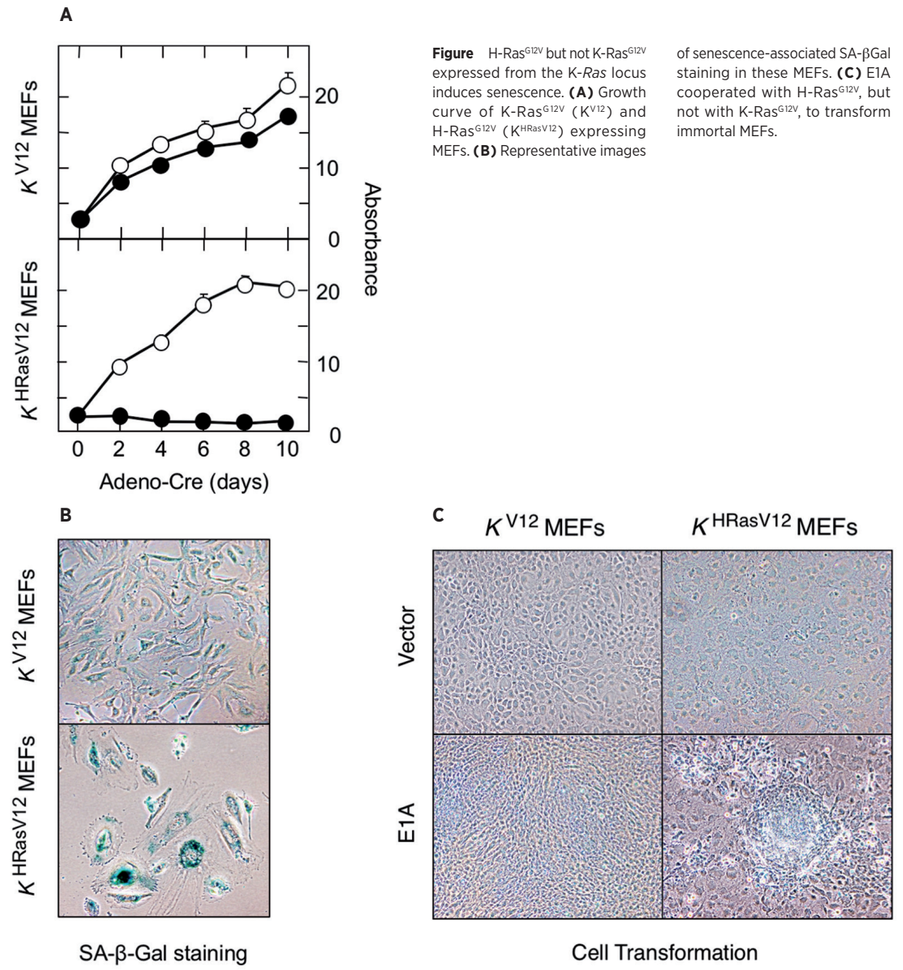

Figure H-Ras^{G12V} but not K-Ras^{G12V} expressed from the K-*Ras* locus induces senescence. **(A)** Growth curve of K-Ras^{G12V} (K^{V12}) and H-Ras^{G12V} (K^{HRasV12}) expressing MEFs. **(B)** Representative images of senescence-associated SA-βGal staining in these MEFs. **(C)** E1A cooperated with H-Ras^{G12V}, but not with K-Ras^{G12V}, to transform immortal MEFs.

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AWARDS AND RECOGNITION

- Advanced Grant of the European Research Council (2016-2021).
- Keynote Speaker, 24th congress of the European Association for Cancer Research, Manchester, UK.
- Keynote Speaker, International Conference on Predictive Cancer Models, Barcelona, Spain.
- Keynote Speaker, 49th Annual Meeting of the European Society of Human Genetics, Barcelona, Spain.
- Meeting Organiser and Session Chair, 28th Pezcoller Symposium, Trento, Italy.
- Honorary Member, Royal Academy of Medicine of Valencia, Spain.

TELOMERES AND TELOMERASE GROUP

Maria A. Blasco
Group Leader

Staff Scientists
Isabel López de Silanes, Rosa M. Marión, Paula Martínez, Marinela Méndez, Águeda M. Tejera, Elisa Varela (until May)

Post-Doctoral Fellow
Kurt Whittemore

Graduate Students
Leire Bejarano, Aksinya Derevyanko, Lole Ferrara, Juan José Montero, Miguel Ángel Muñoz, Juan Manuel Povedano (until June)

Technicians
Rosa M. Serrano, Nora Soberón (until June) (TS)*

Titulado Superior (Advanced Degree)

Student in practice
Ana C. Moises da Silva

OVERVIEW

We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Immortality is one of the most universal characteristics of cancer cells. The enzyme telomerase is present in more than 95% of all types of human cancers and absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes, essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered adult stem cells have a maimed regenerative capacity.

"We have demonstrated that TERRA long non-coding RNAs are essential for telomere protection."

Our research aims are:

- → Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- → Deciphering the interplay between telomeres and DNA repair pathways.
- → Studying the role and regulation of non-coding telomeric RNAs or TERRA.
- → Testing telomerase gene therapy in 'telomere syndromes' and age-related diseases.
- → Elucidating the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

RESEARCH HIGHLIGHTS

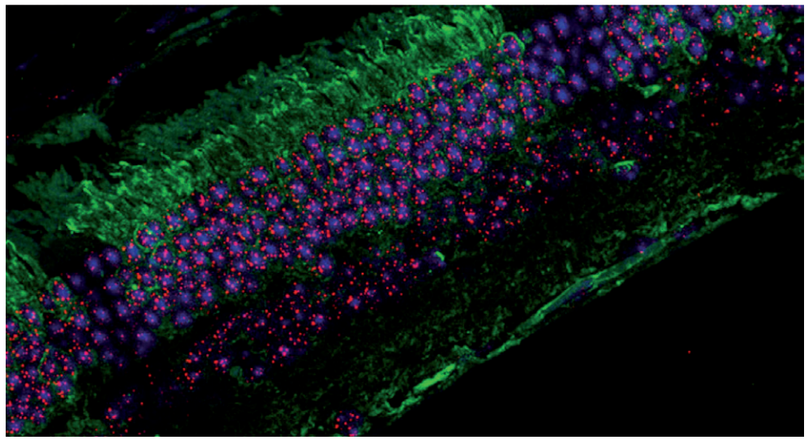

Figure 1 Representative image of an eye of chimaeric mice. Cells bearing hyper-long telomeres are visualised in green. Telomeres appear in red.

Fighting aplasic anaemia using a therapy designed to delay ageing

Aplasic anaemia is a rare, potentially fatal disease of the blood, by which the bone marrow is unable to generate blood cells at the appropriate pace. The disease can be hereditary or acquired and develops at any stage of life. A subgroup of the inherited form is caused by replicative impairment of haematopoietic stem and progenitor cells owing to very short telomeres due to mutations in telomerase and other telomere components. An abnormal telomere shortening is also described in cases of acquired aplasic anaemia. We have tested the efficacy of our telomerase gene therapy, originally designed to delay ageing, in two independent mouse models of aplasic anaemia due to short telomeres. We found that a high dose targets the bone marrow compartment, including haematopoietic stem cells. Telomerase treatment following telomere attrition in bone marrow cells rescues aplasic anaemia and mouse survival. Improved survival is associated with a significant increase in telomere length in peripheral blood and bone marrow cells, as well as improved blood counts. Our telomerase gene therapy represents a novel therapeutic strategy to treat aplasic anaemia provoked or associated with short telomeres.

Mice with hyper-long telomeres and unaltered genes

Telomere length is genetically determined, but in the past we were able to generate mouse embryonic stem (ES) cells with telomeres twice the size of normal ones. We have now used such ES cells with 'hyper-long' telomeres, traceable thanks to the co-expression of green fluorescent protein (GFP), to generate chimaeric mice containing cells with both hyper-long and normal telomeres. We showed that chimaeric mice contain GFP-positive cells – bearing hyper-long telomeres – in all mouse tissues (FIGURE 1), display normal tissue histology, as well as normal survival. Both hyper-long and normal telomeres shorten with age, but GFP-positive cells manage to retain longer telomeres as the mice age. These chimaeric mice also accumulate fewer cells with short telomeres and less DNA damage with age, and express lower levels of p53. Cells with hyper-long telomeres are longitudinally maintained or enriched with age in highly renewing compartments (i.e. blood). We demonstrated that mice with functional, longer and better preserved telomeres can be generated without the need for genetic manipulations, such as telomerase overexpression.

Telomeric RNAs are essential to maintain telomeres

Despite their especially compact structure, which is difficult to access, telomeres transcribe information like the rest of the DNA generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA was one of the unsolved issues of telomere biology in the past decade. This was, in part, due to a lack of knowledge on the TERRA loci, which had prevented functional genetic studies. We had already shown that mouse TERRA arise mainly from the subtelomere of chromosome 18 and to a lesser extent from the subtelomere of chromosome 9. We have now described that long non-coding RNAs with TERRA features are transcribed from the human 20q and Xp subtelomeres. We used the CRISPR-Cas9 technology to delete the 20q locus, which resulted in a dramatic decrease in TERRA levels. The deletion of the Xp locus, on the contrary, does not lead to decreased TERRA levels. These findings demonstrate that, although human TERRA arise from two loci, the 20q locus is the main origin of human TERRA. Thus, both in mice and in humans, TERRA arise from one, or at most two loci (FIGURE 2). Ablation of 20q-TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response. These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres. ■

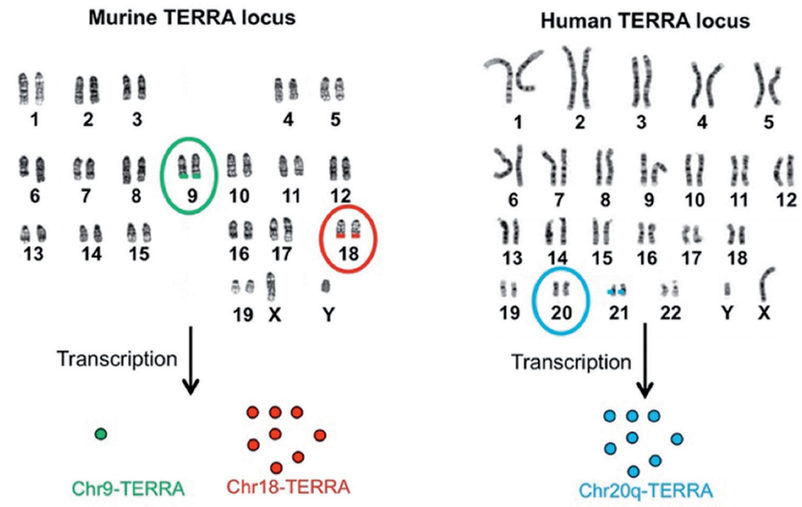

Figure 2 The genomic origin of telomeric RNAs (TERRA). Both in mice and in humans, TERRA arise from one or at most two loci.

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AWARDS AND RECOGNITION

- *Miguel Catalán* Career Achievement Award, Regional Government of Madrid.
- Scientific Committee Member, Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, Brussels, Belgium.
- Member of the External Scientific Board of *Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL)*, Alicante, Spain.
- Member, Alumni Advisory Board, *Universidad Autónoma de Madrid*.
- Founding Editor, *Cell Stress*.
- Editorial Board Member, *Nutrition & Healthy Aging*.

CELL DIVISION AND CANCER GROUP

Marcos Malumbres
Group Leader

Staff Scientists
Mónica Álvarez, Guillermo de Cárcer, Ignacio Pérez de Castro (until February)

Post-Doctoral Fellows
Begoña Hurtado, Carolina Maestre, María Salazar

Graduate Students
Ana F. Batalha Martins, María Maroto, Diego Martínez, Belén Sanz, María Sanz

Technicians
Aicha El Bakkali (since March) (PEJ)*, María Guirola (since March) (TS)**, Elisabet Zapatero (TS)**

**Titulado Superior* (Advanced Degree)

**Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated. During the past few years, we have used different mouse models to understand the relevance of cell cycle regulators, including cell cycle kinases and phosphatases, as well as proteins involved in ubiquitin-dependent degradation, in the control of cell division and tissue physiology. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycle; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understanding the function of microRNAs in cell biology and tumour development, and iv) to understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties. As a final goal, we aim to generate information that may be useful for improving therapeutic strategies against cancer cell proliferation.

"In 2016, we investigated the relevance of several mitotic regulators during cancer progression and therapy, with special focus on kinases that are currently under preclinical and clinical evaluation."

RESEARCH HIGHLIGHTS

Controlling the proper number of cell divisions

The mammalian cell cycle is regulated by at least 2 families of inhibitors, the INK4 and Cip/Kip proteins. While elimination of individual members of these families is a frequent finding in human cancer, the consequences of eliminating this inhibitory mechanism in mammalian cells have not yet been explored. Using a combination of mutant alleles in the mouse, we have now observed that a major physiological function of cell cycle inhibitors is to prevent replicative stress. In a mouse model insensitive to INK4 proteins and deficient in $p21^{Cip1}$ and $p27^{Kip1}$, we observed that these inhibitors prevent the accumulation of DNA damage due to replicative stress in different tissues including the nervous system. Ablation of these inhibitors prevents mouse development. This effect is most likely due to hyperactivation of cyclin-dependent kinases as the replicative stress can be prevented by slightly inhibiting the enzymatic activity of these proteins (Quereda *et al.*, 2016).

Cell cycle kinases as new targets for cancer therapy

Cell cycle progression is controlled by phosphorylation events and cell cycle kinases are currently the focus of multiple therapeutic strategies. Inhibitors of the Aurora and Polo-like kinases are evaluated in clinical trials with promising results, at least in haematopoietic malignancies. Over the last few years, we have generated mouse models with specific mutations in these kinases in order to understand their roles in different tissues and cell types. Our recent data have uncovered an unexpected function of Polo-like kinase 1 (Plk1) in the cardiovascular system; a role that we are studying in detail in order to understand possible toxicities derived from the use of Plk1 inhibitors in patients.

A relatively new serine/threonine kinase, known as MASTL (or Greatwall in flies and *Xenopus*), has been characterised as a critical node in cell division. We have previously shown that MASTL is essential for mouse embryonic development and cell cycle progression (FIGURE). This is due to mitotic collapse after nuclear envelop breakdown (NEB). MASTL is exported from the nucleus to the cytoplasm in a CRM1-dependent manner before NEB. Once at the cytoplasm, Greatwall inhibits the PP2A-B55 complexes to maintain the mitotic state. These findings have therapeutic implications since MASTL acts by blocking the function of the PP2A phosphatase, a tumour suppressor frequently altered in human cancer. This implies that the inhibition of MASTL could, at the same time, slow down cell division and reactivate tumour suppressor PP2A, a protein capable of inhibiting many of the oncogenic molecular pathways involved in cancer development.

Over the past few months, we have tested this hypothesis by studying the relevance of MASTL in tumour cell proliferation and its possible use as a cancer target. In collaboration with Miguel Quintela's Group at the CNIO and Carlos Caldas at Cancer Research UK, we analysed MASTL expression in breast cancer. Our data suggest that this protein is overexpressed in a significant number of hormone-positive and -negative tumours and correlates with poor prognosis. In collaboration with researchers at Pfizer, we used different RNAi and CRISPR techniques to analyse the effect of MASTL knockdown or knockout in breast cancer cells both *in vitro* and *in vivo*. These data indicate that some breast cancer cells require MASTL kinase activity for proliferating, suggesting that a subset of breast tumours may benefit from strategies aimed at inhibiting this kinase. We are currently studying the consequences of inhibiting MASTL in the activity of the PP2A phosphatase. Since MASTL specifically inhibits PP2A-B55 complexes, we are also characterising the relevance of the B55 family members present in the human genome. ■

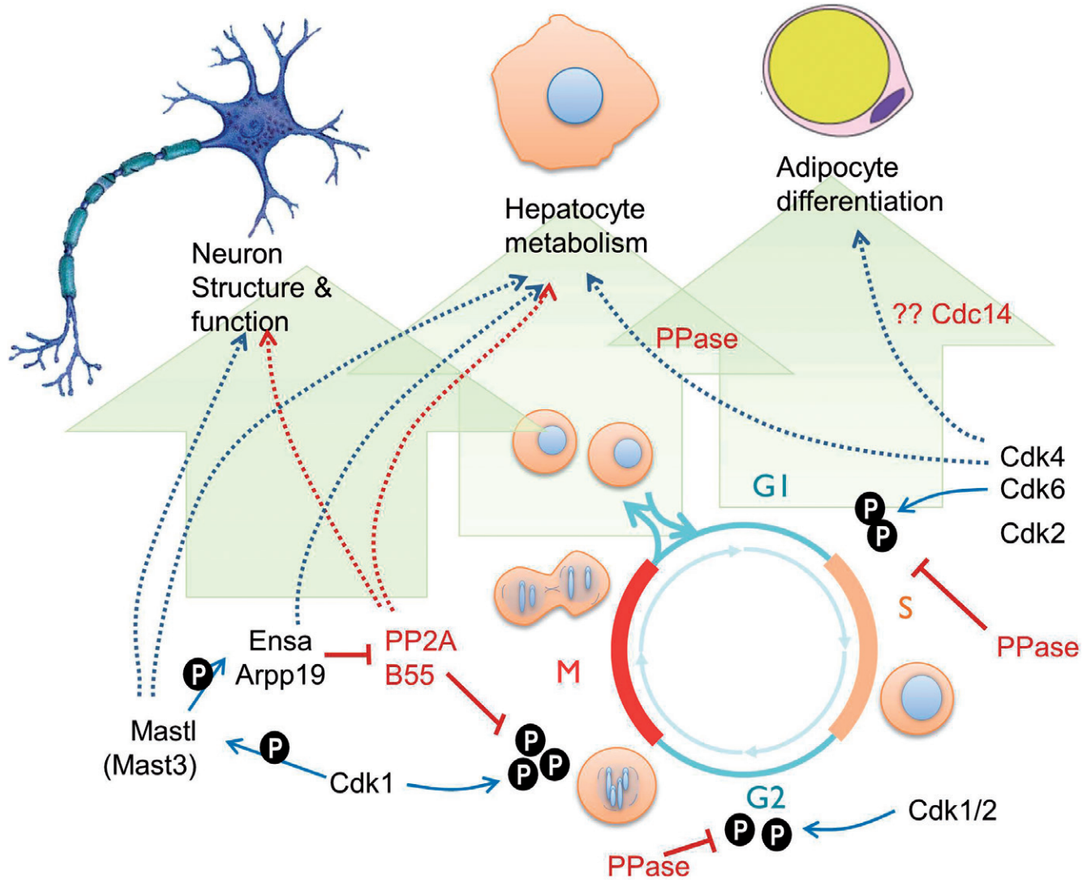

Figure Schematic representation of the control of the mammalian cell cycle by cyclin-dependent kinases (Cdks) and MASTL. MASTL phosphorylates 2 small proteins, known as Ensa and Arpp19, which are both PP2A-B55 inhibitors. The proper balance between cell cycle kinases and phosphatases is crucial, not only for cell cycle progression but also for the structure and function of different tissues.

PUBLICATIONS

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- Malumbres M (2016). CDK4/6 Inhibitors resTORe Therapeutic Sensitivity in HER2(+) Breast Cancer. *Cancer Cell* 29, 243-244.
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AWARDS AND RECOGNITION

- Elected EMBO Member.

GENOMIC INSTABILITY GROUP

Óscar Fernández-Capetillo
Group Leader

Staff Scientists
Emilio Lecona, Matilde Murga, Sergio Ruíz

Post-Doctoral Fellow
Vanesa Lafarga

Graduate Students
Antonio Galarreta, Cristina Mayor, Isabel Morgado, Teresa Olbrich, Federica Schiavoni

Technicians
Marta E. Antón, Patricia Cozar, Alicia González (TS)*, Sara Rodrigo, María Vega

**Titulado Superior* (Advanced Degree)

OVERVIEW

Our laboratory has centred its research on trying to understand how cells respond to 'replicative stress' (RS), which is frequent in cancer and induced by several anticancer agents. In mammals, RS is suppressed by a signalling cascade initiated by ATR and CHK1 kinases. Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of RS. These tools include mice with enhanced or limited ATR-CHK1 function, cell lines in which the pathway can be activated at will, and chemical inhibitors of the ATR kinase. Our studies have revealed the impact of RS on cancer and ageing, have led to drugs that can be used to test our ideas on cancer therapy, and have also unveiled the mechanisms by which these drugs kill cancer cells. Altogether, our main aim is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

"During 2016, we have investigated which tumour types could best benefit from a treatment with ATR inhibitors, the potential mechanisms of resistance to these drugs, as well as new pathways that suppress RS."

RESEARCH HIGHLIGHTS

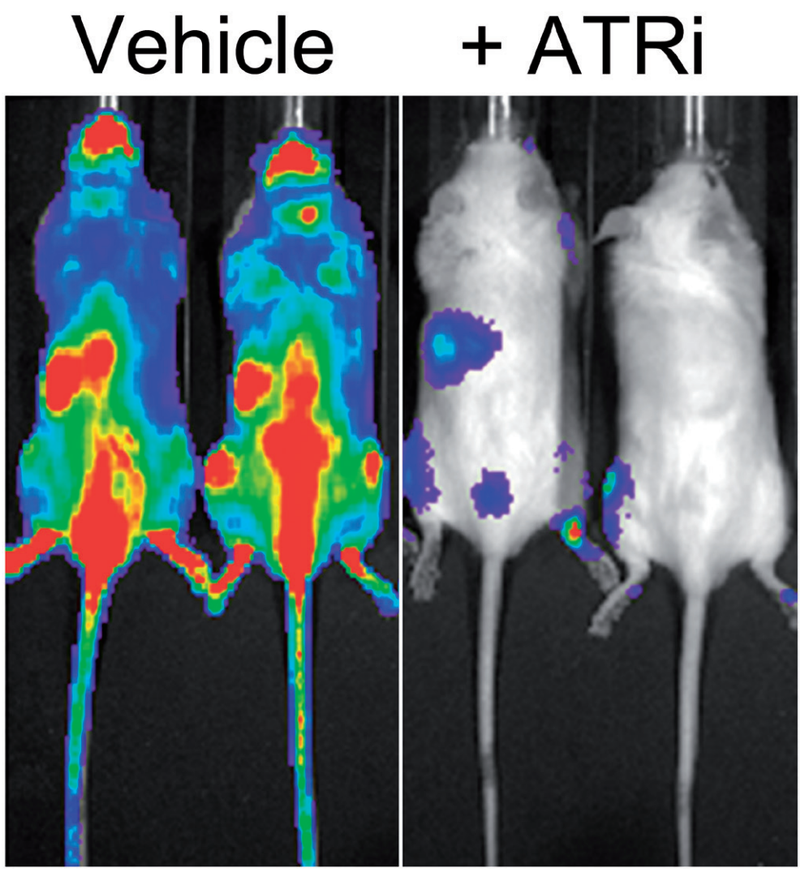

Figure 1 Impact of ATR inhibitors on Acute Myeloid Leukaemia. The images illustrate the efficacy of ATR inhibitors used as monotherapy in a mouse allograft model of Acute Myeloid Leukaemia, driven by an MLL-translocation (Morgado-Palacin *et al.*, *Sci Signaling*, 2016). Note the expansion of the tumour in control mice (left), which can be visualised by a colour gradient, and that is greatly reduced in mice treated with an ATR inhibitor.

Efficacy of ATR inhibition in two preclinical models of cancer

Replicative stress (RS) is a widespread phenomenon in cancer cells that, when persistent, leads to DNA double strand breaks and genomic instability. Besides from the basal level of RS that occurs in every cell division, the presence of oncogenes, or many of the agents used in chemotherapy, are potent inducers of RS. In mammals, RS is sensed and suppressed through a signalling cascade that is initiated with the activation of the ATR kinase. We previously hypothesised that due to the high levels of RS in certain cancers, they could be particularly dependent on a proficient RS-response. In this regard, and in collaboration with the Experimental Therapeutics Programme, we had developed chemical inhibitors of ATR that presented some anti-tumour properties *in vitro*. During 2016, our work in this area was focused on the identification of tumours that are particularly sensitive to ATR inhibition, as well as on the discovery of mechanisms of resistance to these chemicals. For the first area of focus, we have shown efficacy of ATR inhibitors, as monotherapy, in 2 mouse models of Ewing Sarcoma and Acute Myeloid Leukaemia (FIGURE 1). Regarding the second area of focus, we discovered – via genomewide CRISPR-Cas9 screening – that the levels of CDC25A, a key phosphatase controlling mitotic entry, are a key determinant of the sensitivity to ATR inhibitors in mouse and human cells.

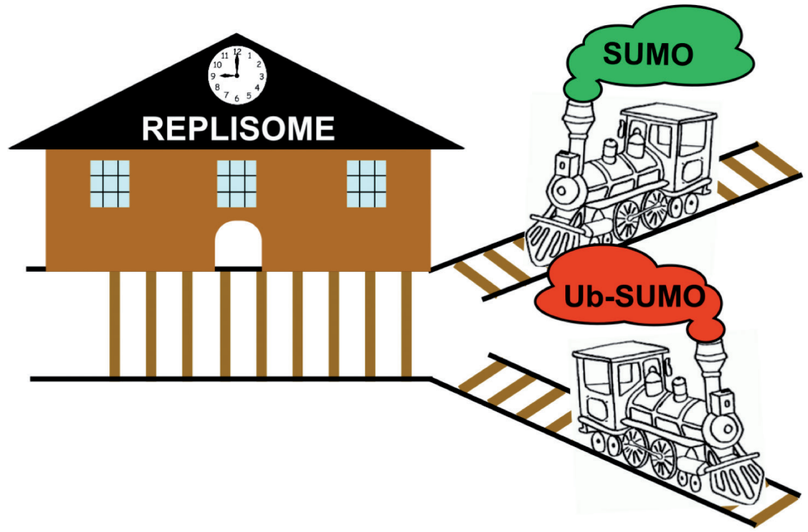

Figure 2 A SUMO and ubiquitin code regulates protein concentration around replisomes. Model derived from our recent work on the USP7 deubiquitinase (Lecona *et al.*, *Nat Struct Mol Biol*, 2016). We propose that, whereas SUMOylation is a signal for recruitment of proteins to the replisome, Ubiquitinated-SUMO constitutes a 'go' signal that drives their eviction.

Two new players that suppress replication stress in mammalian cells

Besides ATR, several other factors participate in limiting the impact of RS in mammalian cells. Recent works have identified that POLD3, a subunit of the DNA polymerase Pold, participates in the repair of the breaks generated by RS, and also suggest that limiting its activity could be specifically deleterious for cancer cells. By developing a novel conditional knockout mouse strain we found that POLD3 deletion is lethal during embryonic development and also when depleted in adult mice. These severe defects were explained by a complete destabilisation of the POLd complex in the absence of POLD3, which abrogates DNA replication, raising serious doubts regarding the potential of POLD3 as an anticancer target. In independent work, we have been investigating how SUMO and ubiquitin participate in the coordination of DNA replication. Here, we identified USP7 as the first chromatin-associated SUMO deubiquitinase (SDUB) and revealed its essential role during DNA replication. Accordingly, USP7 inhibitors lead to RS and DNA damage. By deubiquitinating SUMO and/or SUMOylated proteins, our research revealed that USP7 contributes to keep a SUMO-rich and ubiquitin-poor environment at sites of DNA replication; this is critical to maintain fork progression. Our current view is that USP7 is critical for controlling the traffic of replication factors, by supervising their recruitment or expulsion from replication factories. We propose that SUMOylation constitutes a 'stay' signal that recruits proteins near replication factors, with Ubiquitinated-SUMO being the 'go' signal that leads to their eviction (FIGURE 2). ■

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*co-first authors

AWARDS AND RECOGNITION

- Elected Member of the European Molecular Biology Organization (EMBO).

CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named **cohesin** that is essential for chromosome organisation. Cohesin mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. It also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation, organisation of DNA replication factories and locus rearrangement by recombination. Mutations in cohesin have recently been found in several tumour types, most prominently in bladder cancer and acute myeloid leukaemia. Mutations in cohesin and its regulatory factors are also at the origin of a group of human syndromes collectively known as cohesinopathies.

"We aim to define the specific contributions of cohesin-SA1 and cohesin-SA2 to genome organisation. Our work may uncover vulnerabilities in cancer cells carrying mutations in the gene encoding SA2, which is one of the twelve genes most mutated in cancer."

Our goal is to understand how cohesin works, how it is regulated and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex in somatic cells. We use mouse models carrying *knock out* alleles of genes encoding cohesin subunits to investigate their functional specificity, both at the cellular level and in the context of an organism. We also take advantage of the *Xenopus* egg cell-fee system to explore additional aspects of cohesin regulation.

RESEARCH HIGHLIGHTS

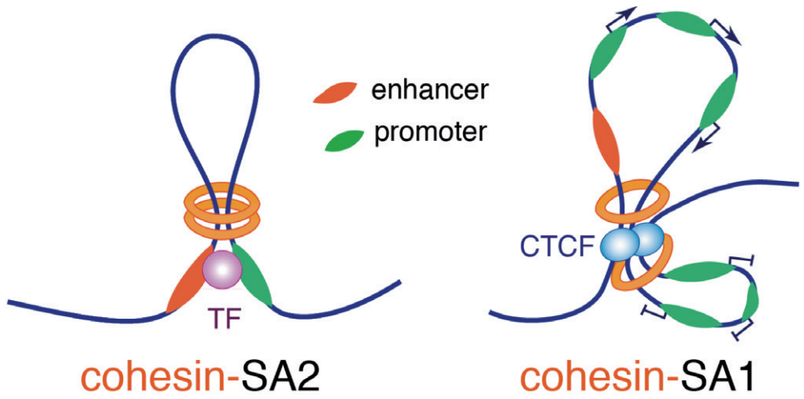

Figure 1 Cohesin-SA1 and cohesin-SA2 have non-overlapping functions in genome organisation and gene regulation. Cohesin-SA2 stabilises local interactions between cis-regulatory elements (enhancers and promoters) mediated by transcription factors (TF) while cohesin-SA1 collaborates with CTCF in the demarcation of domain boundaries.

Cohesin-SA2 regulates transcription independently of CTCF

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. We have previously reported that cohesin-SA1 is required for telomere cohesion, while cohesin-SA2 plays a major role in centromeric cohesion. In terms of transcriptional regulation, much less is known about the potential differences between these two complexes. A study in HeLa cells reported a similar genomic distribution for cohesin-SA1 and cohesin-SA2 that largely overlapped with the distribution of the architectural protein CTCF. Our initial comparison of cohesin distribution and transcriptomes of wild type and SA1-null mouse cells suggested that cohesin-SA1 could be more important for the regulation of transcription than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and the new sites displayed reduced overlap with CTCF and TSS (transcription start sites). Second, we found that in a number of genes whose expression was altered in SA1 null cells, cohesin-SA2 could not replace cohesin-SA1 efficiently since cohesin occupancy was clearly reduced in these cells (Remeseiro, Cuadrado *et al.*, 2012). When we analysed chromatin states in adult mouse brain using histone marks, CTCF and cohesin distribution, we noticed again that cohesin-SA1 complexes co-occurred with CTCF at active enhancers and promoters (Cuadrado *et al.*, 2015). More recently, using a new and better SA2 antibody, we examined the binding of cohesin-SA1 and cohesin-SA2 along the genome of human mammary epithelial cells. We found more than 20,000 positions common for both complexes in which CTCF is also present, but also found around 10,000 cohesin-SA2 specific positions, lacking SA1, in which the overlap with CTCF is significantly lower. Importantly, these cohesin-SA2 specific positions are highly enriched in enhancers and promoters and contain transcription factor binding motifs other than CTCF. Proteomic analysis of immunoprecipitates obtained with SA1 and SA2 antibodies detected several transcription factors among the SA2-specific interactors. Functional analyses, in which we compare changes in gene expression, cohesin distribution as well as chromatin architecture changes after downregulation of SA1, SA2 or CTCF, further support a prominent role of cohesin-SA2 in promoting local interactions between cis-regulatory elements independently of CTCF, while cohesin-SA1 would instead collaborate with CTCF in the demarcation of domain boundaries (FIGURE 1).

Pds5 proteins regulate cohesin dynamics

Two factors are associated with chromatin-bound cohesin, Pds5 and Wapl. Wapl promotes cohesin unloading and in its absence there is an excess of cohesin on chromatin, and chromosome organisation is altered both in interphase and mitosis. The role of Pds5 is less clear. Moreover, there are two versions of Pds5 present in vertebrate cells: Pds5A and Pds5B. In order to explore their specific functions we previously generated murine knock out (KO) alleles for these two genes. We showed that both Pds5A and Pds5B contributed to cohesion establishment during S phase by promoting cohesin acetylation and Sororin binding, with Pds5B being specifically required for cohesion at

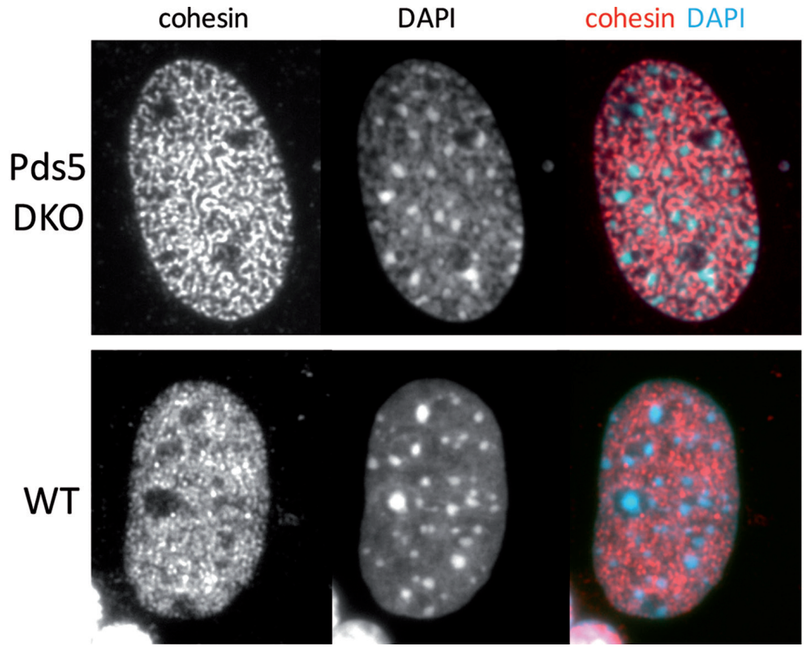

Figure 2 Image of interphase nuclei from mouse embryo fibroblasts lacking both Pds5A and Pds5B (Pds5 DKO, top) or wild type (WT, bottom), fixed and stained with an antibody against cohesin SMC3 and DAPI.

centromeres (Carretero *et al.* 2013). Now we have observed that cells lacking Pds5A, Pds5B, or both, have distinct alterations in their transcriptomes when compared to wild type cells. Genome wide distribution of cohesin is not obviously altered in the absence of either Pds5 protein, but does change in the absence of both. Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Aberrant accumulation of cohesin in axial structures, known as *vermicelli*, previously described in Wapl depleted cells, can be observed only in the absence of the two Pds5 proteins (FIGURE 2). Thus, Pds5 proteins are required for proper cohesin dynamics and cohesin distribution, although no clear specificities can be found for Pds5A and Pds5B, at least globally. The gene expression differences described above might therefore be caused by preferential interactions with chromatin regulators at specific loci. It is also possible that Pds5 proteins have functions independent of cohesin. We are currently exploring both these possibilities. ■

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DNA REPLICATION GROUP

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Karolina Jodkowska, Sergio Muñoz

OVERVIEW

Our laboratory studies the molecular mechanisms that underlie genomic duplication in mammalian cells. The 'replisome' complex in charge of DNA replication encounters natural obstacles (e.g. unusual DNA structures, collisions with transcription proteins), as well as exogenous challenges such as ionising radiation, UV light and chemicals that modify the DNA structure and block DNA polymerases. The situations in which replication forks are forced to slow down, stall or collapse are generically referred to as replicative stress (RS). Our Group investigates the 'DNA damage tolerance' pathways that facilitate DNA replication in the presence of RS or damaged DNA. In recent years, we have identified 2 mechanisms that counteract RS: (1) the conditional activation of dormant replication origins; (2) the participation of PrimPol, a primase-polymerase enzyme, in the restart of stalled forks. We continue to characterise the different cellular responses to RS (FIGURE 1).

"We have developed genetic tools to investigate the physiological impact of defective DNA replication, including mouse strains that suffer from a high incidence of haematological cancers due to their inefficient response to DNA damage during replication."

RESEARCH HIGHLIGHTS

Cellular functions of PrimPol protein

We have continued to characterise PrimPol, a DNA primase-polymerase that participates in DNA damage tolerance during chromosomal replication. With this aim in mind, we have generated human and mouse cells in which PrimPol expression is either downregulated or completely ablated. PrimPol-deficient cells display a marked sensitivity to UV irradiation, including the accumulation of unrepaired Thy dimers (CPDs and 6,4pp) in the DNA. The skin of PrimPol KO mice also presents an inefficient healing response to UV irradiation and a higher frequency of benign papillomas. The importance of PrimPol as a tumour suppressor gene is currently being investigated.

Effects of DNA re-replication *in vivo*

CDC6 and CDT1 proteins are responsible for the loading of MCM2-7, the DNA helicase, at replication origins. After CDC6 and CDT1 execute their 'origin licensing' functions in the G1 phase, their activities are inhibited until mitosis is complete in order to prevent origin reactivation and DNA over-replication within the same cell cycle. However, these strict control mechanisms may be partially overridden in some cancer types, notably non-small cell lung carcinomas, by the overexpression of *Cdc6* and/or *Cdt1* genes.

We have recapitulated the deregulated expression of *Cdc6* and *Cdt1* using mouse strains that allow the inducible expression of both proteins, alone or in combination. While individual deregulation of CDC6 or CDT1 has only mild effects, their combination is lethal for developing embryos and also for adult individuals. Using single-molecule analysis of DNA replication, high-throughput confocal microscopy and histopathology, we have identified origin re-firing events that are sufficient to cause DNA over-replication and DNA damage in different tissues. These mouse models will allow a complete study of the physiological impact of DNA re-replication *in vivo*.

Evidence for replicative stress in early embryonic cell cycles

Replicative stress is normally studied in the context of cells undergoing external challenges. However, it also occurs in the unperturbed S phase when the replication machinery reaches special DNA structures (e.g. G-quadruplexes) that are difficult to replicate, or when it collides with a transcriptional fork. In 2016, we participated in a collaborative study, led by Dr M. Lopes (University of Zurich, Switzerland), that identified unexpected levels of physiological RS. Mouse embryonic stem cells and early embryos at the blastocyst stage display a constitutive accumulation of RPA-covered ssDNA, fork slowing and fork remodelling events, all hallmarks of RS. These characteristics are related to the short duration of the G1 phase in embryonic stem cells and are lost upon the onset of cell differentiation. This result underscores the importance of the G1 phase to fully repair DNA that had been damaged in the previous cell cycle, before entering a new round of replication (Ahuja *et al.*, 2016).

Single-molecule analyses of DNA replication

As replicative stress impinges on many cellular processes, the possibility of analysing DNA replication at the single-molecule level continues to attract the interest of many research groups at the CNIO and other institutions. In 2016, we collaborated in two projects led by Oscar Fernández-Capetillo (CNIO Genomic Instability Group) to demonstrate that USP7 ubiquitin protease targets SUMO and is essential for DNA replication (Lecona *et al.*, 2016), and that PolD3 is haploinsufficient for DNA replication in mice (Murga *et al.*, 2016). In the latter project, the analyses of replication in stretched DNA fibres revealed a striking accumulation of asymmetric forks in the absence of POLD3, a regulatory subunit of DNA polymerase δ (FIGURE 2). Finally, a collaboration with R. Freire (*Hospital Universitario de Canarias*, Tenerife) revealed a novel function for USP37 ubiquitin protease in the control of DNA replication (Hernández-Pérez *et al.*, 2016). ■

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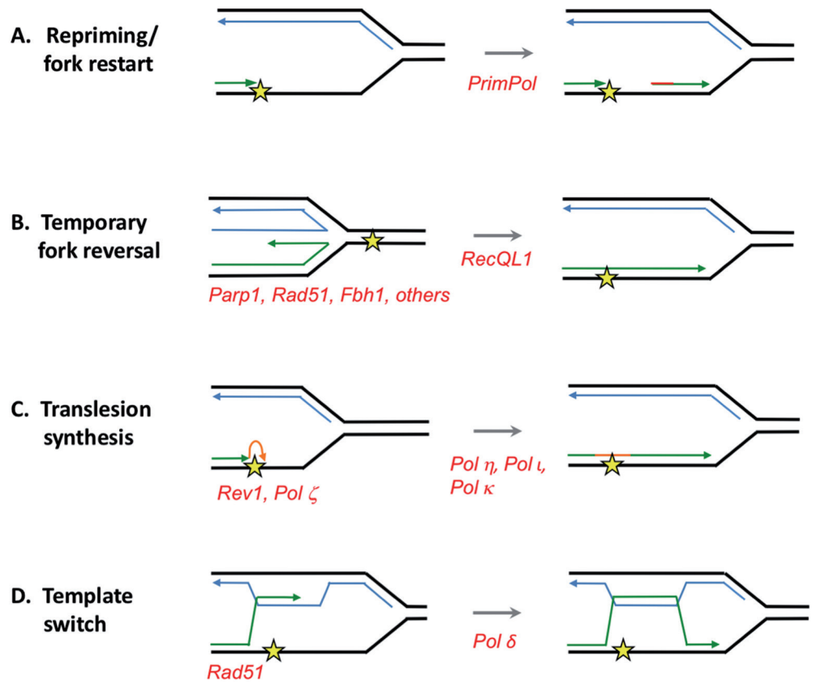

Figure 1 Pathways that facilitate fork progression through damaged DNA. The yellow star represents a polymerase-blocking lesion. The main proteins involved in each pathway are mentioned. (**A**) Repriming downstream of the lesion. (**B**) Fork reversal and restart. (**C**) Translesion synthesis DNA polymerases. (**D**) Lesion skipping by template-switch. Adapted from Muñoz and Méndez (2016).

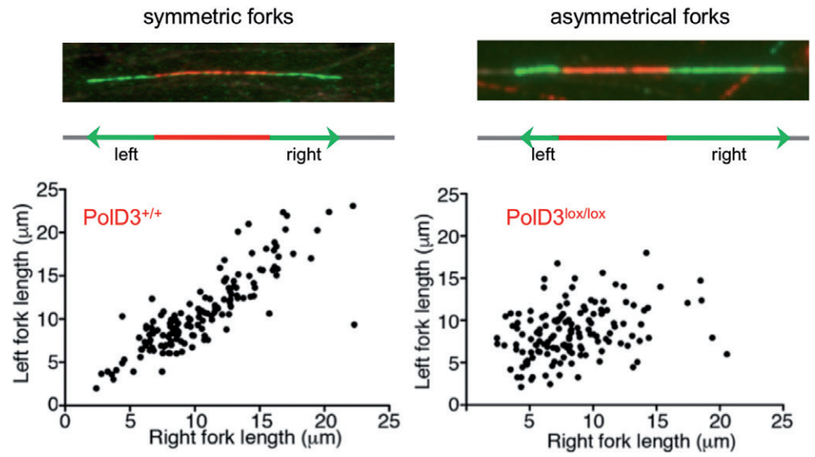

Figure 2 Detection of fork asymmetry in stretched DNA fibres. Top: representative images of replicating DNA molecules labelled with CldU (red) and IdU (green). Each image shows 2 forks moving away from a central origin. Bottom: quantification of fork asymmetry in DNA fibres prepared from B-cells from PolD3-competent (left) or PolD3-deficient (right) mice. Adapted from Murga *et al* (2016).

MELANOMA GROUP

María S. Soengas
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Staff Scientist
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Technicians
Tonantzin Calvo, Estela Cañón (TS)*

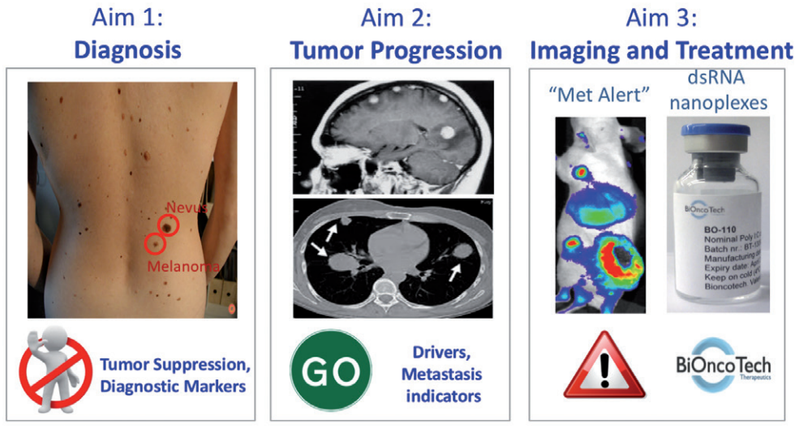
**Titulado Superior* (Advanced Degree)

OVERVIEW

Melanomas are inherently aggressive cancers for which basic and translational research have significantly improved patient prognosis. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. Focusing on stress response programmes involving apoptosis, autophagy and endosome mobilisation, we have discovered lineage-specific oncogenes that define the melanoma 'fingerprint'. Transcriptomic and proteomic analyses of the melanoma secretome have enabled us to define how tumour cells remodel the (lymph)angiogenic vasculature and avoid immune recognition. Moreover, we have generated a unique set of animal models for non-invasive imaging of melanoma progression *in vivo*. These systems have led to the validation of nanoparticle-based treatments that are currently being tested in clinical trials. Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

"We have identified oncogenic cascades that are uniquely deregulated in melanoma and as such, may represent novel targets for therapeutic intervention."

RESEARCH HIGHLIGHTS



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Perez-Guijarro el al. (2016) *Nat Comms.*
Riveiro-Falkenbach et al. (2016) *PCMR.*

Garcia-Fernandez et al. (2016) *Autophagy.*
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Cerezo-Wallis et al. (2016) *Curr Pharm Design.*

Tormo et al. (2009) *Cancer Cell.*
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Maes et al. (2016) *FEBS J.*
Olmeda et al. (Submitted).

Figure 1 Main objectives of the CNIO Melanoma Group aimed at identifying new progression biomarkers and validating more efficient anticancer agents. Indicated are the main experimental systems and representative publications.

CNIO Melanoma Group: objectives and model systems

Melanomas are aggressive solid tumours and provide a prime example of how integrated basic and clinical research have significantly improved patient prognosis. Yet, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge of how melanomas progress and metastasise is still largely incomplete. Therefore, these questions represent key unmet needs, as emphasised by a committee of experts in which M. Soengas participates (Merlino *et al.*, *Pigment Cell Melanoma Res* 2016). In addition, one of the main hurdles slowing progress in this disease is the lack of animal models to monitor melanoma initiation and progression *in vivo*.

To this end, our Group focuses on 3 main areas of research (FIGURE 1):

- → **Aim 1.** Oncogenic pathways, which are selectively deregulated in melanoma and may represent new diagnostic indicators.
- → **Aim 2.** Risk factors and prognostic markers that underlie the unique ability of melanoma to metastasise from seemingly thin lesions.
- → **Aim 3.** Animal models that allow for non-invasive monitoring of premetastatic niches, and as such, may serve as a platform for cost-effective genetic and pharmacological screens.

Lineage-specific oncogenic dependencies in melanoma

One of the long-term objectives of the Melanoma Group is the discovery **of new melanoma drivers**. We previously identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014 and *Oncotarget* 2015). In collaboration with the group of P. Agostinis (University of Leuven, Belgium), we further explored therapeutically-relevant regulatory mechanisms and functions of the endolysosomal machinery in different cell types (Maes et al., *FEBS J* 2016). More recently, we also discovered unique features of autophagy (another key lysosomal-associated process) in melanoma. Employing human melanoma biopsies, combined with newly-generated mouse models, we identified selective heterozygous loses of *ATG5* as a new risk factor for melanoma progression and as a main mediator of the resistance to targeted therapy (García-Fernández et al. *Autophagy*, 2016).

RNA binding proteins and RNA-based anticancer agents in the control of melanoma cell proliferation and metastasis

Melanomas are long-known for being associated with a plethora of changes in mRNA gene expression profiles. Still, the specific contribution of RNA binding proteins (RBPs), particularly, spliceosome modulators, remains virtually unexplored in this disease. We have identified tumour-selective roles of RBPs

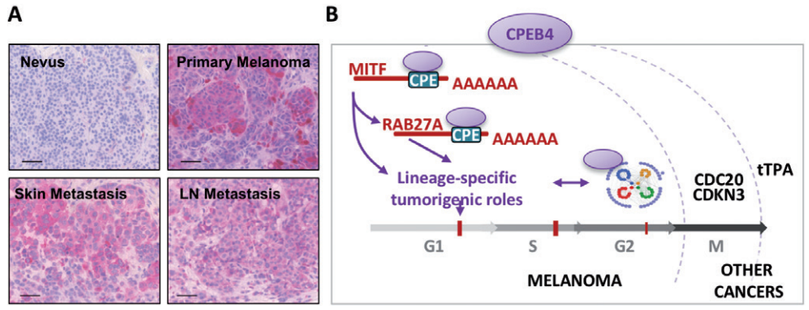

Figure 2 New melanoma drivers: the RNA binding protein CPEB4. (**A**) Upregulation of CPEB4 in malignant melanomas visualised by comparative histological analyses in benign nevi and malignant tumour specimens. CPEB4 is stained in pink. (**B**) Schematic representation of newly identified roles of CPEB4 in the control of lineage-specific tumour drivers (MITF and RAB27). These functions link, for the first time, RNA binding proteins to mechanisms underlying tumour type identity.

CPEB4 and CUGBP1 in the regulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (FIGURE 2). We have also assisted F. Gebauer's laboratory (the Centre for Genomic Regulation, Barcelona) with the identification of pro-metastatic roles of the translation controller UNR in melanoma (Wuth *et al., Cancer Cell* 2016). Similarly, histopathological studies with our long-term collaborators, P. Ortiz-Romero and J.L. Rodríguez-Peralto (*Hospital 12 de Octubre*, Madrid), have validated the chromatin remodeler and RNA binding factor DEK as a risk factor for melanoma metastasis (Riveiro-Falkenbach, *Pigment Cell Melanoma Res*, 2016).

We have also made great progress regarding one of the most pressing needs in the field of melanoma, namely, the mechanisms underlying immune suppression (reviewed in Cerezo-Wallis and Soengas, *Curr Pharm Design* 2016). This was achieved by combining the analysis of human melanoma biopsies with a new class of 'Lymphoreporter' mouse models that we generated in collaboration with Sagrario Ortega's Transgenic Mice Unit at the CNIO. Moreover, we have expanded the use of dsRNA nanoparticles as immunomodulatory agents. This information will be used to support clinical trials that are currently being performed by *Bioncotech Therapeutics*, a biotechnology company cofounded by M. Soengas. ■

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AWARDS AND RECOGNITION

- Outstanding Research Award, the Society for Melanoma Research.
- Elected Board Member of *ASEICA (Asociación Española de Investigación Sobre el Cáncer).*

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Claudia Savini (since November)

Graduate Students
Ana I. Amor, Teresa González (since June), Lucía Robado

OVERVIEW

Our laboratory is focused on understanding metastatic progression. During this process, tumour cells communicate actively with the tumour microenvironment. Among all factors involved in metastasis, our laboratory is specifically interested in defining the role of secreted exosomes during pre-metastatic niche formation. Exosomes are actively involved in cell-cell communication during both physiological and pathological processes. Our data support that tumour-secreted exosomes are involved in: 1) pre-metastatic niche formation and metastatic organotropism depending on the integrin expression profile on their surface; and 2) stromal cell reprogramming by horizontal transfer of molecules (i.e. oncoprotein c-MET) and/or influencing the expression of pro-inflammatory and pro-vasculogenic molecules.

"Exosome secretion by metastatic cells is an adaptive strategy for tumour cells to corrupt the surrounding microenvironment, thereby favouring tumour progression."

Technicians
Marina Mazariegos, Cristina Merino, Sara Sánchez-Redondo (since June)

Visiting Scientist
Olwen Leaman

RESEARCH HIGHLIGHTS

Role of tumour-derived exosomes in lymph node metastasis

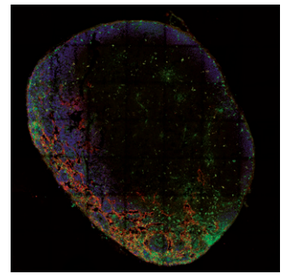
Melanoma-secreted exosomes have been shown to home to specific niches in lymph nodes. We are studying how tumour-secreted exosomes promote cellular and molecular alterations in the lymph node microenvironment, fostering metastasis (FIGURE, A). The goal of the current project is to determine the mechanisms through which tumour-derived exosomes promote lymph node and distal metastasis. Our studies in melanoma patients will be the first ones evaluating the use of circulating vesicles in lymphatic fluid as biomarkers to predict relapse and metastatic potential.

Linking obesity with metastatic risk

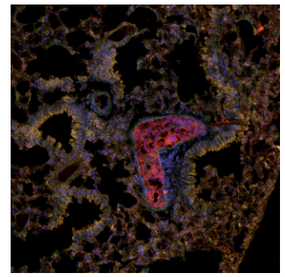
Obesity has been associated with the increased risk of developing metastasis in certain cancers. Although the implication of obesity in cancer is clear, there is, to date, a lack of studies analysing the impact of obesity on metastasis. We are investigating the mechanisms involved in the crosstalk between the adipose tissue, platelets and tumour cells during the metastatic process (FIGURE, B). We are dissecting the systemic effects of tumour-derived exosomes in adipose tissue as well as the involvement of platelets, determining their role in metastasis. Ultimately, we aim to determine specific signatures in circulating exosomes and platelets of cancer patients in order to define new prognostic and therapeutic markers that can be applied in the clinical setting.

Novel pathways involved in neurofibromatosis progression

Although neurofibromatosis is a genetic disorder, in this project we aim to develop a very innovative concept, which focuses on unveiling unknown pathways involved in exosome secretion during neurofibromatosis progression. We are investigating the molecular signature of exosomes secreted from highly metastatic neurofibromatosis models. Our data support that tumour-secreted exosomes carry a specific signature that can be detected in the circulation. This approach will result in the development of new diagnostic tests and therapies to block neurofibromatosis progression. ■



A



B

Figure (A) Analysis of exosome distribution in sentinel lymph nodes. Green-labelled exosomes from B16-F1R2 melanoma cells were injected in the footpad and followed for 16 hours. Analysis of lymph nodes demonstrated that exosomes reach popliteal (sentinel) lymph nodes with a specific distribution found mainly in subcortical areas co-localising with lymphatic endothelial cells (in red). **(B)** Metastasis of breast cancer cell lines in lung metastatic niches. Tumour breast cancer cell lines (in red) were injected by tail vein in mice, in combination with platelets. Analysis of metastasis demonstrates that tumour cells reach metastatic lungs in areas surrounding terminal bronchioles, formerly known as areas where pre-metastatic niches were formed.

PUBLICATIONS

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- García-Silva S, Peinado H (2016). Melanosomes foster a tumour niche by activating CAFs. *Nat Cell Biol* 18, 911-913.
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AWARDS AND RECOGNITION

- *XVII Fundacion Pfizer* Research Award 2016, Spain.
- FERO Grant for Translational Research in Oncology (*XI BECA FERO* 2016), FERO Foundation for Oncology Research, Spain.

BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-doctoral Fellow
Neibla Priego (since April)

Graduate Students
Catia P. Domingues, Maria Figueres (until February), Almudena Saiz (until October), Lucia Zhu (since September)

Technician
Laura E. Doglio (since January)

Students in Practice
Manon Mulders (until June), David Wasilewski (until May), Pablo Sánchez (July-September), Marta Serrano (June-August), Zira Dorado (January-July), Carmen Díaz (May-June)

OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that available therapies (i.e. surgery and radiation) have limited benefits for patients and the disease inevitably progresses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extracranially but not in the brain. In this scenario, cancer cells present at this highly demanding secondary site have additional time to evolve and develop into clinically detectable lesions. In the laboratory, we study why and how cells from different cancer types (breast cancer, lung cancer and melanoma) are able to access the brain, survive and colonise this vital organ. We dissect the biology of these processes *in vivo* using experimental models in order to challenge the current status of this unmet clinical need.

"The Brain Metastasis Group is seeking to identify novel ways to target both cancer cells and the associated microenvironment in order to reduce metastatic burden in the brain."

RESEARCH HIGHLIGHTS

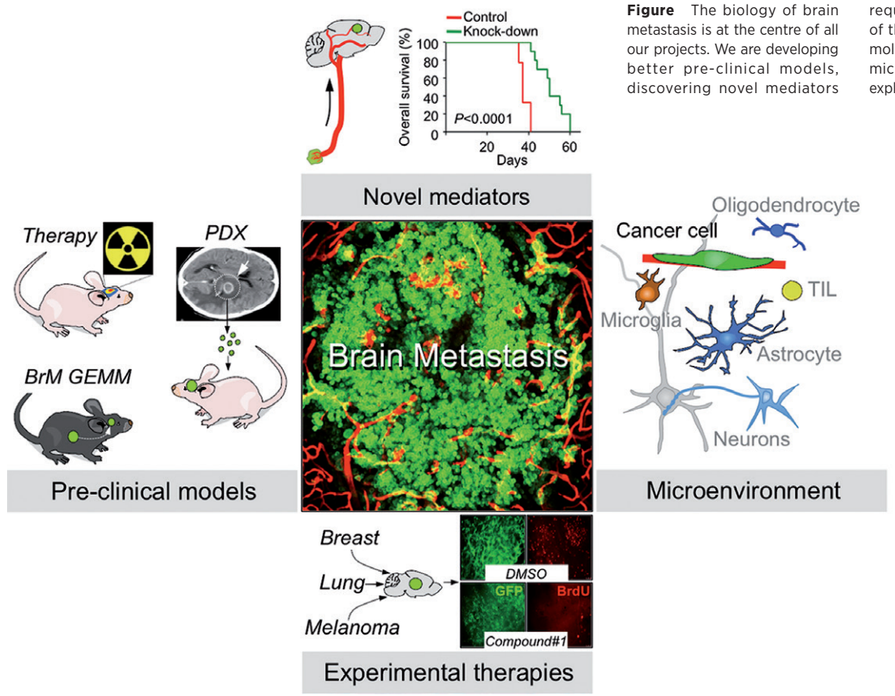

Figure The biology of brain metastasis is at the centre of all our projects. We are developing better pre-clinical models, discovering novel mediators required for the colonisation of the brain and are dissecting molecular interactions with the microenvironment in order to explore new therapies.

The Brain Metastasis Group investigates the progression of cancer to the Central Nervous System (CNS). During 2016, we focused our efforts on various projects:

- → Using a novel medium-throughput drug discovery platform, the laboratory identified **two compounds** with the potential to target established brain metastasis from experimental lung and breast cancer models.
- → We identified **two novel mediators** of brain metastasis that are enabling us to explore the influence of epigenetics on brain colonisation as well as the ability of cancer cells to interact with neurotransmitters.
- → We are evaluating the therapeutic potential of **targeting specific components of the microenvironment** that are only present surrounding metastatic lesions in the brain. Our research suggests that the viability of brain metastasis is highly dependent on altered components of the microenvironment, thus highlighting potential vulnerabilities. ■

PUBLICATIONS AT OTHER INSTITUTIONS

- Chen Q*, Boire A*, Jin X, Valiente M, Er EE, Lopez-Soto A, Patwa R, Sha H, Xu K, Cross JR, Massagué J (2016). Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature* 533, 493-498. *Shared authorship.

AWARDS AND RECOGNITION

- IV "Profesor Durántez"-*Fundación LAIR* Award 2016, Spain.

METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Post-Doctoral Fellow
Ana Ortega

OVERVIEW

In the Metabolism and Cell Signalling Lab, we study the interplay between nutrients, metabolism and cancer. The alarming increase of overweight and obesity over the last decades and the epidemiological links between elevated nutrient levels and human disease calls for a better understanding of the molecular underpinnings of these connections. Conversely, limiting nutrient intake to an extent that does not cause malnutrition is not only protective against diabetes, but also prevents cancer development and delays ageing in most multicellular species by mechanisms that are poorly understood. In the lab, we combine mouse genetics and cell biological tools to gain insight into the cellular processes that become corrupted upon nutrient overabundance, aiming to conceive therapeutic interventions targeting these processes in the context of cancer and the process of ageing.

"By means of novel strains of mice with deregulated nutrient sensing we identified metabolic alterations that drive uncontrolled proliferation of B lymphocytes and nutrient imbalances associated with diabetes."

Graduate Students
Celia de la Calle, Nerea Deleyto

Technician
Ana Sagrera (since August) (TS)*

Student in Practice
Andrew Vandenberg (until August)

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

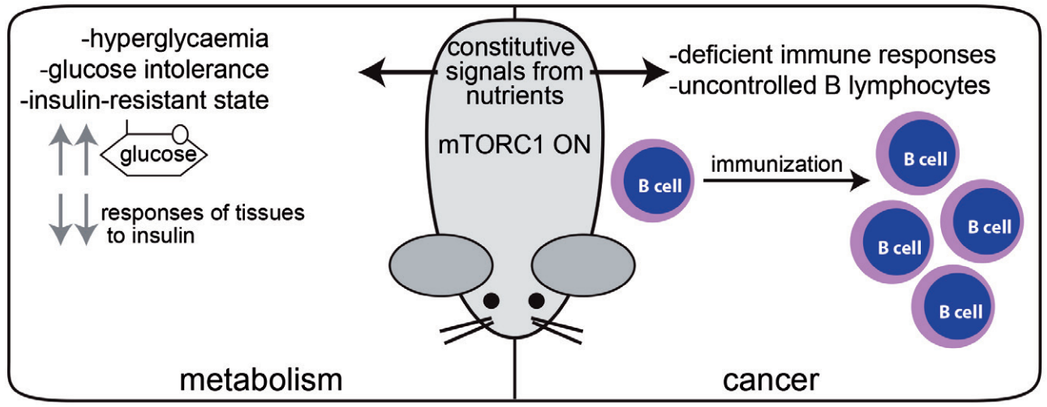

Figure Mice that express mutant variants of the Rag GTPases are genetically unable to respond to a drop in nutrient levels. These mice show metabolic defects that impact on glucose homeostasis and on the control of B lymphocyte behaviour.

Mammals, including humans, have evolved in an environment where the ability to efficiently use limiting nutrient sources has been a key survival adaptation that has shaped all our responses to nutrients. Unprecedented nutrient overabundance is in conflict with our cellular and organismal responses, which are best tuned to operate under scarcity. These aberrant responses not only lie at the core of the pathogenesis of the metabolic alterations observed in diabetes, but are also key in cancer and the process of ageing. We use genetically engineered strains of mice as a physiological framework to understand the molecular bridges from elevated nutrient levels to human disease. In particular, we have genetically modified the RagA and RagC GTPases, key players in the sensing of nutrients that activate a master regulator of metabolism, a kinase called mTOR.

Mice with gain-of-function mutations in RagA – therefore unable to sense a drop in nutrient levels – have an increased glycaemia in spite of a normal food intake and decreased adiposity. Furthermore, these mice show intolerance to glucose, which means that when glucose is administered it remains in the circulation, and peripheral organs (such as liver and skeletal muscle) are unable to uptake it. These perturbations are tightly associated with the development of type 2 diabetes. Indeed, when we examined the ability of peripheral tissues to respond to insulin we observed an impaired response to insulin, also known as insulin resistance, which leads to increased levels of glucose in circulation. We are currently characterising other metabolic imbalances observed in these mice and are performing a genetic dissection of these alterations by deregulating nutrient sensing in an organ-specific manner. ■

PUBLICATIONS AT OTHER INSTITUTIONS

Okosun J, Wolfson RL, Wang J, Araf S, Wilkins L, Castellano BM, Escudero-Ibarz L, Al Seraihi AF, Richter J, Bernhart SH, Efeyan A, Iqbal S, Matthews J, Clear A, Guerra-Assunção JA, Bödör C, Quentmeier H, Mansbridge C, Johnson P, Davies A, Strefford JC, Packham G, Barrans S, Jack A, Du MQ, Calaminici M, Lister TA, Auer R, Montoto S, Gribben JG, Siebert R, Chelala C, Zoncu R, Sabatini DM, Fitzgibbon J (2016). Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. *Nat Genet* 48, 183-188.

CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director

The overall strategic goals of the Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development, progression and metastasis, and to discover molecular mechanisms that could provide a basis for novel therapies. The 5 Groups investigate how tumours grow as 'extrinsic organs'; the spectrum of investigations ranges from epithelial cancers such as liver, skin and intestinal cancer, to bone and brain tumours. The research covers aspects of tumour cell biology, ranging from tumour stem cells, tumour cell interactions with host cells/environment such as tumour-associated cells (like macrophages and fibroblasts), to the role of inflammation, as well as cell adhesion, metabolism and metastasis. Powerful state-of-the-art mouse genetic models, human cellular systems, high-throughput genomic/proteomic and biochemical tools, as well as patient-derived materials, are employed. At present, these aspects are successfully covered and integrated in an interactive and collaborative manner by the complementary research areas of 2 Senior and 3 Junior Groups.

"Our main goal is to keep CNIO globally competitive and to ensure that CNIO remains an international institution. Fourteen different nationalities are represented in our Programme and the goal is to perform first-class cancer cell biology, as well as to train students and postdocs to become the next-generation of promising scientists."

The Senior Group, led by Francisco X. Real, studies epithelial tumours and focuses mainly on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular patho-physiology of these tumours and applies this knowledge in the clinical setting. Mirna Pérez-Moreno's Group investigates the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer. Nabil Djouder's Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development, and in particular to gastro-intestinal cancers. Massimo Squatrito's Group, which is partly supported by the Seve Ballesteros Foundation, studies how brain tumours, mainly glioblastomas and medulloblastomas, develop and how they respond to therapy. Finally, my own Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes, with a strong focus on aspects of inflammation and cancer, e.g. in the liver, lung, skin and bone. We are investigating the role of AP-1 in inflammatory skin diseases, such as psoriasis, but also aim to molecularly define the causes of lung fibrosis. We have continued our efforts to study how the whole organism responds to a locally growing tumour in the context of a complex metabolic impairment in cancer-associated-cachexia.

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

Staff Scientists
Latifa Bakiri, Nuria Gago, María Jiménez, Liliana Fajardo Mellor, Özge Uluçkan

Post-Doctoral Fellows
Albanderi Alfraidi, Kazuhiko Matsuoka, Álvaro Ucero

Graduate Student
Lucía T. Díez

Technicians
Vanessa Bermeo
Ana Guío (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

Our studies aim to analyse gene function in healthy and pathological conditions, e.g. in tumour development, using the mouse as a model organism but also employing patient-derived samples. Specifically, the functions of the AP-1 (Fos/Jun) transcription factor complex regulating cell proliferation, differentiation and oncogenesis, as well as the cross-talk between organs, are being investigated. The goal is to define molecular pathways that lead to disease/cancer development and to identify novel therapeutic targets (FIGURE). We focus on:

- → Elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression, using cell type-specific, switchable genetically engineered mouse models (GEMMs).
- → Developing and characterising new GEMMs for cancer and human diseases, such as bone loss, fibrosis and psoriasis, and applying these to preclinical studies.
- → Using multiple approaches to compare mouse models of disease to human disease and to identify therapeutically relevant targets.

"Our goal is for CNIO to remain an international and competitive institution. At present, 4 out of 5 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Fourteen different nationalities from 4 continents are a testament to our international science culture and we all focus on unravelling the mysteries of inflammation, metabolism and cancer."

RESEARCH HIGHLIGHTS

We have developed a powerful technology for switchable, reversible and tissue-specific ectopic gene expression of specific AP-1 monomers/dimers in the liver, lung, skin and bone. We use mouse and human tissue samples for large-scale studies, such as deep sequencing (RNA-Seq, ChIP-Seq) and mass spectrometry analyses. We evaluate possible biomarkers and therapeutic approaches in small-scale pre-clinical studies based on these screenings.

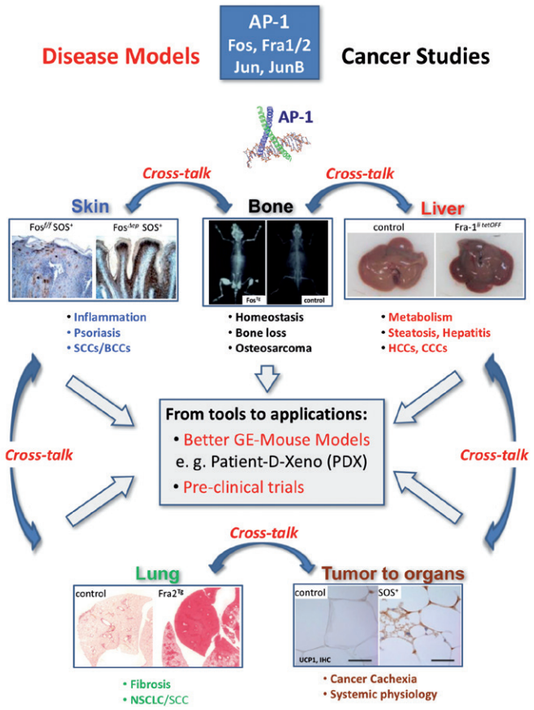

Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease as well as to identify novel targets. Furthermore, we are investigating the systemic response of the mouse organism to a growing tumour in cancer cachexia. Preclinical studies are performed using different genetically engineered mouse models with compounds that target the identified molecules in order to determine the potential of translating our findings for the treatment of human disease.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins and their targets in bone development and disease using loss-(LOF) and gain-of-function (GOF) mouse models. In mice, c-Fos expression leads to osteosarcomas (OSs) and chondrogenic hyperplasias. We found that loss of Wnt signalling delays OS development in c-Fos GOF mice, pointing to a novel mechanism linking c-Fos/AP-1 and OS development.

Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA) and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches. Additionally, we are investigating how crosstalk from other organs, like skin and bone, may contribute to the development and progression of different types of arthritis, as well as whether inflammation generated from the joint, using arthritic models, can influence or induce disease development in adjacent and distant organs.

Liver disease – metabolism, fibrosis, inflammation and cancer

AP-1 proteins are important modulators of hepatic lipid metabolism as specific AP-1 dimers can either activate or repress PPARγ transcription. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, while Fra proteins protect against steatosis, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia in aged mice. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/β-catenin and Myc pathways.

Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas additional inactivation in immune cells abrogates this protective effect. Ectopic c-Fos or expression of Fos-dimers leads to altered cholesterol and bile acid metabolism, inflammation, fibrosis, hepatocyte/bile duct proliferation and tumours with human HCC gene signatures. A robust connection between c-Fos expression and the activity of the LXR/RXR pathway, an important regulator of cholesterol homeostasis, was observed and it most likely contributes to the oncogenic function of c-Fos in hepatocytes.

Cancer-associated cachexia (CAC)

We previously demonstrated that 'browning', a switch from white to brown fat, is a contributor to the wasting process in CAC, and we also documented the importance of IL-6 and β-adrenergic signalling. Using GEMMs, as well as syngeneic mouse models, we are investigating the role of inflammation in CAC and are also studying the systemic events in CAC, such as the role of the neuro-endocrine system, e.g. the renin-angiotensin-aldosterone system. In collaboration with the Medical University of Vienna and Attoquant Diagnostics (Vienna), we are analysing human serum samples from cancer patients to validate the findings from the GEMMs. Our goal is to understand the molecular switch from a local inflammation-associated tumour to the systemic effects of CAC, and to potentially identify novel biomarkers (in collaboration with Drs R. Senaris, Santiago de Compostella, Spain and M. Petruzzelli, Cambridge, UK).

Defining a function for AP-1 in lung disease

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) share the same target organ as well as similar characteristics such as higher incidence in smokers, high morbidity and lack of effective treatments leading to high mortality. Our studies using GEMMs provide experimental tools for studying the important contribution of Fra proteins to these diseases. While Fra-2 is required for the innate immune response associated with disease progression in experimental lung fibrosis, Fra-2 promotes tumour growth in NSCLC. We are currently testing the therapeutic value of Fra-2 inhibition in our pre-clinical models for fibrosis and NSCLC, and are validating our findings using patients' tissue samples. These studies are conducted in collaboration with Daiichi Sankyo Company (Japan) and Mariano Barbacid's Experimental Oncology Group at CNIO, respectively.

Skin cancer, inflammation and human disease

We have demonstrated that loss of epidermal Fra-2 protein results in skin barrier defects. Mechanistically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters. We are currently investigating the targets of Fra-2 in skin that play a role in barrier function and inflammation.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone crosstalk. We have recently reported that IL-17A production in skin causes bone loss by inhibiting Wnt signalling in bone-forming osteoblasts. We have extended our studies and shown that psoriasis patients suffer from bone loss that correlates with IL-17A levels. We are currently evaluating the role of the microbiota in skin inflammation by antibiotic treatments, high-throughput microbiota sequencing and germ-free housing conditions.

High-throughput proteomics and transcriptomic analyses unravelled novel pathways and molecules for targeted therapies, such as S100A8/A9 and complement C3. We have now generated new GEMMs to define the role of these novel target molecules in inflammatory skin disease with a focus on the systemic effects beyond the skin in arthritis and bone loss.

Another angle of research in psoriasis involves the analysis of the role of epidermal stem cells in the disease initiation and progression using state-of-the-art lineage-tracking models. Recent data suggest that a subtype of epidermal stem cells is important for disease progression; we are currently characterising these cells and expanding these studies to patient samples. Finally, we are using GEMMs for Squamous Cell Carcinomas (SCCs), previously generated in the lab, for strategies to prevent skin cancers and to develop novel therapeutic approaches to treat peri-neural invasion with reduced incidence of metastasis. ■

PUBLICATIONS

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- Nowell CS, Odermatt PD, Azzolin L, Hohnel S, Wagner EF, Fantner GE, Lutolf MP, Barrandon Y, Piccolo S, Radtke F (2016). Chronic inflammation imposes aberrant cell fate in regenerating epithelia through mechanotransduction. *Nat Cell Biol* 18, 168-180.
- Uluçkan Ö, Jimenez M, Karbach S, Jeschke A, Graña O, Keller J, Busse B, Croxford AL, Finzel S, Koenders M, van den Berg W, Schinke T, Amling M, Waisman A, Schett G, Wagner EF (2016). Chronic skin inflammation leads to bone loss by IL-17-mediated inhibition of Wnt signaling in osteoblasts. *Sci Transl Med* 8, 330ra37.
- Petruzzelli M, Wagner EF (2016). Mechanisms of metabolic dysfunction in cancer-associated cachexia. *Genes Dev* 30, 489-501.
- Blanpain C, Wagner EF (2016). Editorial Overview: The ins and outs of stem cells in differentiation, inflammation & disease. *Curr Opin Cell Biol* 43, iv-vi.
- Trierweiler C, Hockenjos B, Zatloukal K, Thimme R, Blum HE, Wagner EF, Hasselblatt P (2016). The transcription factor c-JUN/AP-1 promotes HBV-related liver tumorigenesis in mice. *Cell Death Differ* 23, 576-582.
- Puujalka E, Heinz M, Hoesel B, Friedl P, Schweighofer B, Wenzina J, Pirker C, Schmid JA, Loewe R, Wagner EF, Berger W, Petzelbauer P (2016). Opposing Roles of JNK and p38 in Lymphangiogenesis in Melanoma. *J Invest Dermatol* 136, 967-977.
- Shatirishvili M, Burk AS, Franz CM, Pace G, Kastilan T, Breuhahn K, Hinterseer E, Dierich A, Bakiri L, Wagner EF, Ponta H, Hartmann TN, Tanaka M, Orian-Rousseau (2016). Epidermal-specific deletion of CD44 reveals a function in keratinocytes in response to mechanical stress. *Cell Death Dis* 7, e2461.
- Uluçkan Ö, Wagner EF. (2016). Role of IL-17A signalling in psoriasis and associated bone loss. *Clin Exp Rheumatol* 34, 17-20.

EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Staff Scientist
Victor J. Sánchez-Arevalo

Post-Doctoral Fellows
Enrique Carrillo, Eleonora Lapi, Miriam Marqués

Graduate Students
Isidoro Cobo, Francesc Madriles (until March), Catarina Pereira, Mónica Pérez (since September)

Technicians
Itxaso Bellón (TS)*, Natalia Del Pozo, María Tania Lobato, Laia Richart (until September) (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientist
Juan R. Tejedor (*Universitat Pompeu Fabra*, Barcelona)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC), adopting a disease-oriented approach. We use cultured cells, genetically modified mice, and patient samples, giving similar weight to the 3 model systems. Primary observations are made at either of these levels and are then extended through additional work. To translate the findings, we bring this knowledge to a 'population' level, harnessing information and samples from large patient cohorts.

"In UC, we focus on identifying new genes, using them for improved tumour taxonomy, characterising their mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy."

In PDAC, we study cell differentiation as a potent tumour suppressor mechanism acting early during carcinogenesis. We use the excellent genetic mouse models available because these processes cannot be readily studied using human samples. PDAC can originate both in pancreatic progenitors and in acinar cells. Understanding the contribution of these cell types to PDAC is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

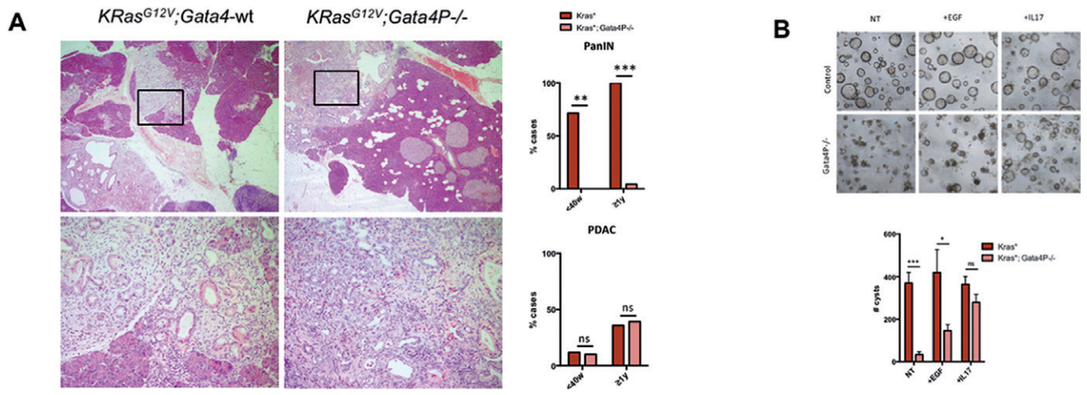

Figure 1 Pancreatic *KRas*^{G12V}*;Gata4*-null mice develop PanIN-less PDAC. Quantification of PanINs and PDAC in young (<40 weeks) and old (>1 year) mice (**A**). Acini from mice lacking Gata4 display reduced cyst formation upon culture in Matrigel. They also show a blunted ADM response to EGF but are able to respond to IL17 (**B**).

Pancreas cancer molecular pathophysiology

We are analysing the tumour suppressive role of several transcription factors involved in pancreatic differentiation. PDAC is characterised by highly prevalent alterations in *KRAS, p16*, *TP53*, and *SMAD4*, and by low-frequency alterations in a plethora of other genes converging in a few critical genetic pathways. The currently accepted progression model proposes that the sequential acquisition of these genetic changes drives the development of PanIN-1, -2, and -3 lesions. We have previously highlighted the weaknesses of this model. Using mutant KRas^{G12V} as the driving oncogene in a pancreatic *Gata4*-null background, we have shown that PDAC can be initiated from pancreatic progenitors or adult acinar cells without the development of acino-ductal metaplasia (ADM) or preneoplastic PanINs (FIGURE 1). These findings, together with recent evidence using whole genome sequencing of human PDAC, suggest the existence of alternative mechanisms of tumour evolution and call for the identification of relevant precursor lesions.

RNA-Seq analyses of *KRas*^{G12V}*;Gata4*-null vs. *KRas*^{G12V}*;Gata4*-wild type pancreata point to differential activation of inflammatory pathways, possibly involved in the PanIN-less phenotype. These findings converge with our data showing the requirement of a full dose of Nr5a2 to suppress inflammation in mouse and human pancreas: reduced Nr5a2 dosage leads to the activation of AP-1 and an epithelial cell-autonomous pre-inflammatory state characterised by production of chemotactic factors. Importantly, Nr5a2 controls both epithelial differentiation and inflammatory programmes, providing mechanistic evidence that these processes are linked at the transcriptional level in pancreatic cells. We are also exploring the potential of enhancing Nr5a2 activity to suppress pancreatitis and tumour development.

This work benefits from a close collaboration with the CNIO Groups of E. Wagner and N. Malats.

Urothelial carcinoma (UC) genetics, biology, and clinical translation

Our goal is to refine current knowledge on the genomic landscape of UC and apply this in the clinical setting. Through exome sequencing we identified *STAG2* and *RBM10* as new UC genes that are more broadly involved in human cancer. *STAG2* codes for a cohesin subunit; its inactivation in UC is not associated with aneuploidy, suggesting that regulation of chromatin architecture and gene expression mediate its tumour suppressor role. Transcriptomic analyses of human tumours and cultured cells, as well as biochemical studies, support a cooperation with transcriptional networks involved in urothelial differentiation. In collaboration with A. Losada (CNIO), we have developed a conditional *Stag2* knockout strain and are analysing the role of cohesin in urothelial cell transformation.

RBM10 codes for a splicing factor and it is mutated in several other epithelial tumours. Inactivation in UC is not associated with stage or grade, but it occurs mainly in tumours with

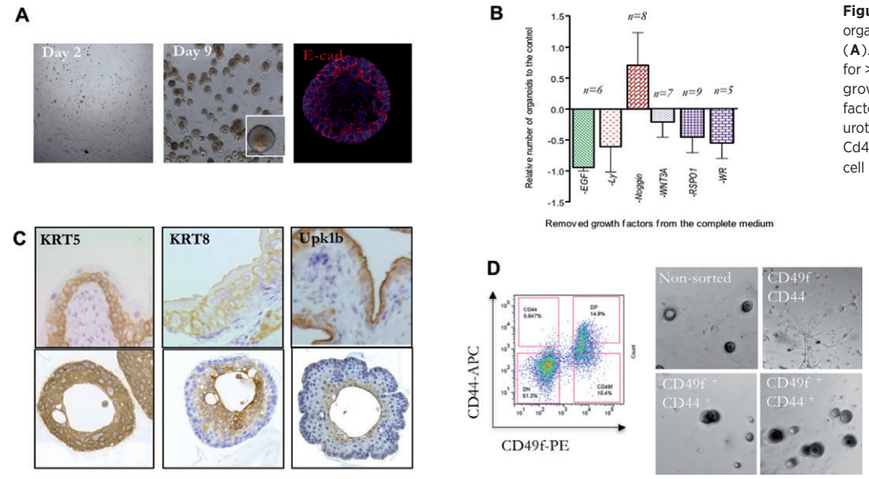

Figure 2 Normal mouse urothelial organoids (NMU-o) express E-cadherin (**A**). Organoids have been passaged for >1 year. Quantification of organoid growth in a 'Leave-one-out growth factor' experiment (**B**). Expression of urothelial differentiation markers (**C**). Cd49f labels a population with stem cell properties (**D**).

urothelial differentiation. In collaboration with J. Valcárcel (CRG, Barcelona), we have generated a conditional *Rbm10* knockout strain and are analysing the molecular mechanisms through which this gene contributes to UC development using a combination of molecular and bioinformatics strategies.

These studies will be complemented with the use of normal urothelial organoids, for which we have established robust culture methods and have shown their strict dependence on EGF and Wnt signalling. We have characterised an organoid cell-of-origin with stem cell properties *in vitro* and have identified conditions promoting urothelial differentiation (FIGURE 2). In addition, we are expanding these studies to human bladder cancers.

Within the context of a project funded by the Spanish Association Against Cancer (AECC), we are analysing the clinical usefulness of the new UC taxonomy. The main aim is to identify predictors of outcome and of response to cisplatin-based therapies in patients receiving perioperative chemotherapy. These studies are linked to the design of clinical trials that include molecular stratification criteria. This work is carried out in collaboration with N. Malats at the CNIO and the SOGUG cooperative group. ■

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AWARDS AND RECOGNITION

- Scientific Advisory Board, *Centre de Recherche des Cordeliers*, Paris, France.

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

Mirna Pérez-Moreno
Junior Group Leader

Post-Doctoral Fellow
Silvia M. Janeiro

OVERVIEW

Tumour cells evolve into a progressively complex interplay between heterogeneous tumour cells and their tissue macroenviroment, which influences their proliferation and malignancy. Identifying the signalling mechanisms and cell types that sustain this complexity is one of the major goals in cancer biology. In adult skin, epithelial progenitor cells have been identified as the cell of origin of skin carcinomas. Several studies have been instrumental for defining regulatory pathways controlling their proliferation and/or differentiation. However, the identification of extrinsic factors modulating stem cell behaviour has not progressed very far to date. Using skin as a model system and employing mouse genetics and human samples, our research aims to understand how the interactions between epithelial progenitor cells, and also the interactions with their surrounding macroenvironment, sustain skin homeostasis, regeneration, and when perturbed lead to cancer. This information may provide insights for the future development of regenerative and anti-cancer therapies.

"During 2016, we continued our efforts to uncover novel events controlling the behaviour of skin stem cells in order to open up new insights into the mechanisms that control their regenerative characteristics, and how when disrupted they can lead to cancer."

Graduate Student
Daniel Peña

Technician
Francesca Antonucci (TS)*
(until March)

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Regulation of epidermal progenitor cells self-renewal and differentiation

During 2016, we continued exploring how tissues acquire an adequate control of cell division and differentiation. In particular, using mouse epidermal development as a model system, we investigated the contributions of mitotic and cytoskeletal proteins in the regulation of skin progenitors' self-renewal through oriented cell divisions.

Contributions of stromal cells to the skin stem cell niche in homeostasis

We have recently identified a novel connection between macrophages and skin progenitor cells, which modulates their stem cell properties and regenerative potential. We are expanding these results to decipher how other signals, and cells from the stroma, are connected with the skin stem cell niche and regulate skin regeneration.

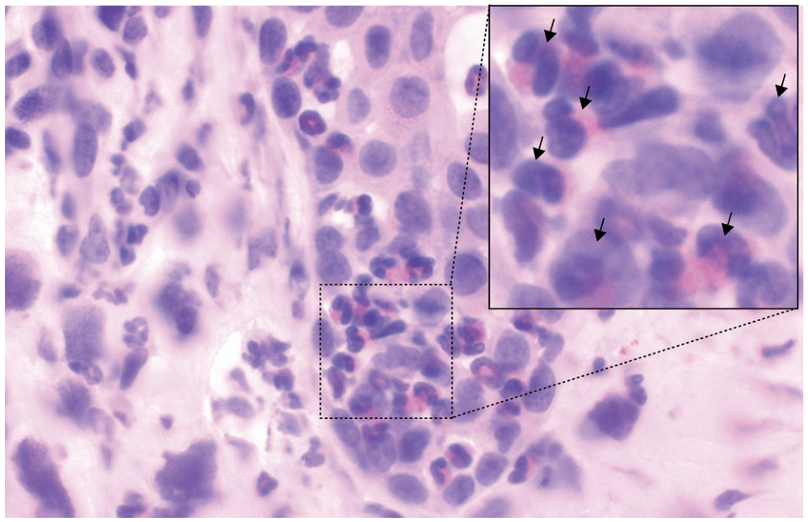
Contributions of stromal cues in cancer stem cell maintenance and tumour progression

The formation of tumours and their progression to malignancy undoubtedly involves the contributions of the tumour macroenvironment. Identifying the signalling mechanisms and cell types that contribute to tumour initiation and progression to malignancy is instrumental for detecting potential targets for clinical applications aimed at eradicating tumours.

The macroenvironment of many tumours is rich in cytokines, chemokines, and inflammatory enzymes. During 2016, we continued exploring the role of diverse cell-derived soluble mediators in modulating proliferation, migration and survival of skin cancer stem cells.

In addition, we focused our efforts on dissecting the contributions of immune cells to the cancer stem cell niche in tumour initiation and development. We are employing conditional loss- and gain-of-function studies in genetically modified mice in order to demonstrate the role of specific cell types and their derived soluble mediators in tumourigenesis; this may provide further insights for the potential development of immunotherapeutic approaches. ■.

Figure Skin carcinoma showing the presence of a high density of inflammatory cells within the tumour. Inset shows a magnification of immune infiltrates within the tumour. *Arrows point to some immune cells



GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Junior Group Leader
Nabil Djouder

Post-Doctoral Fellow
Hugo Bernard

OVERVIEW

The incidence of metabolic disease and cancer has increased to epidemic proportions, possibly due to hypernutrition and a more sedentary life style with less energy expenditure. Our laboratory studies the molecular mechanisms of disease associated with the deregulation of growth factor and nutrient signalling pathways. Identifying new components of the growth factor and nutrient cascades, as well as elucidating their role and functions *in vivo* by generating new genetically engineered mouse models (GEMMs), will help us to better understand how nutrient overload can induce metabolic disorders and cancer.

Thus, using cell biological and biochemical techniques, combined with *in vivo* mouse models and human data, our lab devotes efforts to the development of innovative mechanism-based therapeutics to potentially treat metabolic dysfunctions and cancer.

"Our research focus is to generate mouse models recapitulating human disease associated to nutrient overload in order to guide research perspectives and applications."

Research concepts from our laboratory

- → Metabolic alterations initiate tumorigenesis prior to genomic instability.
- → Inhibition of de novo NAD+ synthesis functions a non-oncogene addiction pathway in liver and pancreas cancer.
- → Oncogene-induced NAD+ depletion in DNA damage.

Graduate Students
Marta Brandt, Almudena Chaves, Amanda Garrido, Ana Teijeiro

Technician
Eva Martínez (since March) (PEJ)*

Student In Practice
Tatiana Grazioso (*Universidad Complutense de Madrid*)

**Plan de Empleo Joven* (Youth Employment Plan)

RESEARCH HIGHLIGHTS

We have a particular interest in studying gastrointestinal track disorders. Our work in this area focuses on metabolic organs such as the liver, intestine and pancreas, as these 3 organs are physiologically interconnected and influenced through their exocrine and/or endocrine functions and microbiota (FIGURE). Our task is thus to generate new mouse models mimicking human disease and to study mechanisms and events iniating disease development. We also use patient-derived xenograft models and organoids to translate our findings into clinical perspectives. Guided by experimental mouse models combined with the use of human data, we aim to provide a comprehensive study for a rational approach towards the development of novel mechanism-based therapeutics to prevent, ameliorate and treat diseases.

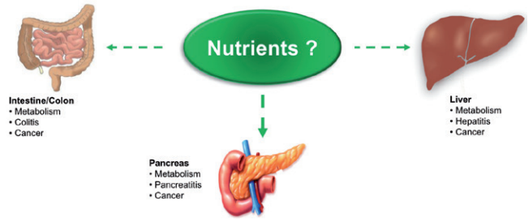


Figure Milestones in growth factors and nutrients research in my laboratory. The scheme illustrates our present and future research. Time and effort are dedicated to better understand how misregulation in growth factor and nutrient signalling pathways can lead to gastrointestinal track disease development.

Identifying new components of growth factor and nutrient signalling cascades

We identified 2 components of the growth factor and nutrient signalling cascades regulating the mTORC1 pathway: Uncoventional prefoldin RPB5 interactor (URI) (Djouder *et al.*, 2007) and Microspherule protein 1 (MCRS1) (Fawal *et al.*, 2015).

Uncoventional prefoldin RPB5 interactor (URI): URI is member of the R2TP/URI-prefoldin like complex, which contains not only prefoldin subunits but also RNA polymerase binding subunit (RPB5), ATPases/helicases RuvB-like protein 2 (RUVBL2, also known as 48- kDa TATA box-binding protein-interacting [TIP48] or reptin) and RuvB-like protein 1 (RUVBL1, also known as 49-kDa TATA box-binding protein-interacting [TIP49] or pontin) and co-chaperones such as heat shock protein 90 (HSP90). URI is a downstream component of the growth factor and nutrient signalling pathways. It is phosphorylated by S6K1 and has an oncogenic role in ovarian cancer and HCC development.

Microspherule protein 1 (MCRS1): MCRS1, in an amino acid-dependent manner, maintains Rheb at lysosome surfaces, connecting Rheb to mTORC1. MCRS1 depletion promotes Rheb/TSC2 interaction, rendering Rheb inactive and delocalising it from lysosomes to recycling endocytic vesicles, leading to mTORC1 inactivation.

Generation of genetically engineered mouse models

- → 2 conditional knock-outs (URI and MCRS1 loss-of-function).
- → 3 knock-ins (over-expression of URI (wt), URI (S371A) and MCRS1).

Research achievements

- → Inflammatory cues and nutrient overloads up-regulate hepatic URI.
- → URI is an oncogene initiating NASH and HCC.
- → Nicotinamide riboside to prevent liver and pancreas cancers.
- → MCRS1 activates mTORC1 in response to amino acids.
- → URI is the first identified OGT regulator in response to glucose fluctuations.
- → Glucose depletion can induce oncogenic signals through OGT/c-MYC regulation.
- → c-MYC is oncogenic and tumour suppressive depending on nutrient availability. ■

PUBLICATIONS

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SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientists
Bárbara Oldrini, Alberto J. Schuhmacher

Graduate Students
Carolina Almeida, Alvaro Curiel, Veronica Matía (since October)

Technician
Claudia S. Troncone

Students in Practice
Paula Nogales (until July), Anna Salamero (until July)

OVERVIEW

Malignant gliomas (astrocytomas, oligodendrogliomas and oligoastrocytomas) are the most frequent form of brain tumours and Glioblastoma Multiforme (GBM), a grade IV astrocytoma, is the most lethal tumour of the central nervous system in the adult. Standard GBM therapy consists of tumour resection and postsurgical treatment with chemotherapy and ionising radiation (IR). Although there have been improvements in surgical and imaging techniques, available treatments for GBMs are still inefficient, most likely due to intrinsic resistance to the current therapeutic modalities and high cellular heterogeneity.

In our laboratory, we use a combination of genomic analysis, mouse models and primary tumour cell cultures, with the main goal of identifying the molecular mechanisms that could provide the basis for novel treatments for GBM patients.

"The main focus of our Group is to uncover the genetic alterations present in GBM patients that are responsible for the aggressiveness and the poor treatment response of this tumour type."

RESEARCH HIGHLIGHTS

Next generation glioma mouse models

A decade of studies has underlined the complexity of the glioma genome, however, the functional significance of the vast majority of the genetic alterations remains elusive. Understanding the genetic events that lead to glioma formation and the mechanisms of resistance to therapy will be instrumental for the development of new treatment modalities for gliomas. To accurately reproduce the high genetic heterogeneity observed in glioma patients, we would have to recreate not just a handful of genetic alterations, but possibly dozens. The advent of the CRISPR/Cas genome editing technology has now made it possible to target almost any candidate cancer gene in the *in vivo* setting. We are actively working to develop the 'next-generation' glioma mouse models that more faithfully recapitulate *in vivo* the complexity of the GBM genome, with a particular interest in tumour suppressor genes and complex gene rearrangements.

Overcoming therapy resistance in GBM

The standard therapies for GBM patients, IR and temozolomide (TMZ), generate double-stranded DNA breaks (DSDBs), the most deleterious form of DNA damage. The DSDBs are then responsible for the initiation of the DNA Damage Response (DDR) and, consequently, the activation of DNA repair pathways and cell-cycle checkpoints. DDR signalling is a very intricate pathway and many of its elements can be altered in a given tumour patient, offering both challenges and opportunities from a treatment perspective. The most frequent resistance mechanism to TMZ treatment is the expression of the DNA-repair gene O^6-methylguanine DNA methyltransferase (MGMT), however, other resistance mechanisms have still to be identified.

Through a variety of genetic approaches (Haploid cells transposone mutagenesis, gRNA and shRNA screenings) we have identified the main signalling pathways that mediate resistance to TMZ. We are currently performing a series of synthetic lethality screenings in order to bypass these mechanisms of resistance. ■

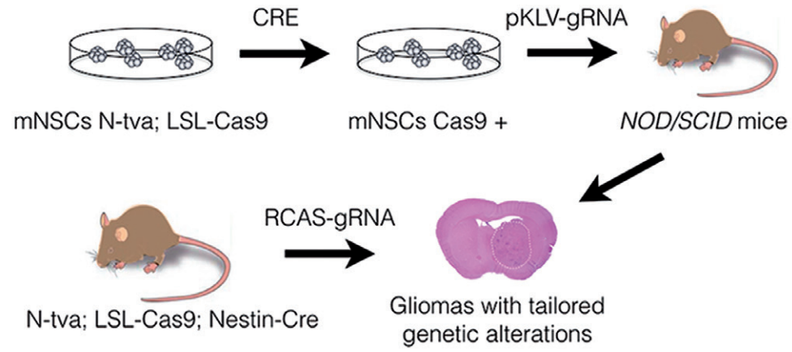

Figure Schematic representation of the RCAS-CRISPR-Cas9 system to generate gliomas with tailored genetic alterations.

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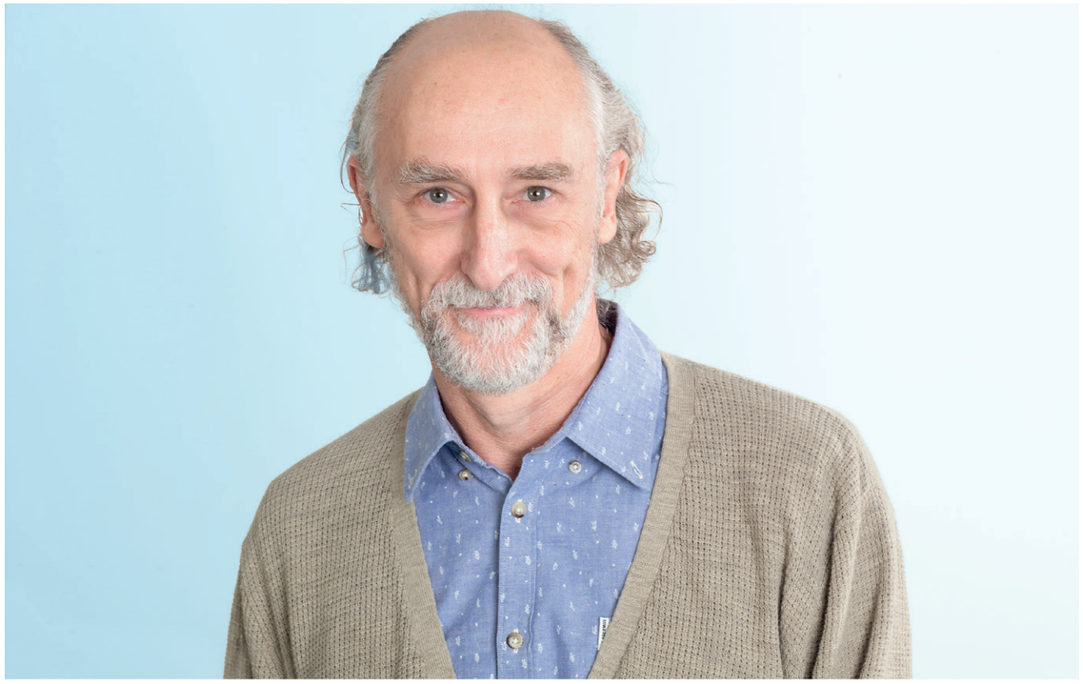
AWARDS AND RECOGNITION

- Alberto J. Schuhmacher has been awarded a Research Contract from the *Ramon y Cajal* Programme (funded by the *Ministerio de Economía, Industria y Competitividad, MEIC*), and the "Eduardo Gallego" Grant of the *Francisco Cobos* Foundation, Spain.
- Álvaro Curiel has received the *Beca de Honor - Colegio Mayor Larraona* given by the University of Navarra, Spain.

STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

ALFONSO VALENCIA Programme Director



The objective of the Structural Biology and Biocomputing Programme is the *mechanistic understanding of key cancer related molecular systems*. The Programme was designed to combine computational and structural approaches, and to collaborate with the CNIO Basic and Translational Research activities.

Our 3 main research goals are to:

- → Reconstruct the structural details of protein complexes that are active in cancer and related processes.
- → Predict the consequences of cancer related alterations; we are focusing on alterations of compensatory nature (co-evolutionary related mutations) as well as those affecting alterative splicing patterns.
- → Contribute to the analysis of cancer epigenomic and genomic information as part of international genome projects.

Currently the Programme includes 3 Research Groups and 6 Core Units that provide support to the CNIO's research activities.

Following the recommendations of the CNIO's External Scientific Advisory Board (SAB), resulting from the 2015 review of the Structural Biology and Biocomputing Programme, we started the process of recruiting additional Groups for the Programme. The selection of candidates for two Junior Group Leader positions was carried out with the help of an *ad-hoc* external and an internal selection committee. Six outstanding candidates were invited to visit the CNIO, defend their work and have a discussion with the corresponding committees. Finally, two candidates were selected covering the areas of biochemistry-structural biology and electron microscopy - protein complexes. These two new Groups will start their work at the CNIO in the first part of 2017. Once these Groups are consolidated, the plan is to re-evaluate the possibility of hiring a senior crystallographer as recommended by the SAB.

On the computational side, the Programme has seen the departure of the Heads of the Bioinformatics and the National Bioinformatics Institute (INB-ISCIII) Units; they have since been replaced by Salvador Capella, as Head of the INB-ISCIII/ELIXIR Unit, and by Fátima Al-shahrour, who will coordinate the Bioinformatics Unit and absorb the activities of the previous Translational Bioinformatics Unit.

Of particular relevance for the CNIO's activities in Computational Biology, was the finalisation of the negotiation with the *Ministerio de Energía, Turismo y Agenda Digital* for the implementation of a biological text mining platform in the framework of the 'Plan de Impulso de las Tecnologías del Lenguaje'. Within the CNIO structure, this activity will fall under and be developed by a new 'Text Mining' Unit headed by Martin Krallinger. This Unit will start operations at the beginning of 2017.

"The Programme is about to undergo an important transition with the addition of two new Groups. Several very positive new activities in the area of data and text mining have also come about. The establishment of the new Groups and the reinforcement of the structural and computational activities at the CNIO remain challenges for the coming year."

Despite these positive developments, the CNIO still needs to reinforce the computational side of the Programme; particularly, research related to the Experimental Therapeutics Programme as well as Computational Cancer Genomics needs to be further strengthened.

STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Alfonso Valencia
Group Leader

Staff Scientists
Andrea Nicole Dölker, Vera Pancaldi, Tirso Pons, Daniel Rico (until July), Michael Tress

Post-Doctoral Fellows
Simone Marsili (until October), Miguel Vázquez (until September)

Graduate Students
Maria Rigau, Juan Rodríguez, Jon Sánchez

Technicians
David A. Juan (TS)*, Martin Krallinger (TS)*, Miguel Madrid (since November) (TS)*, Filipe N. Were (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientists
Dimitrios Morikis (University of California, Riverside, USA), Miguel Vazquez (Norwegian University of Science and Technology, Trondheim, Norway)

OVERVIEW

The main interest of our Group is the study of the molecular bases of cancer by bringing an evolutionary perspective to the study of the interplay between genomics and epigenomics in tumour progression.

Our research is largely carried out in the context of large-scale genome projects, in which we develop new computational methods for the study of genome-cancer relationships.

In this general scenario, the strategic goals of the Structural Computational Biology Group are to:

→ Develop new ideas, methods and software platforms for the extraction, integration and representation of cancer data, including the analysis of molecular, genomic, epigenomic and phenotypic information in collaboration with large-scale genome projects.
→ Include new technologies for data and text mining, together with Machine Learning methods, in our cancer genome analysis framework.
→ Analyse the function, structure and specific interactions of cancer- related proteins.

"This year the initial phase of two large scale projects was completed; i.e. the International Human Epigenome Consoritum (iHEC) and the Pancancer Analysis of Whole Genomes (PCAWG). In both cases, we have contributed to the computational analysis, including the implementation of the data analysis infrastructures and the development of new analysis methods, as well as collaborating towards the interpretation of the biological results."

RESEARCH HIGHLIGHTS

The Group has contributed to several community efforts in different areas:

→ Epigenomics with the BLUEPRINT EU flagship project, which is part of the iHEC consortium; the results from this work were published at the end of 2016.
→ Pancancer Analysis of Whole Genomes (PCAWG), global analysis of 2500 complete cancer genomes; these results will be published in 2017.
→ The BioCreative text mining challenge in chemical compounds resulted in a number of resources and publications that appeared throughout 2016.

We have introduced a new computational method for the prediction of pairs of residues in protein interfaces. This method can help in the analysis of cancer related mutations.

We have also introduced new methods for the analysis of epigenomes at the linear two dimensional level (chromatin states) and three dimensional level (chromatin structure in the nucleus).

The cancer genome analysis system

Our Group is deeply involved in the development of a computational framework for the analysis of human genomes with specific application to the analysis of cancer genomes. Over the years, this framework has been applied to a number of collaborative cancer projects, and it has been particularly instrumental in the CLL-ICGC project.

We have now moved on to a new phase in which the framework is used for the analysis of the large set of full cancer genomes

of the Pancancer Analysis of Whole Genomes (PCAWG); it is one of the four frameworks for data organisation, analysis and exploration used by the consortium.

With regards to the future, given the characteristics of the framework in terms of its modular structure, capacity of integration of new methods in working pipelines, and ease of installation (e.g. adoption of docker and cloud technologies), we consider that it can be the seed of new developments in the overarching analysis of human disease genomes.

Protein structure prediction and cancer genomes

In the context of cancer genome analysis, and as part of the Pan Cancer global effort, we have developed a set of methods to analyse the consequences of mutations in the interface of proteins. The underlying logic is that cellular functions are governed by signals transmitted via protein interactions and protein complexes. In these interactions, the amino acids located in interacting surfaces determine the intensity of the interactions and, very importantly, the specificity of the interactions. The exquisite functioning of cellular systems between proteins depends critically on the pairing of the proteins with their correct partners, and the accuracy of the interactions depends on the correct formation of pairs of residues of the 2 proteins in the interface.

We have shown that cancer associated mutations tend to accumulate in the protein interfaces to the point that, with the information available, it is possible to say that cancer related mutations specifically target protein interfaces. Therefore, understanding the nature of protein-protein interactions is important for understanding the impact of cancer mutations.

We have developed a new methodology able to predict, with high accuracy, a small set of pairs of residues located in the interface of interacting human proteins. The new methodology, based on the study of the co-evolution of the corresponding protein families, does not require any information about the corresponding structures and it is applicable to many human protein complexes for which no other information is available. Furthermore, we have shown that the pairs of residues predicted to interact are very conserved in structural terms (they occupy the same position in space over the lengthy evolutionary time), which is indicative of their importance in the organisation of the corresponding interfaces.

Based on these results, we are now exploring the use of the newly developed computational methods as an alternative approach for the interpretation of the consequences of cancer related mutations.

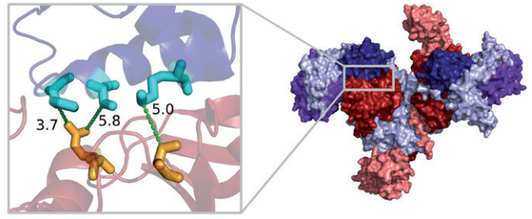


Figure 1 Co-evolution based correct prediction of pairs of residues in the interface of 2 domains of the human cytosolic phenylalanine tRNA synthetase (α subunit in dark red, B5 and B3/4 domains in β subunit in purple and dark blue, respectively); taken from Rodriguez-Rivas *et al.*, 2016.

EPIGENOME analysis infrastructure and portal

In the context of the BLUEPRINT iHEC project we have designed a system for the comparative analysis of epigenetics data (the BluePrint analysis portal http://blueprint-data.bsc.es/release_2016-08/, developed in collaboration with the BSC-CNS and EBI-EMBL). This portal is now the main point of access for the project's results (e.g. chromatin states, ChIP-Seq positions of histone modifications), enabling the direct comparison of the epigenetic structure of different cell types.

Based on the information provided by the Blueprint Analysis Portal, we have developed the methodology to compare epigenomes at the level of their organisation in functional segments (chromatin states). The initial results show that the system is not only able to reproduce the structure of the lineage differentiation during haematopoiesis, but also to detect what the main potential epigenetic driving factors of the differentiation are. The method, initially developed for the Blueprint data sets, is now being extended to other data types provided by the iHEC consortium.

Alternative splicing at the protein level

In 2016, we continued our work on alternative splicing in the context of the NIH-funded GENCODE project. Our results, summarised in a review published in *TIBS* (Tress *et al.*, 2016), show that in light of combined approaches, including protein modelling, proteomics and evolutionary analysis, there is little evidence to demonstrate that alternative isoforms are expressed at the protein level in detectable quantities. In other words, the only available evidence is that normal proteins are coded by the principal isoform of each gene and not by any of the potential alternative forms that are undoubtedly produced at the mRNA level. Even if this observation is in line with recent results of the large scale analysis of gene expression in human tissues (publications of the ENCODE/GTEx -www.gtexportal.org), it is still somewhat controversial since it indicates a big unexplained discrepancy

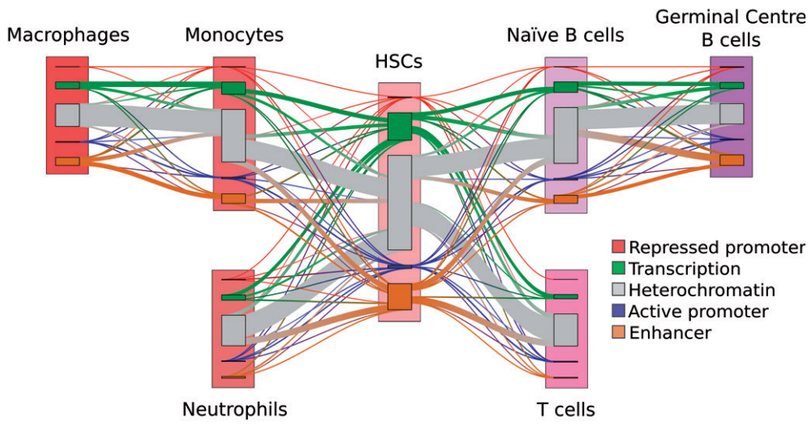


Figure 2 Representation of chromatin state transitions during haematopoietic differentiation. Boxes represent chromatin states and lines represent the observed transitions between cell types.

between the results obtained at the level of gene and protein expression; a discrepancy that might have profound implications for our understanding of the role of mRNA in cells and the overall understanding of the biological function of processed RNAs.

Biological Text Mining

Text mining, an important part of the Group's activity, has broad implications in Biomedicine. In 2016, we completed this year an exhaustive review of the application of text mining to the area of chemistry (Krallinger *et al.*, this work has been submitted to *Chem Rev*); this review was based on our experience in the analysis of text mining systems and the results in the context of the 2015 BioCreative Chemdner challenge (http://www.biocreative.org/tasks/biocreative-iv/chemdner/).

During 2016, we reached an agreement with the *Ministerio de Energía, Turismo y Agenda Digital* for the implementation of a biological text mining platform in the framework of the 'Plan de Impulso de las Tecnologías del Lenguaje'; this project is to develop tools and procedures in line with the recommendations of the European e-Infrastructure in text mining OpenMinted, in which we also participate. ■

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CELL SIGNALLING AND ADHESION JUNIOR GROUP

Daniel Lietha
Junior Group Leader

Post-Doctoral Fellows
Iván Acebrón, Johanne Le Coq

Graduate Students
Marta Acebrón, Marta Camacho (until May), José Vicente Velázquez

Technician
Pilar Redondo

OVERVIEW

Our Group studies regulatory mechanisms of key signalling switches controlling growth and adhesion signals. Such signals regulate important cellular processes such as proliferation, adhesion and survival. We use structural techniques, such as X-ray crystallography and electron microscopy, in combination with biochemical and functional studies to understand these mechanisms at atomic detail and to rationalise how oncogenic events result in their deregulation. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

"We obtain detailed structural and mechanistic insights in order to understand how growth and adhesion signals are triggered to cause tumour invasion, and we use this information for allosteric targeting."

We focus on mechanisms of growth and adhesion signalling that occur at the plasma membrane and involve specific phosphoinositides. In particular, we aim to answer two main questions: (i) what are the events occurring at the cell membrane at integrin adhesion sites that trigger Focal Adhesion Kinase signalling; and (ii) how are phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) levels regulated to affect signalling of the Akt pathway.

RESEARCH HIGHLIGHTS

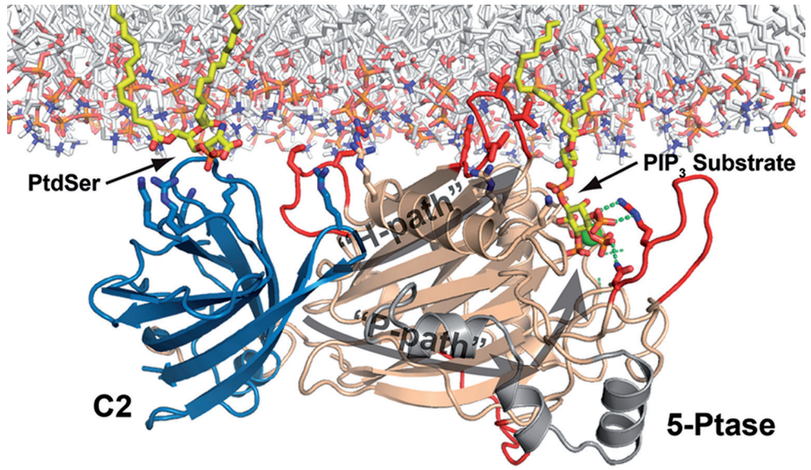

Figure Using a multidisciplinary approach we defined two allosteric paths emanating from the interface between the phosphatase (5-Ptase) and C2 domains of SHIP2, which via hydrophobic regions ('H-path') or a connection of polar residues ('P-path') affect different substrate binding regions in the active site.

We showed that Focal adhesion Kinase (FAK) interacts with PIP_2 lipids at cell adhesion sites and that this interaction induces FAK clustering and conformational changes, which trigger FAK autophosphorylation. Following this, Src is recruited to FAK and, in turn, phosphorylates the FAK kinase to induce full FAK activation. Currently, we are studying the architecture of FAK clusters bound to lipid membranes by electron microscopy, and we are investigating how force, induced at adhesion sites by actomyosin contraction, induces changes to these structures in order to activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ experimental and virtual screening, using fragment based approaches, to identify allosteric ligands, and then use a structure based approach to develop these fragments into inhibitory lead compounds.

SH2-domain-containing inositol 5-phosphatases (SHIP) remove the 5-phosphate from PIP_3 and thereby, like PTEN, negatively regulate PIP_3 levels. Despite their importance, little is known about the mechanisms of SHIP regulation. We solved a crystal structure containing the catalytic and C2 domains of SHIP2, showing an extensive interface between the two domains. We have shown that the C2 domain of SHIP2 binds phosphatidylserine, and hence the rigid C2 interaction efficiently positions the active site towards its substrate (FIGURE). Although the C2 domain interacts with the phosphatase domain far from the active site, we show that the C2 interaction greatly enhances the catalytic activity of SHIP2. We employed molecular dynamics simulations to guide a mutagenesis study that has identified distinct allosteric signalling pathways emanating from hydrophobic or polar interdomain interactions, which differentially affect lipid chain or head group regions of the substrate. Furthermore, we confirmed via cell biology experiments that mutations at the domain interface affect downstream signalling to Akt. ■

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STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

Santiago Ramón-Maiques
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and to prevent cancer. Our Group is interested in understanding central cellular processes that affect the integrity of the genome, such as the metabolism of nucleotides, DNA recombination or the maintenance and recognition of chromatin architecture. These processes depend on the assembly of large and dynamic macromolecular complexes. We combine protein engineering, X-ray crystallography, nuclear magnetic resonance (NMR) and single-particle electron microscopy (EM), together with biochemical and functional studies, in order to decipher the structure of these protein-protein and protein-DNA complexes, as well as to understand their catalysis and regulatory mechanisms at the atomic level. This knowledge should guide the design of compounds to modulate protein activity and provide novel opportunities for fighting tumours.

"We obtained an atomic view of the ATC domain of human CAD – a metabolic gatekeeper controlling cell proliferation – bound to the anti-tumour drug PALA, and localised CAD within the cell."

Graduate Students
Francisco Del Caño, Alba Ruiz (until February)

Technicians
Araceli Grande (TS)*, Igor Yefimenko (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Unmasking CAD, a metabolic gatekeeper of cell proliferation

CAD is a 1.5 MDa multi-enzymatic complex formed by hexameric association of a ~240 kDa polypeptide with four functional domains: glutaminase (GLNase), carbamoyl phosphate synthetase (CPSase), aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). Each domain catalyses one of the initiating steps in the *de novo* biosynthesis of pyrimidine nucleotides. CAD is tightly regulated by allosteric effectors and by phosphorylation through different signalling cascades, and its activity is key to fuel the high demand of pyrimidines during cell growth and proliferation. Despite its central role in metabolism and its potential as an anti-tumour target, there is no detailed information about the architecture of CAD or about the structure of any of its functional domains. We aim to decipher the structure of the complex and to understand its catalytic and regulatory mechanisms at the atomic level.

Structure and functioning of the ATC domain of human CAD

We resolved the crystal structure of the ATCase domain of human CAD – free or bound to carbamoyl phosphate, or to the anti-tumour drug PALA – confirming its overall similarity with bacterial homologues (Ruiz-Ramos *et al.*, 2016). Unexpectedly, we found a decreasing affinity for PALA that could help to understand tumour resistance to this drug. Mutagenic and biochemical analysis linked the lowered PALA affinity to the communication of conformational changes between the ATCase subunits. The mutation of one key residue in this mechanism was recently found by others to cause the first CAD-related human disease (Ng B.C. *et al., Hum Mol Genet*, 2015).

Using CRISPR to understand the functioning of CAD *in vivo*

We generated fluorescent recombinant chimeras and used CRISPR to introduce green fluorescent protein (GFP) into the endogenous CAD gene and to knockout CAD in human cell lines. These tools enable us to interrogate important aspects of CAD functioning *in vivo*. By tracking the subcellular localisation of CAD in mammalian cells we demonstrated that, contrary to previous reports, CAD is located exclusively at the cytosol and does not translocate into the nucleus during the cell cycle. These engineered proteins and gene edited cells are also proving to be instrumental for the identification of interacting protein partners and for the testing of the disease-causing potential of newly identified clinical mutations in CAD. ■

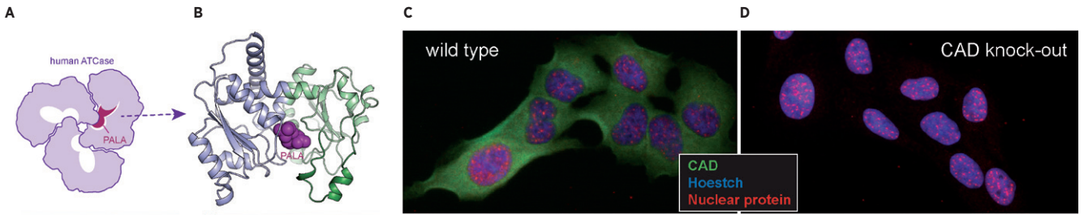

Figure (**A**) Cartoon representation of human ATCase trimer. (**B**) Crystal structure of human ATCase bound to the anti-tumour drug PALA. (**C, D**) Subcellular localisation of CAD using fluorescence microscopy in U2-OS wild-type cells (C) and in CRISPR-generated CAD knock out cells (D).

PUBLICATION

- Ruiz-Ramos A, Velázquez-Campoy A, Grande-García A, Moreno-Morcillo M, Ramón-Maiques S (2016). Structure and functional characterization of human aspartate transcarbamoylase, the target of the anti-tumoral drug PALA. *Structure* 24, 1081-1094.

SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and of other biophysical instrumentation available through the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of spectroscopic and biophysical techniques. This includes the application of NMR to the *in vitro* characterisation of the structure and dynamics of biomolecules (proteins in particular) and their interactions with other biopolymers, as well as with small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media and cell and tissue extracts from both animal models of cancer and human samples.

"In 2016, we quantified metabolites from cell media, mice blood and liver extracts, thereby contributing to the understanding of the cellular and physiological metabolic responses to fasting and to oncogene activation, which are important aspects of tumour biology."

RESEARCH HIGHLIGHTS

Our Core Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, a multi-angle static light scattering apparatus, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2016. For example, we reported the results of a multidisciplinary intra-Programme collaboration this year (with the former Computational Biophysics Group), illustrated in the FIGURE, which combines various NMR and SPR experiments with enhanced sampling molecular dynamics simulations to shed light on the conformational dynamics associated with the binding of Imatinib to the proto-oncogene c-Src. We found that both conformational selection and induced fit play a role in the binding mechanism, reconciling opposing views held in the literature.

The Unit hosts a 700 MHz NMR spectrometer, which is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provided the required throughput for screening small molecule protein binders (together with the CNIO's Structural Biology and Biocomputing and Experimental Therapeutics -ETP- Programmes), as well as for metabonomics measurements that were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Tumour Suppression Group (from the Molecular Oncology Programme), as well as the Genes, Development and Disease and the Growth Factors, Nutrients and Cancer Groups (from the Cancer Cell Biology Programme). Collectively, with these and previous groups, we implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples, as illustrated by two important publications. ■

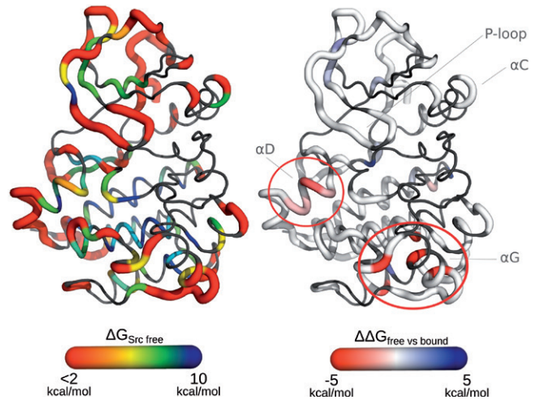

Figure Free energies for local unfolding represented on the backbone structure of the kinase domain of Src in its free form (left) as well as their variation upon binding to Imatinib (right). The indicated values are coded both in the colour and in the thickness of the backbone coil. They were derived from the rates of exchange of backbone amide protons measured from H/D exchange NMR measurements for all but grey-coloured residues. Red circles indicate α-helices αD and αG; the regions with increased exposure to the solvent in the bound structure.

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Book Chapter

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BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Post-Doctoral Fellow
Hector Tejero

OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and, therefore, essential for the future of cancer therapeutics. Bioinformatics-based approaches have the ability to transform the huge amount of biological data into comprehensive models that provide an in-depth understanding of cancer disease and the complex relationships between genotype and phenotype that are needed to identify cancer driver molecular alterations and new therapeutic targets.

"We have developed SATIE, a tool that enables us to predict sequential treatments in cancer. SATIE can propose sensitising treatments, second-line therapies or therapeutic interventions for acquired drug resistance."

Our Unit has several goals:

- → To provide bioinformatics support with data analysis and interpretation using computational and statistical methods.
- → To achieve genome analysis in cancer patients' data in order to identify new biomarkers and mechanisms of drug response.
- → To develop new computational methodologies and bioinformatics tools for cancer research.
- → To maintain CNIO's scientific computing facilities and to provide training in bioinformatics tools and methods.

Graduate Student
Javier Perales

Technicians
Ángel Carro (TS)*, Coral Fustero (PEJ-L)**, Gonzalo Gómez (TS)*, Osvaldo Graña (TS)*, Elena Piñeiro (TS)*, Miriam Rubio (TS)*, Kevin Troulé (PEJ-L)**

**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)*

RESEARCH HIGHLIGHTS

During 2016, the Bioinformatics Unit (former Head, David G. Pisano), and the Translational Bioinformatics Unit headed by Fátima Al-Shahrour from the Clinical Research Programme, were reorganised and merged into one single Bioinformatics Unit (BU). BU was established with the aim of providing resources to enable the integration of biological and clinical data, using computational biology approaches, as well as to contribute to research projects in need of bioinformatics support.

In 2016, BU published 22 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations with CNIO Research Groups as well as other national and international research institutions. We developed several bioinformatics tools for the analysis of next-generation sequencing data in collaboration with the SING group from the University of Vigo – RubioSeq+ (Rubio-Camarillo *et al.*, 2017), nextpresso (Graña *et al.*, 2016) – and 2 web tools to guide the selection of therapies from genome-wide studies in cancer disease – PanDrugs (http://pandrugs.bioinfo.cnio.es) and SATIE (http://satie.bioinfo.cnio.es). RubioSeq+ has been used in several projects, such as the analysis of the lynx genome (Abascal *et al.*, 2016), and for the whole-exome sequencing analysis of patient-derived xenografts for lung cancer (Pereira *et al.*, 2016).

Regarding our scientific collaborations, we helped to unveil mechanisms of cellular reprogramming and senescence (Mosteiro *et al.*, 2016), and also to describe the role of p21 in fasting adaptation (Lopez-Guadamillas *et al.*, 2016) in collaboration with Manuel Serrano's Group (CNIO). Other bioinformatics analyses were performed together with Mariano Barbacid's Group (CNIO) (Ambrogio *et al.*, 2016); these identified DDR1/Notch inhibition as a novel therapy for KRAS-driven lung adenocarcinoma. Finally, within the context of our international collaborations with Harvard associated institutions, we have contributed to the study of leukaemia stem cells in AML (Puram et al. 2016) and the mechanisms of CALR mutations in MPN cells (Elf *et al.*, 2016). ■

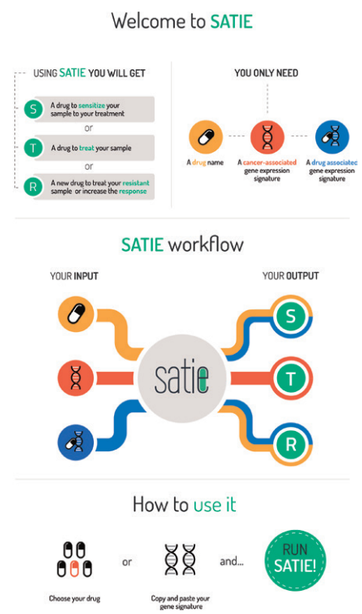

Figure SATIE-Sequential Antitumour Treatment Inference and Enrichment tool. http://satie.bioinfo.cnio.es

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*please see BU's web site for a list of all publications.

NATIONAL BIOINFORMATICS INSTITUTE UNIT

Salvador J. Capella Gutierrez (since June)
Unit Head

Technicians
Andrés Cañada (TS)*, José M. Fernández (TS)*, José M. Rodríguez (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

The Spanish National Bioinformatics Institute (*Instituto Nacional de Bioinformática, INB*) is a component of the National Infrastructure of Biomolecular and Bioinformatics Resources Platform (*Plataforma en Red de Recursos Biomoleculares y Bioinformáticos, PRB2*) of the Spanish National Institute of Health Carlos III (*Instituto de Salud Carlos III, ISCIII*). The INB is the Spanish Node of ELIXIR, the permanent European Infrastructure for Life Sciences. The INB is composed of 10 working nodes distributed across 9 different research centres. The INB Unit at the CNIO undertakes the coordination of the institute. As the coordination node, the goals of the INB Unit are to:

→ Coordinate the Spanish participation in ELIXIR. Promote the implementation and adoption of ELIXIR guidelines among the Spanish bioinformatics community.

"The INB Unit has actively participated in the management of data portals for big research consortia like BLUEPRINT, ICGC, and PanCancer, aiming to understand the genetic bases of cancer."

→ Design, promote and ensure the execution of the INB's scientific-technical and training programmes, undertaken with the support of all nodes.

→ Promote the collaboration between INB nodes and third parties, including research consortia, other research infrastructures, small and medium enterprises (SMEs), and the industry.

RESEARCH HIGHLIGHTS

Despite this Unit's coordination role, the INB actively participates in different Work Packages (WP) of the ELIXIR-EXCELERATE programme. It is also involved in other major projects such as RD-Connect, BLUEPRINT and eTOX. The Unit's contribution can be divided into three main areas:

Data resources and Bio-computing

The storage and processing of data have become fundamental tasks within almost all of the current research projects. Through a collaboration model, the Unit participates in several research projects studying the data requirements and developing solutions to store and process the data. An example of this is the BLUEPRINT data-portal (http://dcc.blueprint-epigenome.eu). BLUEPRINT is a high impact FP7 project aimed at producing epigenomes of haemopoetic cell lines from healthy and non-healthy human donors. In the current version, the data portal provides an epigenomic analysis, obtained from 1,019 samples, to the scientific community. Their associated epigenomes are characterised by: gene and transcript expression (from RNA-Seq experiments), hyper and hypo methylated regions (derived from WGBS experiments), chromatin accessibility (DNAse-Seq), and 7 Histone marks binding activity (ChIP-Seq). Recently, a scientific article was published illustrating the possibilities offered by this portal (Fernandez JM *et al*, 2016).

Infrastructure development

Within the infrastructure development aspect, there is a clear focus on developing a **text-mining infrastructure** for the processing of biomedical texts. The LiMTox system (http://limtox.bioinfo.cnio.es) is the first text mining approach designed to extract associations between compounds and a particular toxicological end point at various levels of granularity and evidence types, all inspired by the content of toxicology reports. During this time, a second end-point (http://melanomamine.bioinfo.cipf.es) has been built using the same system and focusing on the study of different aspects of melanomas.

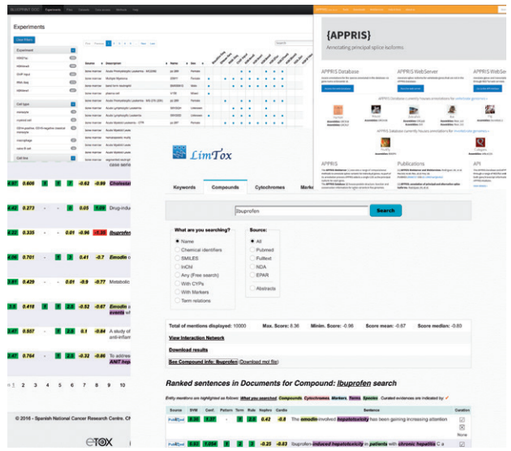

Figure A collage of the different services developed by the INB Unit. Top left shows the experimental information available at BLUEPRINT. Top right shows the information available at the APPRIS home-page. Bottom panels feature information found for *Ibuprofen* using the LimTox system.

End users applications and services

The Unit actively contributes to the creation of an integrated platform that connects databases, registries, biobanks and clinical bioinformatics for research on rare diseases. RD-Connect (http://rd-connect.eu) allows researchers and clinicians to explore the possible genetic causes of these diseases by combining genomic data with phenotypic information from patients across Europe, in a clear attempt to have enough statistical power to support findings. The INB unit has also developed APPRIS (http://appris.bioinfo.cnio.es) and keeps improving it in order to annotate genes and identify the principal isoform of every single gene. Currently, the GENCODE consortium uses APPRIS to annotate the principal human and mouse isoforms. ■

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ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Technicians
Alberto Buscató (until July) (PEJ-L)*, Carlos Rodríguez (since September) (PEJ-L)*

**Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

The Electron Microscopy (EM) Unit is a central core facility and a research laboratory that provides CNIO researchers, as well as the broader research community, with access to Transmission Electron Microscopy and also provides expertise in EM image analysis. As a core facility, we offer standard specimen preparation techniques for proteins, protein complexes and vesicles, data collection and data processing tailored to the particular needs of the users. We also offer training for regular users on the use of equipment, as well as guidance regarding specimen preparation.

"We have used single-particle electron microscopy to elucidate the molecular architecture of full-length TRF1 and to demonstrate how it assists its interaction with other proteins and telomeric DNA."

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit is a research facility that supports biological scientific projects ranging from the cellular to the macromolecular level. The EM Unit performs sample preparation protocols, negative staining, cryo-EM, and data collection methods, as well as 2D and 3D data processing.

In collaboration with CNIO's Telomeres and Telomerase Group (Molecular Oncology Programme) and the Crystallography and Protein Engineering Unit (Structural Biology and Biocomputing Programme), we used the single-particle electron microscopy technique to obtain the first low resolution structures of full-length TRF1 dimer (shelterin component) and its structure in complex with telomeric DNA. We contributed to the understanding of the molecular mechanism that protects the ends of chromosomes: our results demonstrate that full-length TRF1 presents a molecular architecture that assists its interaction with telomeric DNA and at the same time makes TRFH domains accessible to other TRF1 binding partners. Furthermore, our studies suggest hypothetical models on how other proteins such as TIN2 and tankyrase contribute to regulate TRF1 function.

In collaboration with Iván Ventoso, from the *Centro de Biología Molecular* 'Severo Ochoa' (CSIC-UAM) and the *Departamento de Biología Molecular, Universidad Autónoma de Madrid* (UAM), the EM Unit participated in the novel findings that illustrate how viral mRNA is threaded into the 40S subunit during the scanning process. Based on structural and functional data, we generated new insights into the scanning process, describing how a stem-loop in the proximal region of viral mRNA can promote a Eukaryotic Initiation Factor 2 (eIF2)-less translation initiation by trapping in RNA extensions of the ribosomal 40S subunit.

We continued our collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) on PI(4,5)P_2-mediated induction of Focal Adhesion Kinase (FAK) clustering at the cell membrane, applying 2D electron crystallography. ■

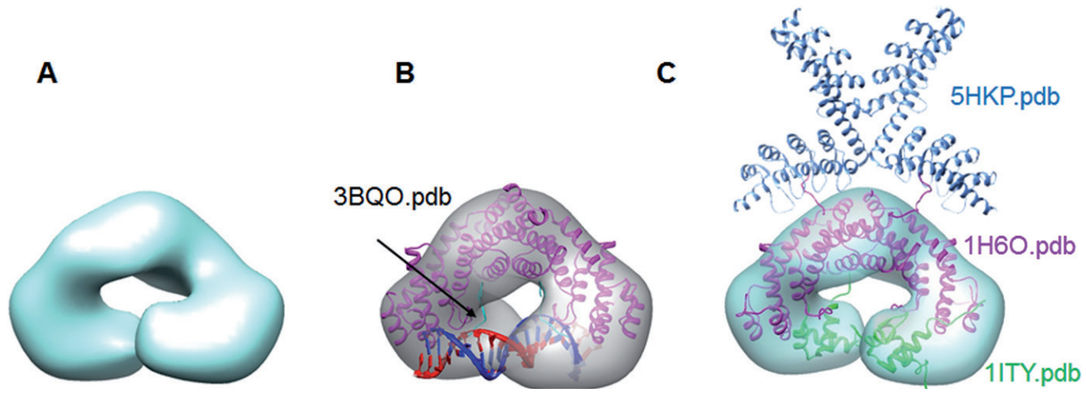

Figure TRF1 structure (A) and model for TRF1 DNA binding (B) and release (C). The interaction with TIN2 stabilises the complex with DNA through direct interaction of TIN2 with DNA. Tankyrase 1 engages the TRF1 dimer on two opposite sides of the molecule, introducing the PARylation and the release of TRF1.

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CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
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Student in Practice
Silvia L. Gomes

Technicians
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Plan de Empleo Joven-Licenciado (Youth Employment Plan-Graduate)
***Titulado Superior* (Advanced Degree)

OVERVIEW

Nowadays, knowledge of the three-dimensional (3D) structure of a protein is critical in order to gain a full understanding of its function. The structures of proteins, alone or in complex with other biological partners, reveal functional networks thereby providing a better understanding of the behaviour of the cell's molecular machinery. This implies knowing how proteins move, detecting their interacting partners, and comprehending the changes that they undergo. Close images facilitated by 3D structures provide the possibility of introducing rationally designed mutants that alter their affinity and specificity towards interacting molecules, aiding in the recognition of the physicochemical mechanisms that govern their function. This is why structural data has become crucial in guiding the drug design process, and the results have proven to be relevant for the development of novel therapies.

To achieve this goal, the Crystallography and Protein Engineering Unit provides services at different levels in order to meet the demands of research groups at the CNIO and outside our institute. At the structural determination level, the Unit offers state-of-the-art, high-throughput protein crystallisation screening facilities that include sophisticated equipment for the identification of protein crystals, as well as a full-service offering of X-ray crystallography and small-angle x-ray (SAXS) analyses. As an academic Unit, we have access to high-tech European infrastructures such as the synchrotron light sources. At the protein production level, we have at our disposal a wide array of instrumentation and technical support for the design and purification of soluble recombinant proteins required in large amounts up to milligram quantities, for structural, biophysical or biochemical characterisation, and also for antibody production.

RESEARCH HIGHLIGHTS

This has been a year of growth and exciting changes for this 'new' Crystallography and Protein Engineering Unit. It is the result of the inclusion of CNIO's protein production facility, previously integrated in the Proteomics Unit (Biotechnology Programme), in the Crystallography Unit. The Unit continues to be shared between the Structural Biology and Biocomputing Programme and the Experimental Therapeutics Programme.

Throughout 2016, we have worked closely with the Experimental Therapeutics Programme on several projects, some of them also in collaboration with other CNIO Groups. The scaling up of the production of proteins, like full-length human MASTL, has permitted a wide range of biochemical experiments to take place. Other projects were directly focused on structural characterisation by x-ray crystallography in support of drug discovery, as in the case of the human proteins HASPIN and CDK8/CyclinC complex where we obtained several crystal structures of the protein-ligand complexes (FIGURE). Especially relevant was our continuous work on the production of proteins for the generation of antibodies by the CNIO Monoclonal Antibody Unit (Biotechnology Programme). During 2016, this smooth collaboration has led to the production of several proteins involved in cancer such as CDC25A, IDO1, TDO2, IL11, PDL1, PDL2 or NOMO1.

The Unit also undertakes several collaborations with different CNIO groups. It is noteworthy to mention the collaborations established with CNIO's Telomeres and Telomerase Group, the Gastrointestinal Cancer Clinical Research Unit, the Epithelial Carcinogenesis Group and the Structural Computational Biology Group. Additionally, the Unit maintains external collaborations with groups at the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CIB-CSIC), the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA), the Department of Biomedicine (University of Bergen, Norway), and the Department of Molecular Engineering (Åarhus University, Denmark).

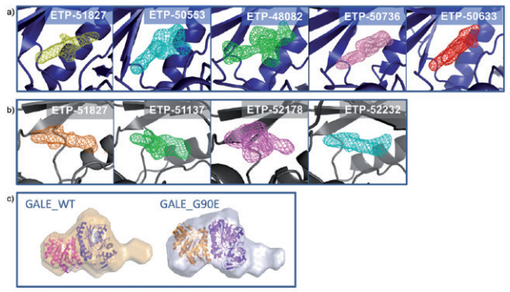

Figure Close views of the active sites from the x-ray structures of HASPIN (**A**) and CDK8/CyclinC (**B**). The coloured omit maps correspond to the electron density of the bound compounds (synthesised in CNIO's Medicinal Chemistry Section). (**C**) SAXS *ab initio* shape reconstructions of wild type UDP-galactose 4'-epimerase (GALE) and its mutant G90E, superimposed on the crystal structure. The data explains the drastic conformational changes that reduce NAD+ binding affinity in the mutant, causing type III galactosemia. This work was done in collaboration with the Physical Chemistry Department (University of Granada, Spain).

Finally, the Unit has continued the study of the role of ephrinB2 in different pathologies. This was done by blocking its activity with specific recombinant antibodies generated by us, in collaboration with groups from the MRC Clinical Sciences Centre (UK) and the NCI Center for Cancer Research (USA). ■

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Vice-Direction of Translational Research

ÓSCAR FERNÁNDEZ-CAPETILLO
Vice-Director of Translational Research

“With our translational research efforts, at the CNIO we are trying to reduce the gap between research laboratories and cancer patients.”

The ultimate goal of the CNIO is to contribute to the global effort in the fight against cancer. This involves the work of many different laboratories and individuals, who collectively help to achieve this mission. During 2016, the CNIO maintained an important focus on translational research, placing significant importance on those research aspects that are in close proximity to the patients. These included the identification of novel mutations and altered pathways in samples from cancer patients that could perhaps be used to guide novel treatments. It is noteworthy to mention that a lot of this work was done under the framework of large international Consortia in which we participated, again underscoring CNIO’s active contribution towards the worldwide effort in cancer research. One example of our key role in collaborative efforts is the important function that the CNIO Biobank carries out in coordinating the Spanish National Biobank Network, which manages patient samples from 52 institutions across Spain. Finally, and in addition to our work in the discovery of molecular alterations that are present in cancer patients, the Clinical Research Programme tries to capitalise on these discoveries (as well as others from all around the world) by bringing them to the clinic. Besides from their efforts in trying to implement new clinical trials, the research from our clinical groups has revealed new insights that can be used to benefit cancer patients, such as novel strategies directed towards overcoming resistance to antiangiogenic agents. Finally, through our collaborative agreement with *Hospital 12 de Octubre*, two Clinical Research Units established by investigators from that hospital are also housed at the CNIO, in an effort to further strengthen our links with neighbouring hospitals.

HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Director

The Human Cancer Genetics Programme is currently composed of three Research Groups: Human Genetics, Endocrine Cancer, and Genetic and Molecular Epidemiology Groups; and three Units: Human Genotyping-CEGEN and Molecular Cytogenetics and Genome Editing Units; and the Familial Cancer Clinical Unit. In addition, there is a Familial Cancer Consultancy for the evaluation of families with cancer and the provision of genetic counselling. The Consultancy is located in the *Hospital Universitario de Fuenlabrada* and works in close collaboration with the Oncology Service at that Hospital. The number of consultancy days and amount of families attended have increased since we set it up five years ago. Currently, we are attending to around 350 families/year. This increase of families has resulted in a higher number of genetic and genomic diagnosis studies, which have been made possible thanks to the incorporation of a massive sequencing platform. This platform has been operational over the past year.

"The programme continues with carrying out its translational work, connecting clinicians with scientists, studying human cancers and helping and advising professionals about the new genetic results generated by novel technologies."

The Programme's core goals are centred on research, training and diagnosis. The genetic and cytogenetic study of tumours, genome editing, genetic interactions, data integration, the search for diagnostic and prognostic markers, the discovery of novel cancer-related genes and environmental factors that confer cancer susceptibility, are our main research priorities. A further complementary area of work is the application of Pharmacogenetics and Pharmacogenomics to identify genes that modify drug response. This research line focuses on a wide variety of tumours, taking advantage of the high-throughput genotyping technologies provided by the Genotyping Unit.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote in training and education. During this year, the Programme's groups have hosted 8 residents from different Spanish hospitals for 3-month periods. We also offer professionals from different international research centres the opportunity to join us, either as visitors or for longer training visits consisting of short-term stays of 1-3 months (a total of 3 international visitors from Latin America were hosted in 2016).

In terms of education, since the beginning of 2016, 1 foreign and 3 national Erasmus Master's students and 9 national and 2 international PhD students have worked on their research projects, 1 of whom has already successfully defended her thesis.

We participate in many international and national consortia. This enables us to apply for international projects, hold international meetings and publish in the best journals. Likewise, a good collaboration with other CNIO Groups and Units is one of our main characteristics, allowing us to benefit from the internal exchange connecting people, techniques, technology and knowledge.

Milestones and major achievements of the Programme in 2016 include:

- The co-organisation of the 6th Familial Cancer Conference in collaboration with the European School of Oncology and *Nature Reviews Clinical Oncology*.
- Mercedes Robledo, Head of the Hereditary Endocrine Cancer Group, was awarded the International Medal bestowed by the Society for Endocrinology.
- Núria Malats, Head of the Genetic and Molecular Epidemiology Group, was elected Chair of the EUPancreas COST Action.
- Co-coordinating the BDebate on the Human Microbiome. CaixaForum, Barcelona, 29 June - 1 July 2016.
- The co-direction of the CNIO Canceromatics III - Tumor Heterogeneity Conference, November 2016.

HUMAN GENETICS GROUP

Javier Benítez
Group Leader

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Post-Doctoral Fellows
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Technicians
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**Titulado Superior* (Advanced Degree)

OVERVIEW

The Human Genetics Group is working on the study of human cancer from a genetic, cytogenetic and epidemiologic point of view. We want to understand why the inherited susceptibility to cancer doesn't follow a mathematical model in people, but rather 'an apparently random model', and why there are families with a large number of members suffering from cancer.

For these studies we work with individuals, families and the affected and normal population, trying to perform a correct diagnosis with known genes as well as looking for new genes that could explain cancer susceptibility in specific families. Our main objective is to work with every family by raising their awareness in regards to their own risk of developing cancer and how to prevent it. To this primary level, we have to add a secondary level of prevention, which will facilitate an important risk reduction in the population, through the development of non-invasive and non-genetic but yet extremely effective measures.

"During 2016, we showed how inhibitors, other than PARPi, could be used in patients with *BRCA* mutations. We started working on the identification of new treatments for cardiac tumours based on transcriptome analysis, and finally, we are also exploring a polygenic inheritance model in families with testicular cancer that is based on more than 25 identified genes associated with this disease."

RESEARCH HIGHLIGHTS

Breast cancer: PARP1 and OGG1 inhibitors in *BRCA1* mutation carriers

We have demonstrated that certain missense mutations in *BRCA1* seem to make cells more sensitive to Poly (ADP-ribose) Polymerase (PARP) inhibitors than those mutations that give rise to the absence of the protein (frameshift mutations) (T. Valclová, *Hum Mol Genet* 2016). We are currently investigating the mechanisms underlying these differences with the aim of identifying new markers of sensitivity or resistance to these agents.

In parallel, we recently showed that the Single Nucleotide Polymorphism (SNP) rs2304277, located in the 3' untranslated region (UTR) of the *OGG1* DNA glycosylase gene of the Base Excision Repair pathway (BER), modified cancer risk in patients harbouring mutations in *BRCA1* (Osorio A. *et al.*, *Plos Genetics*, 2014). We have identified that the SNP is associated with a constitutive *hOGG1* transcriptional downregulation, which leads to a high genome and telomere instability in those patients harbouring *BRCA1* and *BRCA2* mutations, thereby explaining the contribution of this polymorphism to cancer risk. This association is most likely explained by a synthetic lethal/sick interaction between these 2 genetic events. (Benitez-Buelga C. *et al.*, *Oncotarget*, 2016). In order to take an in-depth look at the biological link between BER and the homologous recombination (HR) DNA repair pathway, we tested the pharmacological inhibition of OGG1 in a set of *BRCA1* and *BRCA2* deficient cancer cell lines. We found that OGG1 inhibition is effective, leading to 1) an accumulation of telomere oxidation (genomic instability), and 2) an alteration in the normal proliferation of *BRCA1* deficient cell lines, pointing to a synthetic lethal interaction between OGG1 and BRCA1 (FIGURE 1).

Familial cancer exome project

This project started several years ago with the objective of identifying new high susceptibility genes that explain families

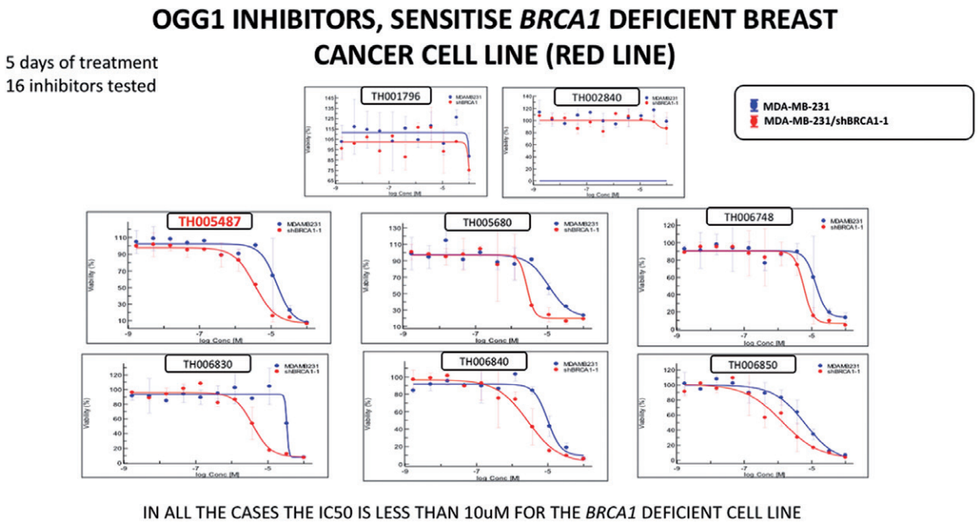

Figure 1 Different effect of OGG1 inhibitors in BRCA1 vs control cell lines.

with rare tumours as well as deciphering the genetic heterogeneity present in some of them:

→ In 2015, we identified *ATP4a* as being responsible for families with gastric neuroendocrine tumours. We are currently searching for new genes in two families that cannot be explained by mutations in *ATP4a*.

→ A second gene, *POT1*, which was published in 2015 as being associated with familial cardiac angiosarcoma, also explains some families with Li Fraumeni-like syndrome. Analysis of tumour samples by Next Generation Sequencing (NGS) has shown an over-representation of the angiogenic pathway, which may be useful in clinical trials. In collaboration with M. Blasco's Telomeres and Telomerase Group, we are generating a knock-in mouse with the aim of recapitulating the disease, as well as enabling us to work with antiangiogenic drugs.

→ We are currently investigating a large family with meningiomas across 3 generations. Analysis of the data generated using bioinformatics tools has shown the existence of 2 candidate genes that could be responsible for this familial tumour. We are starting functional studies and are recruiting more families.

→ Ovarian cancer families are rare and are usually associated with breast cancer. We sequenced the exomes of 9 patients from 5 families and identified 33 rare variants in 28 genes potentially implicated in ovarian cancer risk. By conducting a case control association study we narrowed down the number of candidate missense variants to 10. These, together with 5 high-impact variants (protein truncating or splicing variants), will be evaluated in a larger international case control study to finally define their role in ovarian cancer susceptibility. In parallel, we selected a non-described *RAD51C* missense variant among the identified candidates, and through functional characterisation we were able to determine its pathogenicity and its probable involvement in ovarian cancer risk in one of the families. This finding not only has implications for genetic counselling but also for the potential treatment of affected carriers with PARPi.

→ *Breast cancer*. We have performed whole-exome sequencing (WES) in 3 BRCAX families (familial breast cancer families negative for mutations in BRCA1/2). One of the families was found to harbour a deleterious mutation in the known breast cancer susceptibility gene *ATM*. A complete screening of this gene in a set of 400 Spanish breast cancer families showed a prevalence of almost 2% of mutations in *ATM*, higher than that reported in other populations (Tavera-Tapia *et al., BCRT* 2016). In another family, we found an excellent candidate breast cancer gene that is currently being screened by targeted NGS in a series of 700 BRCAX families and 700 controls. The third family is still under analysis.

→ *Male breast cancer*. We performed WES in a male breast cancer family with an apparently recessive model of inheritance. We have found 7 candidate variants that are currently being validated in a series of 1000 male breast cancer cases and 1000 controls; this is undertaken in collaboration with Nick Orr's lab at the Institute of Cancer Research in London.

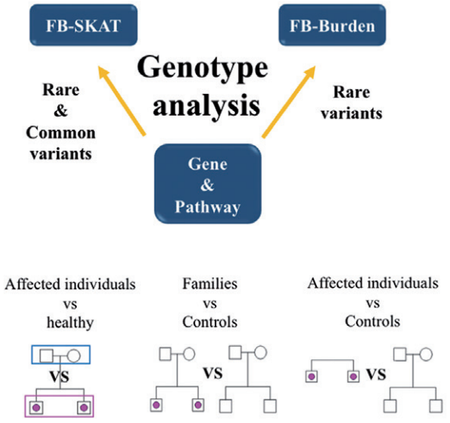

Figure 2 Different strategies used in the polygenic analysis of families with testicular cancer.

→ *Testicular cancer*. Testicular cancer follows a polygenic model of inheritance. We have studied, by NGS, 35 families with 2 or 3 first degree relatives affected by the disease. The results have been classified according to different inheritance models; different methods of analysis have been conducted in order to select some candidate genes (FIGURE 2). The candidate variants are currently being genotyped in a set of more than 500 sporadic testicular cancer cases and 500 controls in order to know how many of them could be considered as candidates to be associated with the disease. ■

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AWARDS AND RECOGNITION

- Ana Osorio has been awarded the madri+d Science Communication Award from the Madri+d Knowledge Foundation for the article 'Is cancer hereditary?'
- Oriol Calvete has won the *Juan Letona* accésit prize awarded by the HM Hospitals Research Foundation for the best clinical translational medicine paper.

HEREDITARY ENDOCRINE CANCER GROUP

Mercedes Robledo
Group Leader

Staff Scientists
Alberto Cascón, Cristina Rodríguez

Post-Doctoral Fellow
Cristina Montero

Graduate Students
María V. Apellániz, Bruna Calsina, María Curras, Lucía Inglada, Veronika Mancikova (until April), Laura Remacha, Juan M. Roldán (since February)

Technicians
Rocío León, Rafael Torres (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features we have been able to propose diagnostic and prognostic markers, to identify altered pathways that could be therapeutically targeted, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying targeted and whole-exome next-generation sequencing to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

"We identified a new major susceptibility gene for paraganglioma, a gene-net regulated by methylation in medullary thyroid carcinoma, and germline variants and tumour microRNAs that predict outcomes in cancer therapies."

RESEARCH HIGHLIGHTS

A gain-of-function mutation in DNMT3A causes paraganglioma

The high percentage of patients carrying germline mutations makes pheochromocytomas (PCC) and paragangliomas (PGL) the most heritable of all tumours. However, there are still cases that are not explained by mutations in the known susceptibility genes. We aimed to identify the genetic cause in patients strongly suspected of having hereditary tumours. Whole-exome sequencing was applied to the germline of a parent-proband trio (FIGURE). Genome-wide methylome analysis of mutated tissues and targeted deep sequencing of 112 additional samples were also performed. Exome sequencing identified a single, novel *de novo* mutation in *DNMT3A*, DNA (Cytosine-5-)-Methyltransferase 3 Alpha, affecting a highly conserved residue located close to the aromatic cage responsible for binding the protein to trimethylated histone H3. *DNMT3A*-mutated tumour and blood tissue from the patient exhibited significant (FDR<0.15) hypermethylation of homeobox-containing genes, providing evidence that the mutation plays an activating role. Targeted deep sequencing revealed the presence of subclonal mutations affecting the same residue in six additional PGLs, all of which exhibited positive staining for H3K9me3. The case described herein not only increases the number of known PCC/PGL susceptibility genes, but also represents, to the best of our knowledge, the first example of a gain-of-function mutation affecting a DNA methyl transferase gene involved in cancer predisposition.

Multilayer OMIC data in Medullary Thyroid carcinoma identifies the STAT3 pathway as a potential therapeutic target in RET^{M918T} tumours

Medullary thyroid carcinoma (MTC) is a rare disease with few genetic drivers that, when diagnosed at an advanced stage, remains incurable. Due to its rarity, its genomic dissection has not been comprehensively explored. Exploiting multilayer genomic data, considering the transcriptome, miRNome and methylome, it was possible to uncover genes negatively regulated by methylation, such as *DKK4*, *PLCB2*, *MMP20*, miR-10a, miR-30a and miR-200c, using MZ-CRC-1 and TT cell lines. Moreover, hypomethylation may induce activation of key pathways related to the malignant behaviour of *RET*^{M918T}-related MTCs. Functional annotation enrichment analysis identified the JAK/Stat pathway as a specific hallmark of *RET*^{M918T}-harbouring MTCs. *In vitro* studies with MTC cell models pointed to a *RET*^{M918T} genetic class-specific proliferative dependency on STAT3 activity. Remarkably, the inhibition of STAT3 increased the sensitivity of *RET*^{M918T} -bearing MTC cells to the FDA-approved RET inhibitor Vandetanib. This combinational treatment could potentially overcome the adverse effects encountered in clinical practice when Vandetanib monotherapy is applied.

Identification of germline genetic variants and tumour microRNAs to predict outcomes in cancer therapies

Personalised cancer treatment is of enormous clinical and social relevance since it can lead to safer and more efficient therapies. This year we focused our efforts on applying next generation sequencing to: i) understand how low frequency genetic variants impact paclitaxel-induced neuropathy, and ii) identify microRNAs predictive of the antiangiogenic drug response in renal cancer patients. Peripheral neuropathy diminishes the quality of life of many cancer patients, sometimes permanently, and limits the dose and efficacy of many cancer drugs. We found that low frequency variants in *EPHA6*, *EPHA5* and *EPHA8* genes contribute to the susceptibility to paclitaxel-induced neuropathy. Furthermore, EPHAs neuronal injury repair function suggests that these genes might constitute important neuropathy markers for many neurotoxic drugs. Regarding antiangiogenic therapies, these have drastically improved the survival of kidney cancer patients; however, a fraction of the patients are refractory to these drugs. The first miRNome deep-sequencing study on an exceptional series of patients treated with sunitinib revealed microRNAs predictive of sunitinib response. Furthermore, a two microRNA-based classifier discriminated individuals with progressive disease upon sunitinib treatment (P=1.3x10-4) with better predictive value than the commonly used clinicopathological risk factors. Thus, we provide new relevant markers that can help rationalise cancer treatment. ■

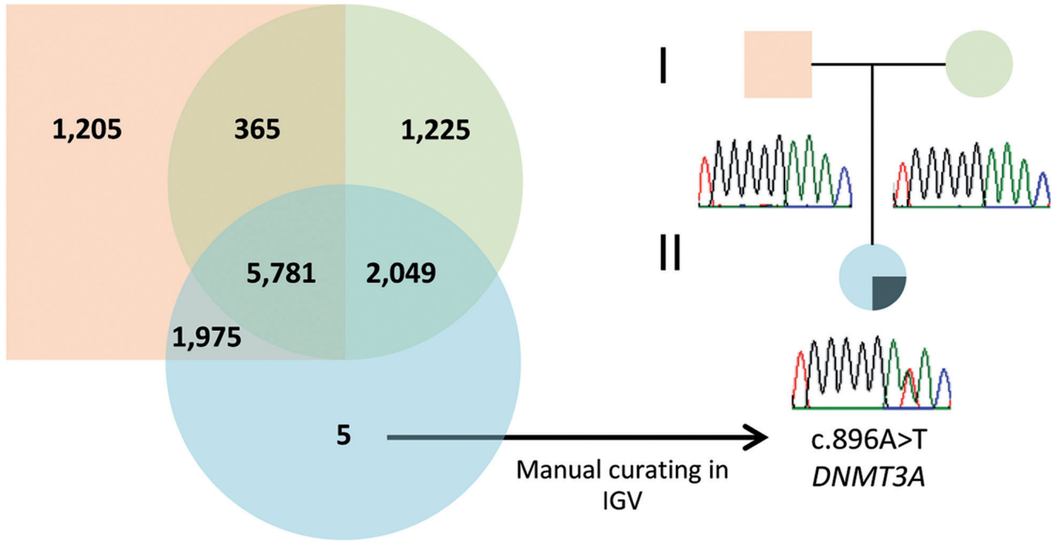

Figure Schematic representation of the coding variants passing all filtering steps (with a genotype quality ≥ 90) that were found in the father (orange square), the mother (green circle), and the patient (blue circle) exomes, respectively. Manual curating by the integrative genomic viewer (IGV) excluded 4 of the 5 variants because they were either found in at least one of the progenitors or because they were probably the result of a sequencing artefact. The single *de novo* variant found (c.896A>T in *DNMT3*) was validated by Sanger sequencing.

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AWARDS AND RECOGNITION

- Mercedes Robledo has received the International Medal awarded by the Society for Endocrinology.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Núria Malats
Group Leader

Staff Scientist
M. Evangelina López De Maturana

Post-Doctoral Fellows
Paulina Gómez, M. Esther Molina

Graduate Student
Silvia Pineda (until March)

Technicians
Ana Alfaro, Lola Alonso (TS)*, Isabel Alonso (since April), Lidia Estudillo (since April), Mirari Márquez (TS)*, Marta Rava (TS)*, Rebeca Sánchez, Virginia Sebastián (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Rianne Boenink (Radboud University, Nijmegen, The Netherlands), Isabel A. Martín (National Institute of Health Carlos III, Madrid, Spain),

Ramón Tejedor (National Institute of Health Carlos III, Madrid, Spain), Mónica Cabrera (Pompeu Fabra University, Barcelona, Spain)

Visiting Scientists
M. Ángeles Jiménez (until June) (*Centro Nacional de Microbiología*, National Institute of Health Carlos III, Spain), Kristel Van Steen (Liège University, Belgium)

OVERVIEW

The scope of research carried out by our Group ranges from the identification of aetiological agents and mechanisms, to the translation of the findings into the clinical and Public Health domains, focusing on bladder, pancreatic, and breast cancers.

We employ a wide variety of biomarkers to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. *Omics* data provide a unique opportunity in this regard and the Group explores its integration in epidemiologic studies.

The strategic goals of the Group are to:

- → Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression and with its molecular/*omics* subphenotypes.
- → Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer by integrating epidemiologic with *omics* information.
- → Assess clinical and public health strategies for cancer control using current genomic tests and data.

"We have undertaken in-depth analyses integrating *omics* and non-*omics* data to predict pancreatic and bladder cancer risk and outcome, and have assessed the challenges that epidemiology faces in this endeavour."

RESEARCH HIGHLIGHTS

Research findings

During 2016, the Group mainly focussed its research on pancreatic and bladder cancers.

Regarding **pancreatic cancer** (PC), we have further analysed the epidemiological and clinical data from the PanGenEU Study and have characterised the risk of PC associated with diabetes, multimorbidity patterns and family history of cancer, among others. We have completed the genome-wide association study (GWAS) and, in collaboration with the international consortia, we are now replicating the primary findings. We are exploring, in collaboration with experts in the field, genome-wide methylation data generated with the Illumina 850K array in cases and controls. We also participated in a study that identified 3 new pancreatic cancer susceptibility signals on chromosomes 1q32.1, 5p15.33 and 8q24.21. Regarding **bladder cancer** (BC), we showed that common SNPs have a limited role in predicting BC outcomes and reported, for the first time, a heritability estimate for disease outcome by assessing the predictive ability of the models, including up to 171,304 SNPs for tumour recurrence and progression. We have also provided proof of concept for the joint effect of genetic variants in improving the discriminative ability of clinical prognostic models by using innovative analytic approaches, and demonstrated that SNPs in inflammatory-related genes were associated with BC prognosis (FIGURE 1). Through international collaborations, the Group has participated in the exploration of common germline variants in the APOBEC3 region associated with BC and breast cancer risk, and observed a tissue-specific role of environmental oncogenic triggers. In line with this study, mutations in cancer driver genes were primarily found in high-risk BC, together with APOBEC-related mutational signatures. We also participated in the development of a urine-based peptide biomarker and a combined methylation&mutation panel for detecting both primary and recurrent BC.

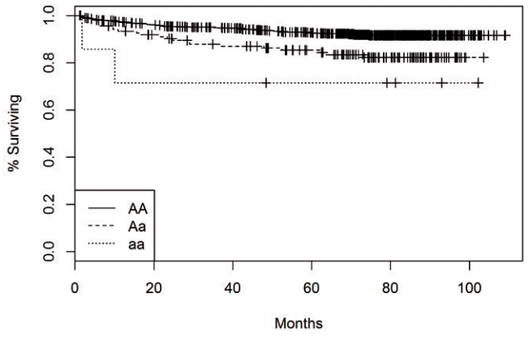

Figure 1 Progression-free survival of the 822 non-muscle invasive BC patients according to CD3G-rs3212262 genotypes. Five-year progression free survival was 92% for AA, 85% for Aa and 71% for aa genotypes (log rank p-value=8.4x10-4, adjusted Cox p-value = 0.023).

Methodological contributions

We have proposed an epidemiological-based integration of *omics* and non-*omics* data by considering the 'massive' inclusion of variables in the risk assessment and predictive models (FIGURE 2). We also discussed the numerous challenges imbedding this type of research and have proposed analytical strategies that allow considering both *omics* and non-*omics* data used in the models towards a personalised prevention. Furthermore, we have adapted Bayesian sequential threshold models in combination with LASSO and applied them to time-to-event and the censoring nature of data, in order to study 822 BC patients followed-up more than 10 years.

Translational activities

We coordinate the COST Action BM1204 *EUPancreas (www.eupancreas.com).* This Action includes 250 multidisciplinary members from 22 EU countries, EU governmental and nongovernmental institutions, and private companies. Several scientific, training, and dissemination activities have been conducted during 2016. By endorsing the Pancreatic Cancer Europe (PCE) multi-stakeholder platform, we have actively participated in several activities aimed at increasing the awareness of PC in the general population, the medical community, and among health policy makers. The Group has actively participated in setting up a European-based clinical registry of PC (PancreOS) jointly with the EPC, the Joint Research Centre from the European Community, and the European Network of Cancer Registries. The Group has also contributed to the development of recommendations for a state strategy for personalised/precision medicine, led by the Roche Institute. Another area our Group contributed to was the identification of different sources of big data and the importance of unstructured data for potential future uses in drug discovery; the main practical and ethical challenges to unravel the full potential of big data in healthcare were discussed. ■

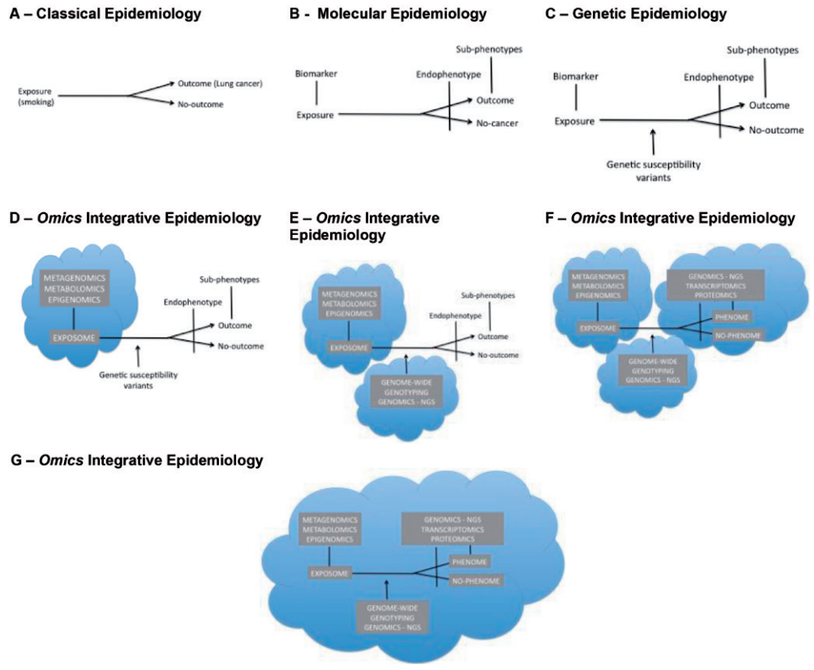

Figure 2 Conceptual association models applied in classical (**A**), molecular (**B**), genetic (**C**) and *omics* integrative epidemiology (**D**, **E**, **F**, and **G**).

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PATENT

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AWARDS AND RECOGNITION

- Founder and Board Member of Pancreatic Cancer Europe.
- Member of the Working Group 'Recomendaciones para un plan de Medicina de Precisión', *Fundación Instituto Roche*, Spain.

FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste
Clinical Unit Head

Graduate Student
Laura Pena

Technicians
Maika González, Fátima Mercadillo

OVERVIEW

Individuals that present with an uncommon malignancy or with cancer at an early age of onset deserve special attention because they are more likely to harbour an inherited predisposition and may require unique treatment strategies. Identification of a heritable cancer predisposition syndrome is not only essential for genetic counselling and for the design of a surveillance scheme for both the patient and his/her relatives, but also for facilitating the refinement and optimisation of treatment strategies so as to minimise toxicity and maximise efficacy. Vigilance of these syndromes can significantly enhance the quality and comprehensiveness of clinical management of cancer.

In addition, the evaluation of inherited cancer predisposition is changing with the routine use of NGS. Despite the promise of NGS, the utility of testing multiple genes with different modes of inheritance and with varying levels of penetrance has been questioned due to the increasing costs of surveillance and unnecessary treatments, and the uncertain consequences of the identification of variants of unknown significance. More than ever it is necessary to underline that NGS testing should only be offered in the context of expert genetic counselling.

In the Cancer Genetics Consultation of the Familial Cancer Clinical Unit (FCCU) we work together with Fuenlabrada Hospital clinicians, as well as health-care providers from other Madrid hospitals and other Autonomous Communities, in order to heighten the vigilance of hereditary cancer syndromes and to better adapt the genetic counselling process in alignment with the introduction of new technologies.

CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The FCCU evaluates individuals and families who are at an increased risk of developing cancer at our cancer genetics consultancy in the Medical Oncology Service of the *Hospital Universitario de Fuenlabrada* (HUF). The referral system, surveillance and treatment measures are discussed with medical oncologists and other clinicians during the monthly sessions conducted by the hospital's Hereditary Cancer Clinical Committee. During 2016, our consultancy at HUF was visited by 408 patients, a 21% increase over 2015. Moreover, 352 genetic diagnostic studies were performed in the FCCU laboratory (306 in 2015). We also tested patients with multiple gene panels; this enables us to offer results on genes of interest in just a few weeks' time. The FCCU has continued to actively contribute to unravel the complexity of genetic cancer predisposition and to help refine tools for a better evaluation of patients and families. FCCU members collaborate with the 'Lynch Syndrome prediction model validation study group' to define the most precise tools for the evaluation of families with hereditary colorectal cancer as well as to identify the best candidates for genetic studies. In collaboration with other research groups, the FCCU has defined the role of the *UNC5C* gene in hereditary forms of colorectal cancer and in polyposis, as well as the role of *OGG1* as a cancer risk modifier in *BRCA1* and *2* mutation carriers.

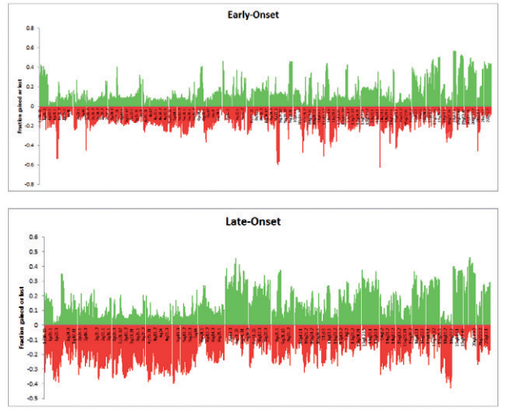

Figure Copy number gains and losses in ≤45 y-o and ≥70 y-o colorectal cancers.

Genetic susceptibility to colorectal cancer is a key area focus for the FCCU's research activities. Familial or hereditary forms of colorectal cancer, early-onset colorectal cancer (EOCC), and synchronous or metachronous colorectal tumours are our main topics of interest. We have continued the characterisation of EOCC, on the premise that the carcinogenetic mechanism and the progression of these tumours may differ in comparison with late-onset colorectal cancer (LOCC) (FIGURE). The *APC* gene status, wild-type or mutated, seems to be a marker of prognosis in colorectal cancer with microsatellite stability (MSS), but the prognosis would have a different sign in EOCC and LOCC. In MSS-EOCC, the worst prognosis was associated with *APC*-mutated tumours and distal location. However, in the MSS-LOCC group, the worst prognosis was observed among proximally located tumours with *APC*-wild type. These results not only continue to suggest a different behaviour according to the age of onset, but also define different groups in relation to the tumour location.

During 2016 the FCCU has maintained a fruitful relationship with *AEAS*. Several members of the association have received genetic counselling in our consultancy, and the study of sarcoma predisposition genes (mainly *TP53*, *POT1* and *CDKN2A*) was also carried out in our laboratory. These activities are part of our ongoing collaborations with cancer patients associations. Recently, we have designed a new survey that will be distributed among members of the *AEFAT* with the aim of identifying those families with an increased susceptibility to cancer. ■

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Book chapter

- Rueda Fernández D, Urioste Azcorra M (2016). *Biología Molecular en el cáncer hereditario. In: Manual de Genética y Biología Molecular.* Ed: *AEBM - Medicina de Laboratorio*. Madrid. ISBN: 978-84-617-4821-1.

PATENT

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MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

Juan C. Cigudosa
Unit Head

Staff Scientist
Sandra Rodríguez

OVERVIEW

Chromosomal translocations are very common events involved in the development of several cancers, especially in sarcomas and haematological malignancies. The research activity of the Molecular Cytogenetics and Genome Editing Unit covers the main topics related to cancer cytogenetics and genome engineering: from classical cytogenetics techniques to new genome engineering tools, including the CRISPR-Cas9 system. We are focusing on the implementation and development of new technologies to enhance knowledge about the biology of tumours and to discover new potential therapeutic targets. With the combined use of CRISPR-Cas9 genome editing and cellular technologies, we are creating *in vitro* models that recapitulate chromosomal and genetic cancer alterations. Members of the Unit also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutes.

"By way of different molecular approaches, we generate human cancer cell models carrying tumour-associated chromosomal translocations in order to study their functional contribution to oncogenesis."

Post-Doctoral Fellow
Raúl Torres

Technicians
Angelo Bertini (until April) (TS)*, M. Carmen Carralero, M. Carmen Martín, Marta Martínez-Lage (since June) (TS)*, Francisco J. Moya (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

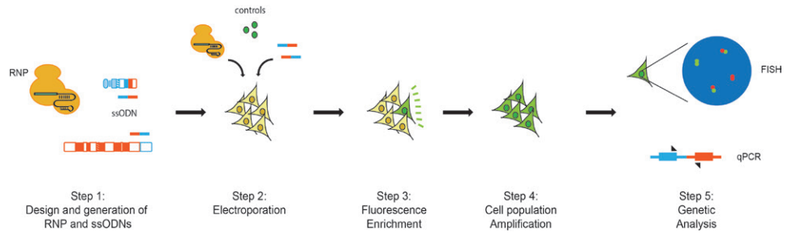

Figure Targeted chromosomal translocations workflow using CRISPR ribonucleoprotein (RNP), GFP molecule controls and single-stranded oligodeoxynucleotides (ssODNs) transfection approach.

Optimising CRISPR-Cas9 to model cancer aberrations in primary cells

In vitro modelling of complex tumour-associated chromosome translocations at native loci is feasible with CRISPR. However, the generation of translocations must be optimised, especially for mimicking events in human primary cells. We have optimised our CRISPR protocol to efficiently obtain those cells, thereby enabling the rescue of translocation+ populations of human primary cells, including induced pluripotent stem (iPS) cells and mesenchymal stem cells (MSCs). These models can surely help us to understand the molecular mechanisms underlying the initiation of human cancers, and can also be used for high-throughput drug screening, toxicological testing and biomarker identification.

From the patient's chromosome translocations to their functional effects

We have worked on the oncogenic role of the translocation t(8;21)(q22;q22)/*RUNX1-RUNX1T1*, which occurs in 4% of acute myeloid leukaemia patients. We deciphered a new function for the activation of MAPK8, observed in t(8;21)+ cells, which is responsible for the stabilisation of SP1. Our data show the essential role of SP1 in t(8;21)+ cell maintenance through the regulation of key genes, such as *CDKN1A*. These results provide new evidence for the inclusion of pharmacological approaches leading to degradation of SP1 in the treatment of these patients.

Technological and translational activities

We provide state-of-the-art molecular cytogenetics and genome editing services. The Unit makes available various techniques to the CNIO Research Groups; these techniques provide more sensitive and accurate tools to analyse cancer cells, such as RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific FISH analysis to detect genomic integration sites of small constructs including LV particles. In 2016, we carried out over 1,000 assays for experimental and clinically-oriented projects. ■

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HUMAN GENOTYPING-*CEGEN* UNIT

Anna González Neira
Unit Head

Graduate Student
Sara Ruiz

Technicians
Charo Alonso, Núria Álvarez, Belén Herráez, Tais Moreno (until October) (TS)*, Guillermo Pita (TS)*, Rocío Nuñez (since October) (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

The most abundant types of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Association studies involving the large-scale analysis of both SNVs and CNVs in thousands of patients can help to identify genes underlying complex diseases such as cancer, and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one to millions of SNVs and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in the Unit. Complementarily, research focused on the identification of biomarkers for precision medicine is also undertaken.

"Advances in understanding patients' responses to therapy will help to individualise cancer patient care."

RESEARCH HIGHLIGHTS

Identification of genetic variants associated with docetaxel and anthracycline efficacy

Taxanes and anthracyclines are widely used in the treatment of breast cancer, despite the benefit being limited to a small proportion of patients and that preoperative biomarkers, which are predictive of clinical outcome, still remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer, previously enrolled in a phase 2 randomised clinical trial (NCT00123929), in which patients were randomly assigned to receive doxorubicin (anthracycline) or docetaxel (taxane) neoadjuvant chemotherapy. We assessed whether genetic variants in 15 key transport or metabolism genes relevant to doxorubicin and docetaxel drugs could play a role as predictive biomarkers. We identified a genetic variant located in the promoter of ABCC2 as the strongest association with tumour response in patients treated with doxorubicin (P=0.009). We also identified a significant association for an intronic variant located in CYP1B1 associated with docetaxel tumour response ($P=2.15x10^{-4}$). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers of treatment outcome in breast cancer patients.

New low-frequency variant loci associated with anthracycline-induced cardiotoxicity (AIC) in cancer patients by Illumina HumanExome Beadchip

Anthracycline chemotherapeutic agents are widely used in the treatment of cancer; however, chronic anthracycline-induced cardiotoxicity (AIC) is a serious long-term complication leading to substantial morbidity. Our aim was to identify new genes and low-frequency variants influencing the susceptibility to AIC. We studied the association of variants on the Illumina HumanExome BeadChip array in a discovery cohort of breast cancer anthracycline-treated patients. Using gene-based tests (SKAT-O) that have greater statistical power to detect rare variant associations and that can evaluate the cumulative effect of multiple genetic variants, we identified novel significant associations in a gene with a major role in mitochondrial fatty acid β-oxidation and the respiratory chain, involved in anthracycline-related toxicity via an oxidative stress mechanism. We replicated our association results in another cohort of anthracycline treated paediatric cancer patients from Spain.

Functional characterisation at the 20q13.33 risk locus for capecitabine-induced hand-foot syndrome (CiHFS)

Capecitabine is a chemotherapy drug widely used in breast and colorectal cancer; the most frequent adverse drug reaction to this treatment (in 30% of the patients) is CiHFS, a cause of dose reductions and dose delays. By genome-wide association studies (GWAS), we identified four linked *CDH4* regulatory variants (h2=risk haplotype) associated with the risk of CiHFS appearance ($HR=2.48\ p=1.43x10^{-8}$). The *CDH4* gene encodes R-Cadherin, which is localised in the granular layer of the epidermis and is involved in the cohesiveness of epithelial layers. We demonstrated that these regulatory variants are able to mediate chromatin structural changes in chromatin organisation, which results in the presence of the risk alleles and in decreased expression levels of CDH4 mRNA and R-Cadherin protein. Additional functional experiments are being performed. The study has been carried out in collaboration with CNIO's Chromosome Dynamics Group and the Epithelial Cell Biology Group. ■

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CLINICAL RESEARCH PROGRAMME

MANUEL HIDALGO (Until December) Programme Director

The Clinical Research Programme (CRP) aims to translate advances in cancer research into the prevention, diagnosis, and treatment of patients. The major goals of the CRP are the conduction of early clinical trials with novel drugs, the discovery of biomarkers of drug action and disease outcome, the implementation of a strategy for personalised medicine, and the launching of a training programme in drug development.

"The Clinical Research Programme focuses on developing novel and more effective treatments against cancer."

The CRP is composed of 5 Clinical Research Units (CRU) and 1 support Unit. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo until December 2016, studies novel therapeutics and personalised medicine in pancreatic cancer. Miguel Quintela-Fandino leads the Breast Cancer CRU that works on the development of kinase and angiogenesis inhibitors in breast cancer, as well as on the understanding of the molecular taxonomy and metabolic vulnerabilities of this disease. The Prostate Cancer CRU, led by David Olmos, explores novel therapeutics and biomarkers of the disease, with a particular interest in understanding DNA damage repair deficiency mechanisms in prostate cancer. The Lung Cancer CRU, headed by Luis Paz-Ares, and the Haematological Malignancies CRU, led by Joaquín Martínez-López – both established as part of an agreement with the *Hospital Universitario 12 de Octubre* – focus on molecular and preclinical studies in non-small cell lung cancer and in multiple myeloma, respectively. The Molecular Diagnostics Unit, led by Luis Lombardía, provides support to medical professionals of the National Health system and the CRP through the provision of a wide variety of molecular tests that determine alterations in biomarkers involved in cancer. In 2016, the Programme continued the expansion of its clinical trials activities in collaboration with several hospitals in Spain.

GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

Manuel Hidalgo (until December)
Clinical Research Unit Head

Staff Scientists
Rodrigo De Almeida, Pedro P. López, Sofía Perea, Begoña Vázquez (since April)

Clinical Investigators
M José De Miguel (until June), Laura Medina (TS)*

Post-Doctoral Fellows
Lucía Fernández (until June), Camino Menéndez, María Vela (until February)

Graduate Students
Spas Dimitrov, Beatriz Salvador

Technicians
Carolina Alonso (since December), Soraya Ardila (until March) (TS)*, Natalia Baños, Victoria B.bonilla, Yolanda Durán, Manuel Muñoz, Gemma M. Sánchez (TS)*, Francesca Sarno (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientists
Raul Calero (CNIO-HNJ Clinical Research Unit, *Hospital Infantil Univesitario Niño Jesús*, Madrid), Lucas Moreno (CNIO-HNJ Clinical Research Unit, *Hospital Infantil Univesitario Niño Jesús*, Madrid)

OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development of novel therapeutics for patients with cancers of the gastrointestinal tract as well as personalised medicine approaches for these patients. The work of the Group combines the preclinical assessment of novel anticancer agents in 'Avatar' mouse models with the design, conduction, and analysis of clinical trials with novel anticancer agents in patients with gastrointestinal tumours. Over the last few years the Group has implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action.

Key to the work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. The Group has developed and has characterised the largest collection of these models in pancreatic cancer. Avatar models are used in 3 critical applications: (i) the screening of new anticancer agents; (ii) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response/resistance; and (iii) finally, the Avatar models for personalised cancer treatment integrated with next generation sequencing. ■

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BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Clinical Research Unit Head

Staff Scientists
María José Bueno, Silvana A. Mouron

OVERVIEW

The Breast Cancer Clinical Research Unit (BCCRU) focuses on the translational interface of therapeutic development. Breast cancer is a heterogeneous disease, and thus there are large inter-patient variations in terms of disease course, prognosis, relapse and resistance to conventional or targeted therapeutics. Our activities are directed towards personalised treatment, and range from preclinical models to the sponsoring of multicentric clinical trials. Specifically, our research areas are:

→ Discovery of new targets for breast cancer prevention: role of fatty acid synthase (FASN).
→ Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biologic features that constitute therapeutic targets.
→ Study of the mechanisms of resistance against targeted therapies.
→ Conduct investigator- initiated clinical trials.

"In 2016, the Breast Cancer Group has tackled the mechanisms of resistance against antiangiogenics, implementing these findings into clinical trials."

Clinical Research Fellow
Laura M. Medina (until July)

Post-Doctoral Fellow
Franciso J. Blanco (until June)

Graduate Students
Sara Fernández, Gonzalo Pérez, Ivana Zagorac

Technicians
Verónica Jiménez, José Francisco López (TS)*, Esperanza Martín

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

In the field of functional taxonomy, we have completed our study in triple-negative breast cancer. We have interrogated the disease from the bimodal relapse pattern point of view, and performed a phosphoproteomic screening that would reduce the countless patterns of genomic, epigenomic and transcriptomic aberrations into a discrete number of patterns of hardwired signalling pathways. We found 6 kinases whose hyperactivity accounted for 94% of the relapsed cases. These kinases were grouped into a maximum number of 34 patterns, the largest of which (25%) was virtually associated with cure. This taxonomy was also useful because all the kinases in the final 'relapse signature' were also targetable nodes.

Regarding the study of targeted therapies, we have observed that the generally assumed hypothesis of vascular normalisation upon exposure to antiangiogenics is not always true. In fact, resistance against antiangiogenics can originate after a vascular normalising or 'abnormalising' response. Whether a tumour experiences the former or the latter depends on the tumour type and the type of agent. What is quite important from the clinical point of view is that we can track, individually, whether a tumour experiences a normalising or an abnormalising response after less than 2 weeks of exposure to the agent, using a non-invasive imaging test with 18F-fluoromisonidazole. This has been demonstrated in animals and in patients. The applicability of this finding lies in the fact is that we have also unravelled the mechanisms of resistance depending on whether the tumour reacts with normalisation or abnormalisation against antiangiogenics: in the first case, the tumour switches from glycolytic to mitochondrial metabolism,

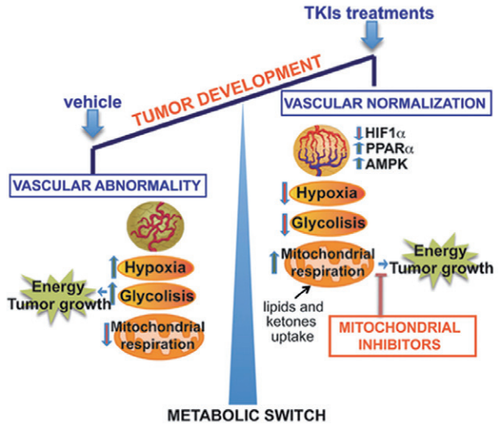

Figure Depiction of the metabolic adaptation of tumours when experiencing vascular normalisation upon exposure to antiangiogenics. An alternative response, increased vascular abnormality, occurs in roughly 30% of the cases. This response is coupled with immune reprogramming.

which is reversible by mitochondrial inhibitors. In the latter, the tumour experiences an immune-switch. Since both mechanisms are targetable, we can now individually track which pathway a tumour is undergoing upon exposure to antiangiogenics and tailor which synergistic agent that patient would need. ■

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PATENT

- Gold D.P., Quintela-Fandiño M.A. (2016). Combination Therapies. *WO/2016/126618*.

AWARDS AND RECOGNITION

- 2016 AstraZeneca Award for Young Investigators, Spain.

PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro

Clinical Research Fellow
Nuria Romero

OVERVIEW

Prostate cancer (PrCa) is the most common cancer and the 2nd leading cause of cancer mortality among men in Western countries. Despite advances in diagnosis and early-disease treatment, up to 30% of PrCa patients will develop metastasis at some point and succumb after the acquisition of a castration-resistant status (mCRPrCa). The early identification of PrCa patients with greater predisposition to develop aggressive mCRPC could lead to the development of novel treatment strategies and improved outcomes. In addition to AR aberrations following androgen-deprivation therapy leading to resistance to current treatment options, DNA repair defects have been identified in about 5% and 25% of early PrCa and mCRPC, respectively. Seminal work from our Group, and others, has established that some alterations, e.g. germline *BRCA1/BRCA2* deleterious mutations, are linked to poor outcomes. Currently, we lack the adequate biological knowledge and reliable biomarkers to select the right treatment for the right patient at the right time.

RESEARCH HIGHLIGHTS

PROCURE biomarkers platform

This network was started by our Group in 2013; it currently has 5 ongoing prospective studies (PROREPAIR, PROSTAC, PROSABI, PROSENZA, PRORADIUM) in mCRPC in 63 participating centres with over 900 enrolled patients.

PROREPAIR study

This is a prospective multicentre cohort study involving 50 Spanish centres within the PROCURE network. By April 2016, 432 mCRPC patients were enrolled to evaluate the prevalence and impact of DNA repair germline mutations in mCRPC survival and the response to systemic treatments for mCRPC. Germline mutations were analysed in the following genes: *ATM, ATR, BARD1, BRCA2, BRCA1, BRIP1, CHEK2, GEN1, MLH1, MRE11A, MSH2, MSH6, NBN, PALB2, PMS2, RAD51C, RAD51D and XRCC2*. Current results suggest that up to 12% of the patients in this series harbour a germline deleterious mutation. Analyses of the clinical impact of germline and somatic mutations in outcomes are still undergoing. BRCARAD and BRCAPROS studies, although in a retrospective fashion, will address similar questions at an early prostate cancer stage.

SWITCH Phase II study

In 2016, we also completed the enrolment and follow-up of our first clinical trial, 'Phase II pilot study of the prednisone to dexamethasone switch in mCRPC patients with progression on abiraterone and prednisone', aimed at analysing the role of certain steroids in the resistance and response to novel androgen-synthesis inhibitors in 26 patients. A simple change in prednisone to dexamethasone rescued the sensitivity to abiraterone and prolonged the time benefiting from this treatment in 40% of the patients; such responses could be linked to AR mutations detected in ctDNA.

Biological characterisation of *BRCA2* and *ATM* mutated tumours

Initial results from human tumour characterisation and mouse models conducted by our Group support that BRCA2 germline and/or somatic alterations may occur early in cancer progression, and that ATM aberrations will favour cancer progression and early intratumour heterogeneity. ■

Graduate Students
Ylenia Cendón, Lorena Magraner (since April), Paz Nombela, Floortje Van De Poll (until May)

Technician
Vanessa Cañadilla

Student in practice
Noemi Hernández (since September)

Visiting scientists
Teresa Garcés (since February), Gala Grau (since June), Ana M. Gutiérrez (since May), Fernando López, Maria I. Pacheco, Leticia Rivera (since May)

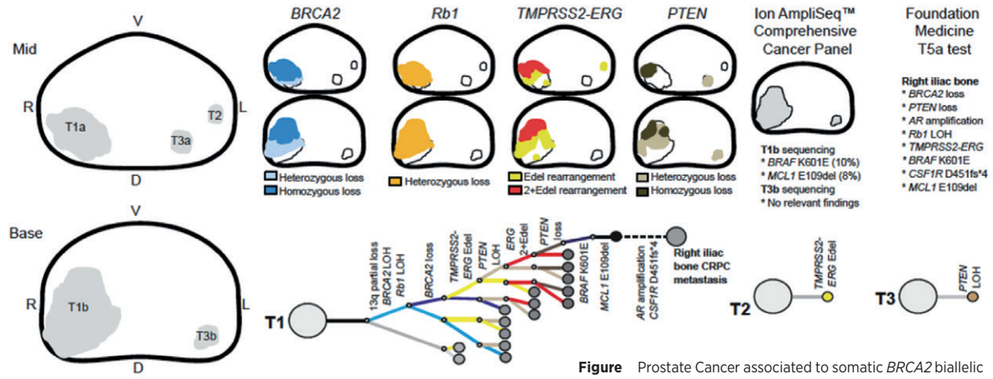

Figure Prostate Cancer associated to somatic *BRCA2* biallelic loss. Heterogeneity and clonal diversity was established based on the frequency and distribution of different dominant events for Prostate Cancer by FISH, as well as targeted sequencing focused on primary prostate cancer and a CRPC bone metastasis.

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- Castro E *et al.* (incl. Olmos D) (2016). The Role of PARP Inhibition in the Treatment of Castration-Resistant Prostate Cancer. *Cancer J* 22, 353-356.
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AWARDS AND RECOGNITION

- Research contract from the *Ramón y Cajal* Programme, *Ministerio de Economía, Industria y Competitividad* (*MEIC*).
- Scientific Committee Member, ESMO Congress, Copenhagen, Denmark.
- Faculty Board Member, EORTC-ECCO-AACR-ESMO Methods in Clinical Cancer Research Workshop, Zeist, Netherlands.
- Nuria Romero was awarded the 'Best Communication' Award, 2nd Androgen Project Meeting in Prostate Cancer, Spain.
- Elena Castro was the recipient of the Best ESMO Fellowship Project (ESMO Congress, Denmark) and the *Juan de la Cierva* Research Contract (*MEIC*, Spain).

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

OVERVIEW

The Molecular Diagnostics Unit (MDU) is mainly dedicated to developing, implementing, standardising and making available a wide variety of highly sensitive and specific molecular diagnostics assays that are scarcely available in the Hospitals of the Spanish National Health System. MDU's portfolio of genetic tests enables the determination of alterations in the sequence or expression levels of key genes involved in cancer. In turn, these assays can be used for the early diagnosis of neoplasias, the detection of minimal residual disease in patients showing clinical remission, for monitoring the response to therapy in patients, as well as for facilitating decision-making amongst different treatment options. Furthermore, the Unit also provides support to the research needs of CNIO's Clinical Research Units and Research Groups by checking their samples for alterations in the biomarkers included in our portfolio. Finally, MDU is very much committed to disseminating knowledge in the field of molecular diagnostics by hosting and mentoring biomedical students.

"In this transition phase of precision medicine, MDU is increasingly focused on the implementation of assays for the detection of biomarker alterations that could grant a more selective diagnosis for cancer patients."

RESEARCH HIGHLIGHTS

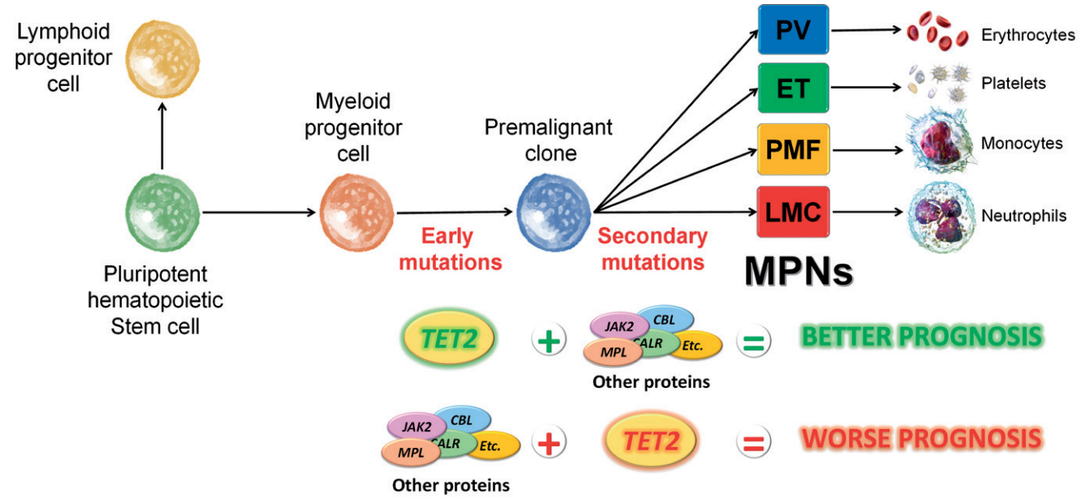

Figure The detection of mutations in *TET2* will improve the diagnostics algorithm by allowing prediction of the prognosis of patients with MPNs (MPN: myeloproliferative neoplasm; CML: chronic myeloid leukaemia; PV: polycythaemia vera; ET: essential thrombocythaemia; PMF: primary myelofibrosis).

Strengthening our support

During 2016, our catalogue has grown with the addition of a new molecular diagnostics test based on the detection, by bi-directional Sanger sequencing, of mutations in exons 4 and 5 of the *MYD88* gene. Waldenström's macroglobulinemia (WM) is a rare form of blood cancer that is characterised by an excess of malignant white blood cells (lymphoplasmacytic cells) in the bone marrow. It has been shown that WM is the result of a multistep transformation process that accumulates sequential oncogenic alterations. The most prominent is the *L265P* somatic activating mutation in the *MYD88* gene (present in 90% of WM). Hence, its detection would enable us to differentiate WM (but also diffuse large B-cell vitreoretinal lymphoma or marginal zone lymphomas) from indolent B-cell or other chronic lymphoproliferative disorders.

Additionally, because identification of several gene alterations involved in the onset of myeloproliferative neoplasms (MPNs) has revealed the huge complexity of these diseases and has challenged their accurate differential diagnosis, we started working on the implementation and validation of a new assay that will enable us to detect mutations in the *TET2* gene; this will complement the diagnosis of MPNs patients. Mutations in this tumour suppressor gene (present in 13% of MPNs) lead to genomic instability via epigenetic modifications and foster cancer progression. Recent studies have revealed that the order in which these mutations are acquired is critical. Thus, patients with early mutations in *TET2* were more likely to have better prognosis compared to patients who had previous mutations in others genes linked to MPNs (FIGURE).

Lastly, we have completed the initial experimental phase of a clinical trial sub project, FRAGANCE, led by the CNIO Gastrointestinal Cancer Clinical Research Unit, which is geared towards precision medicine for fragile patients with advanced pancreatic cancer.

Tutoring

MDU has also upheld its policy regarding training programmes in 2016 by welcoming one medical resident and one undergraduate student. ■

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

OVERVIEW

The Haematological Malignancies Clinical Research Unit focuses on 3 main objectives:

- → *Molecular research of haematological cancer*: the study of cancer-induced changes at the proteomic and genomic levels. We aim to: i) find new genomic and proteomic biomarkers for a better diagnosis of these haematological diseases; ii) identify new molecular alterations as predictors of response to treatment, e.g. to study minimal residual disease; and iii) study immune mechanisms of cancer control, with a special focus on NK cells.
- → *In vitro research*: i) to establish the effects of new anticancer molecules in *in vitro* models of the disease; ii) to determine the mechanisms of resistance to anticancer drugs.
- → *Clinical research*: to translate preclinical findings to the patients through a phase I clinical trials unit.

"We contribute towards redefining the response criteria for Multiple Myeloma (MM) through the usage of new molecular techniques. In 2016, we published the first reports sequencing complete exomes of amyloidosis. Finally, we reported a new cell therapy approach based on infusion of NK cells in MM."

Staff Scientists
Rosa Ayala, Teresa Cedena, Inmaculada Rapado, Beatriz Sánchez-Vega

Post-Doctoral Fellows
Lucía Fernández (since July), María Linares, Ricardo Sánchez, Antonio Valeri

Graduate Students
Alicia Arenas, Isabel Cuenca, Alejandra Leivas, M. Luz Morales (since June), Esther Onecha, Alejandra Ortíz (since May), Yanira Ruíz

Technicians
Alba García, Ana I. Sánchez

RESEARCH HIGHLIGHTS

The most relevant achievements of our Group in 2016 were:

- → We reported a phase I clinical trial based on an innovative cell therapy approach using activated and expanded NK cells for Multiple Myeloma (MM). The results of exploring this approach in phase II and III clinical trials are promising.
- → We published the first report of exome sequencing in amyloidosis. We have not found any recurrent mutation.
- → Finally, we redefined the role of stringent complete response by next generation sequencing in Multiple Myeloma. ■

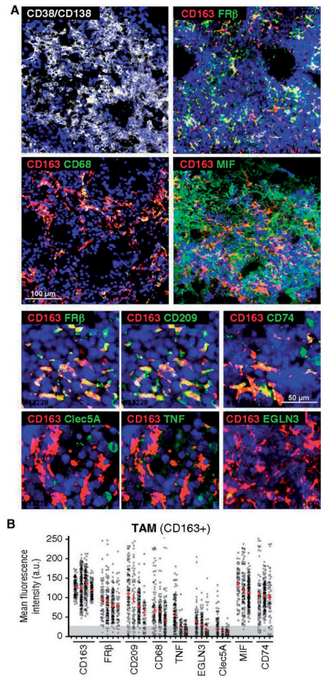

Figure Phenotyping of multiple myeloma macrophages (MM-MØ) from Bone Marrow (BM) patient samples. (**A**) Multi-coloured staining of BM aspirates containing particles from active disease MM patients, as indicated. Upper panels represent panoramic views, whereas bottom panels are magnified ones. Nuclear-49,6-diamidino-2-phenylindole appears in blue in all cases. (**B**) Plot showing the mean fluorescence intensity for each marker in CD1631 tumour associated macrophages (TAM; n = 10 cases). Cells > 25 arbitrary units (a.u.) are considered positive, relative to negative control. Scale bars as indicated.

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- Cross NC et al. (incl. Martinez-López J) (2016). Development and evaluation of a secondary reference panel for BCR-ABL1 quantitation on the International Scale. *Leukemia* 30, 1844-1852.
- Santos-Lozano A, Morales-Gonzalez A, Sanchis-Gomar F, Cristi-Montero C, Fiuza-Luces C, Pareja-Galeano H, Martínez-López J, Garatachea N, Lucia A (2016). Response rate to the treatment of Waldenström macroglobulinemia: A meta-analysis of the results of clinical trials. *Crit Rev Oncol Hematol* 105, 118-126.

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- Paiva B, Puig N, Cedena MT, de Jong BG, Ruiz Y, Rapado I, Martinez-Lopez J, Cordon L, Alignani D, Delgado JA, van Zelm MC, Van Dongen JJ, Pascual M, Aguirre X, Prosper F, Martín-Subero JI, Vidriales MB, Gutierrez NC, Hernandez MT, Oriol A, Echeveste MA, Gonzalez Y, Johnson SK, Epstein J, Barlogie B, Morgan GJ, Orfao A, Blade J, Mateos MV, Lahuerta JJ, Miguel JF (2016). Differentiation stage of myeloma plasma cells: biological and clinical significance. *Leukemia*. PMID: 27479184.
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- Mateos MV, Martínez-López J, Hernández MT, Ocio EM, Rosiñol L, Martínez R, Teruel AI, Gutiérrez NC, Martín Ramos ML, Oriol A, Bargay J, Bengoechea E, González Y, Pérez de Oteyza J, Gironella M, Encinas C, Martín J, Cabrera C, Paiva B, Cedena MT, Puig N, Bladé J, Lahuerta JJ, San-Miguel J (2016). Sequential versus alternating administration of VMP and Rd in elderly patients with newly diagnosed MM. *Blood* 127, 420-425.
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H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
Clinical Research Unit Head

Staff Scientists
M. Teresa Argullo, Daniel E. Castellano, Irene Ferrer, Rocio García, Blanca Homet (until September), Lara C. Iglesias, Sonia Molina, Santiago Ponce, Jon Zugazagoitia

Post-Doctoral Fellow
Beatriz Soldevilla (since June)

Graduate Students
Ángela Marrugal, Laura Ojeda, Álvaro Quintanal, Patricia Yagüe (since October)

Technicians
Laura García, Rocío Suárez, M. José Durán (since July), Virginia Pardo (since February)

OVERVIEW

Our Group combines basic preclinical studies with clinical and translational research, mainly in lung cancer and other solid tumours. In summary, the main research areas of our Group focus on 2 modalities: (1) the identification of new molecular biomarkers that can be used in the clinic for diagnostic, prognostic, predictive and pharmacogenomic purposes; and (2) developing novel treatment strategies. For example, we have comprehensively profiled bronchoalveloar lavage (BLA) fluids of COPD and lung cancer patients, showing a differential mi-RNA, protein and inflammatory cytokine expression between both diseases and different subtypes of lung cancer. On the other hand, we have developed a patient-derived xenograft (PDX) platform of non-small-cell lung cancers to test new drugs/targets. We are also developing PDXs of small-cell lung cancers. Finally, our Group has extensive experience in the development of new drugs, as well as in conducting practice-changing phase II/III trials in the fields of precision oncology and immunooncology.

"Our Group has significantly contributed to the discovery of biomarkers as well as to the early development of new drugs tailoring novel targets. We have co-led randomised clinical trials with biological therapies and immunotherapy in lung cancer and other solid tumours."

RESEARCH HIGHLIGHTS

New drug development and early clinical trials

Our Group has been actively involved in pharmacogenomic, pharmacokinetic, translational and clinical studies with novel antitumour agents in several types of solid tumours, particularly lung cancer. Our principal clinical research area has been immunotherapy and immune-based early clinical trials. As a first relevant example we can mention the CheckMate CA 209-032 trial testing nivolumab +/- ipilimumab in recurrent or extensive-stage small-cell lung cancer, which was fully recruited in 2016, with a substantial contribution by investigators from our Group. These important data have been recently published in *The Lancet Oncology*. In addition, Luis Paz-Ares is the principal investigator of a phase I trial (JDVF) testing a novel combination of pembrolizumab plus ramurirumab in different types of solid tumours. Encouraging preliminary clinical data were presented at ASCO 2016 in the cohort of non-small-cell lung cancer, showing a response rate of 35 % and 7-months of progression-free survival in pretreated patients. Finally, a first-in-human trial with a novel T-cell bispecific antibody targeting carcinoembryonic antigen (CEA) expressed on tumour cells and CD3 on T-cells was initiated and is actively recruiting patients.

Conducting practice changing randomised controlled trials

Our Group has also made a substantial contribution in conducting pivotal trials with immune checkpoint inhibitors. In particular, an important phase III trial, led by Dr Paz-Ares (the international principal investigator), with pembrolizumab in completely resected non-small cell lung cancer patients is actively recruiting participants. Furthermore, the first randomised trial comparing second-generation (afatinib) versus first generation (gefitinib) tyrosine-kinase inhibitors in patients with EGFR-mutant lung cancers, also internationally led by Dr Paz Ares, was completed in 2016 and its results were recently published in *The Lancet Oncology*.

Novel biomarker development and translation

IL-11 and CCL-1 have been proposed as novel diagnostic biomarkers of lung adenocarcinoma in bronchoalveolar lavage fluid. This finding has potential implications in early lung cancer diagnosis. Moreover, different members of our Group contributed towards providing further insights into the role of PD-L1 expression and other potential immune biomarkers for the benefit of immune checkpoint inhibitors. ■

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- Diekstra MH *et al.* (incl. Castellano D, Rodríguez-Antona C) (2016). Sunitinib induced hypertension in CYP3A4 rs4646437 A-allele carriers with metastatic renal cell carcinoma. *Pharmacogenomics J.* PMID: 26810136.
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BIOBANK

Manuel M. Morente
Director

Technicians
Nuria Ajenjo (TS)*, Inmaculada Almenara, M. Jesús Artiga (TS), Francisco De Luna (TS)

**Titulado Superior* (Advanced Degree)

OVERVIEW

The CNIO Biobank is a cross-service platform for CNIO researchers, as well as the general scientific community, and is geared towards the promotion of biomedical research in cancer and related diseases. The CNIO Biobank facilitates access to human samples for researchers, ensuring that both the acquisition and use of human samples complies with all the legal and ethical principles that protect donors' rights.

The CNIO Biobank is a 'biobank for biomedical research purposes', as defined by the Spanish Law 14/2007 on Biomedical Research and the Royal Decree RD 1716/2011. It is therefore defined as a public, non-profit organisation that hosts several collections of human biological samples for biomedical research.

The biobank is organised as a technical unit with strict criteria for quality, order and purpose, regardless of whether or not it hosts other collections of biological samples for different purposes. Samples and their associated information are managed in compliance with Spanish legislation and international recommendations; all of this is consistent with quality criteria for sample collection and its subsequent management.

The Biobank has been authorised by the Health Authorities of the *Comunidad Autónoma de Madrid* – in accordance with the regulation established by RD1716/2011 – and is registered in the National Registry of Biobanks with reference B.000848.

RESEARCH HIGHLIGHTS

Biobanking

- → Collection, management, manipulation and custody of human biological samples and associated documentation, in accordance with the legal framework for biobanking.
- → Transfer of samples and clinical biomedical information to research projects, under the approval of the corresponding scientific and ethical committees.

Management of other collections

- → Custody service of collections of biological samples and/or information related to biomedical research as promoted by the CNIO or other external research groups.
- → Coordination of sample collections in multicentre studies.
- → Processing of products derived from human samples for research (tissue arrays, DNA, RNA, etc.).
- → Researchers who want to deposit their collections at the CNIO-Biobank facilities, or who wish to request samples, must sign an MTA (Material Transfer Agreement) that specifies the terms and conditions under which the Biobank will custody the samples and data.

Ethico-legal advice for CNIO researchers regarding the use of human samples in biomedical research

- → Technical, scientific and ethical advice regarding the collection, storage and management of human samples used for biomedical research, as well as in regards to the creation and management of new collections that are beyond the Biobank's scope.

Other services

- → Collaboration with CNIO researchers in human pathology.
- → Collaboration in diagnostic activities as specialists in human pathology.

During 2016, the CNIO-Biobank has supported 9 tissue requests from scientific research projects with 180 single cases and 303 tissue microarrays transferred. Additionally, as the Spanish National Biobank Network Coordination Office, we have managed 37 scientific research projects of high complexity.

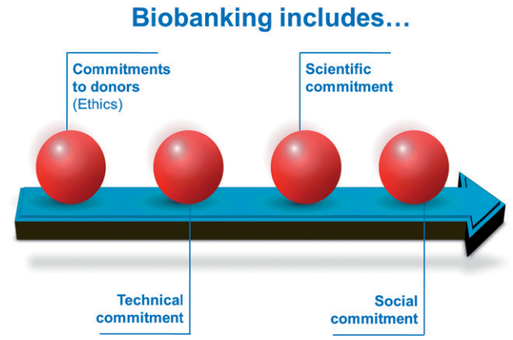

Figure Biobanking is a transversal activity based on four basic commitments: respect for donors' rights, technical excellence, adherence to the scientific requirements, and strict compliance with Spanish legislation.

The mean impact factor of the 10 publications published in 2016, for which our Unit provided support was 11.384. We also provided sample and/or documental support for the familial cancer activities of the CNIO Human Cancer Genetics Programme.

The CNIO Biobank participates in and coordinates the Spanish National Biobank Network. This nationwide platform of services integrates 52 institutions (www.redbiobancos.es) and is an initiative of the *Instituto de Salud Carlos III* (*ISCIII*).

Finally, the Unit has spearheaded many activities in the national and international biobanking scene through its participation and leadership in numerous forums, working groups and national and international scientific societies. These include the European, Middle Eastern and African Society for Biopreservation and Biobanking (ESBB), the International Society for Biological and Environmental Repositories (ISBER), international think tanks such as the Marble Arch International Working Group on Clinical Biobanking, BC-Net IARC-WHO/NCI initiative, EurocanPlatform (7th FP), and others. ■

PUBLICATIONS

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- Doucet M *et al.* (incl. Morente M) (2016). Quality Matters: 2016 Annual Conference of the National Infrastructures for Biobanking. *Biopreserv Biobank.* PMID: 27992240.
- Suárez AE *et al.* (incl. Artiga MJ) (2016). Angioimmunoblastic T-cell lymphoma with a clonal plasma cell proliferation that underwent immunoglobulin isotype switch in the skin, coinciding with cutaneous disease progression. *J Cutan Pathol* 43, 1203-1210.
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AWARDS AND RECOGNITION

- Member, Evaluation panel 'Enabling German Biobank Sites to Connect to BBMRI-ERIC', Federal Ministry of Education and Research, Germany.

Direction of Innovation

ÓSCAR FERNÁNDEZ-CAPETILLO
Director of Innovation

“Innovation and research constitute both sides of the same coin. At the CNIO, we are making our effort so that the products of our research end up benefiting society.”

Scientific research often brings about the generation of knowledge and/or products that can be of use beyond the boundaries of academic research. These can include novel technologies, animal models, antibodies or chemical entities with interesting biomedical properties, among others. At the CNIO, we aim to bridge the gap between our researchers and potential outsources that could potentially be interested in further developing our inventions. These initiatives have materialised in the form of contracts with industry or in the licensing out of several of our products. Importantly, while the royalties deriving from these activities mostly benefit the CNIO as an Institution, they also circle back to the scientific Groups to fund their research as well as to the inventors themselves. To date, more than 40 investigators from 11 groups have benefited from this initiative.

A Programme that deserves to be singled out in terms of innovation is that of the Experimental Therapeutics Programme (ETP), a whole department that aims to develop chemical entities that could potentially lead to new anticancer therapies. With several of these molecules already licensed out to the Pharmaceutical industry, their current portfolio of projects includes several at an advanced stage. The Direction of Innovation promotes a pipeline based on collaborative drug development between ETP and the rest of the CNIO Groups, so that drug-development capitalises on the excellent research conducted by our basic scientists.

Accordingly, all of our current early stage drug development projects have emerged from active collaborations with scientific research groups. In addition, we are trying to consolidate ETP as a strong node for anticancer drug development in Spain, so that our expertise can contribute towards the development of new therapies together with other cancer researchers in our country.

An important strength of our innovation activities derives from the excellent support provided by the Biotechnology Programme. Their work is not only vital for the progress of our scientific projects, but has also led to the realisation of important products and technologies that have contributed to our innovation portfolio. Some of the antibodies developed at the CNIO are contributing towards important aspects of current innovative treatments, such as those based on immunotherapies. The accreditation of our Animal Facility by the *Association for Assessment and Accreditation of Laboratory Animal Care International* (AAALAC) also represents a key milestone in 2016 for the Biotechnology Programme.

Finally, several initiatives continue to be in place with the aim of fostering an innovation culture among our scientists, such as our continuous agreement with the prestigious *IE Business School*, through which many of our investigators have already obtained training in market-oriented innovation strategies.

BIOTECHNOLOGY PROGRAMME

FERNANDO PELÁEZ Programme Director

The main mission of the Biotechnology Programme is to provide expert technical support and advice to CNIO Research Groups in a number of disciplines and technologies widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and reagents for cancer research. The Programme is currently composed of nine Core Units covering major areas in Biotechnology, namely, Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Transgenic Mice, as well as an Animal Facility. Although the Core Units are mainly focused on meeting the internal demand and collaborating with the CNIO Research Groups, they also provide support and collaborate with groups from other public institutions, as well as with private companies.

In 2016, the Programme was significantly reinforced with the recruitment of 9 young technicians who are funded for a 2-year period by the programme *Ayudas para la Promoción de Empleo Joven e Implantación de la Garantía Juvenil en I+D+i del Ministerio de Economía y Competitividad.*

This year, the CNIO Animal Facility obtained full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC); this recognition reflects the high standards achieved by the CNIO with regards to the use and care of animals for experimentation.

Faithful to its mission, a number of different technological innovations have been explored or implemented by the Core Units during this last year, often in collaboration with CNIO Groups. Noteworthy examples include the application of CRISPR technology for the generation of mouse models, the generation of monoclonal antibodies against small molecules, the application of proteomic approaches to the study of exosomes, the development of immuno-PET approaches for tumour imaging, the expansion of multicolour capabilities in flow cytometry studies, and the application of microfluidics-based setups to advanced microscopy, to name a few.

In 2016, the Programme and its Core Units have been particularly active in networking activities. This included the participation of several of our Programme members in the Core Technologies for Life Sciences (CTLS) meeting at EMBL in Heidelberg, Germany, where the first steps were taken towards the organisation of a new scientific association addressing core facilities issues (CTLS). In addition, several Unit Heads were very active in participating in networks and scientific societies from their corresponding fields. Also, the Programme Director was voted as President-Elect of the Spanish Society of Biotechnology (SEBiot), highlighting the prominent role of the CNIO in this area.

"The best possible partner for enabling CNIO's scientists to achieve their research goals is a strong Biotechnology Programme with state-of-the-art Core Facilities."

Also, as an indication of our high commitment to training and education, the Programme has been involved in the organisation of courses, workshops and specialised meetings. Moreover, an increasing number of our staff members undertook Masters and other training activities, at the CNIO and elsewhere.

This year, the Core Units were particularly successful in attracting funding from external sources through activities related to innovation; several contracts and agreements with private companies and public institutions, based on the technologies mastered by several of our Core Units, were formalised. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit have grown by about 16% over the previous year, reaching a new historical maximum.

Last but not least, 2016 has once again been a very productive year, scientifically, for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in nearly 30 publications, many of them appearing in top journals.

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Guadalupe Luengo, Jorge Monsech, Ana Belén Moreno (PEJ)*, Ángeles Rubio, Delfina Sanguino

**Plan de Empleo Joven* (Youth Employment Plan)

"The Genomics Unit, with its toolbox for DNA and RNA analyses, helps CNIO scientists to understand the molecular processes underlying cancer in a large number of basic and applied research projects."

OVERVIEW

Genomics is the discipline that studies the structure and dynamics of the genome, its features, its regulation and expression. The genome is the core of life, the ensemble of the genetic material that conserves the assembly instructions of the species. Each cell in an individual keeps a copy of it deep in its nucleus. Chemically made of linear DNA macromolecules and distributed into chromosomes, it is packed with and interpreted by a myriad of protein cohorts acting in concert. It is expressed into RNA transcripts; some are functional in and of themselves, and others, constitute an intermediate step leading to the functional proteins that govern the cell. While less than a 2% fraction of a mammalian genome codes for protein, a vast majority of it (80%) has been found to participate in biochemical events. The genome is not immutable, it can suffer alterations. A chance for biological evolution or for damage. In fact, cancer derives from the accumulation of such alterations. Cells with a damaged genome can transform and develop into a tumour. The field of Genomics sheds light on this world of complexity.

RESEARCH HIGHLIGHTS

All tumours, even those of the same type and sharing a similar fate, are molecularly different and heterogeneous at the molecular level. By employing a distinct set of powerful methodologies, Genomics reveals the genetic diversity of cancer and helps to dissect molecular mechanisms. These methodologies have the capacity to interrogate a wide number of genetic loci, or even a whole genome in a single assay. Some tools detect modifications at a structural level: mutations, binding of protein factors, variations in chromatin folding. Others are suitable for observing functional choreographies, transcriptomic changes – for example, in response to treatments – that may uncover therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of complexity. The genomic wide level is addressed by both deep-sequencing and microarray technologies. Deep-sequencing permits a variety of applications, such as whole-genome or whole-exome tumour sequencing, transcriptome analyses by RNAseq, or location of interacting protein factors on chromosomal DNA by ChIPseq. As a novel sample type, the Unit successfully participated in the exome sequencing of *cell free* DNA obtained from cancer patients' blood. This year has seen a 40% increase in the overall demand and in the number of samples processed.

On the other hand, the DNA microarray platform can be efficiently used for transcriptome determinations or for the detection of chromosomal copy number abnormalities. At the single locus level other offers are available. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyzer from Applied Biosystems, is being used to find and confirm mutations in candidate genes as well as for the verification of cloned genes or inserts. The Unit also provides a transgenic mouse genotyping service, based on allele-specific quantitative PCR for a quick and efficient turnaround time. With a current, but continuously growing, catalogue of over 30 genetic modifications, the demand for genotyping services this year has almost doubled in comparison to former years. ■

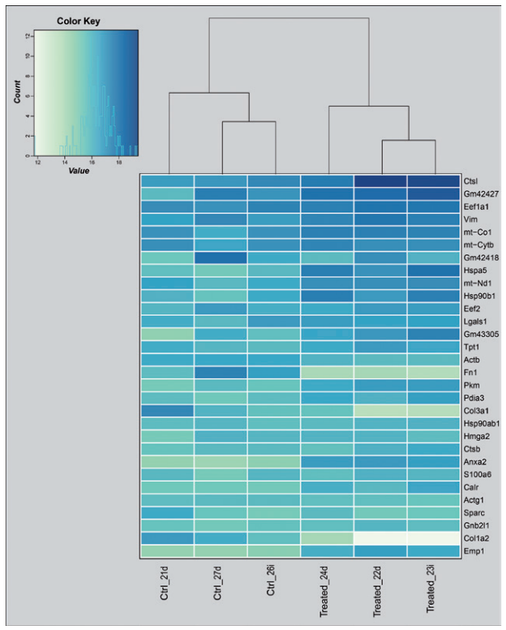

Figure An RNAseq experiment reveals the effects of a treatment at the RNA level. A blind analysis properly clusters samples of the same type together in columns. Genes whose transcription is significantly affected by treatment are shown in rows. Colour intensity reflects the transcriptional level. Some gene expression variability is apparent among replicates from the same condition.

TRANSGENIC MICE CORE UNIT

Sagrario Ortega
Core Unit Head

Student in Practice
Aleida Pujol

Technicians
Estefania Ayala, M. Carmen Gómez, Jaime A. Muñoz (TS)*, Lucía M. Pérez De Ayala (PEJ-L)**, Patricia Prieto (TS)*, Pierfrancesco Vargiu (TS)*

**Titulado Superior* (Advanced Degree)

***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

Genetically engineered mice are an essential tool for analysing the molecular mechanisms underlying tumour development and cancer biology. Modelling cancer by modifying the germ line of the mouse has become a crucial component of drug discovery as well as for the assessment of experimental therapies at the preclinical stage. The Transgenic Mice Unit at the CNIO offers state-of-the-art technology for the manipulation of the mouse genome. Using classical transgenesis, homologous recombination in embryonic stem cells and genome editing by targeted nucleases, the Unit has generated more than 300 mutant alleles of cancer related genes in the mouse germ line. The Unit also provides support and collaborates with CNIO researchers in many aspects related to research with embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and embryo- and mouse model-based research. Finally, the Unit also leads its own research projects focused on the generation of mouse models to study tumour biology, as well as on the screening of cancer-related genes.

"In 2016, the Unit generated over a dozen GEM strains containing knockout and knockin mutations, using the CRISPR/Cas9 system of *S. Pyogenes*. The Unit contributed to 8 peer-reviewed articles, in collaboration with CNIO and external groups, including the description of a new mouse strain for conditional gene targeting of the lymphatic system."

RESEARCH HIGHLIGHTS

The CNIO Transgenic Mice Unit is dedicated to the generation, cryopreservation and derivation of genetically engineered mouse strains. We have created over 200 mutant strains, including knockout, knockin and conditional alleles, by gene targeting in embryonic stem (ES) cells, and over 100 mouse strains by conventional transgenesis. The Unit currently maintains a cryopreserved stock of over 1000 mouse strains, frozen at the Unit as sperm or embryos. This stock represents an invaluable resource of engineered strains for modelling and studying cancer in the mouse. Through our Unit, the CNIO shares part of this stock with EMMA (the European Mouse Mutant Archive) in order to make these models more accessible to the wider scientific community. We acknowledge the CNIO Animal Facility for their constant help and collaboration to make all these achievements possible.

The CRISPR/Cas9 system of *Streptococcus pyogenes* has expanded the currently available set of mammalian genome engineering tools, providing an easy, efficient, flexible and versatile method for creating targeted mutations in mammalian genes. We use the CRISPR/Cas9 system to generate knockout and knockin mice by introducing the components of the system, the guide CRISPR RNA and the Cas9 nuclease (either as messenger RNA or as protein) directly into mouse zygotes (FIGURE). In our experience, this system has proven to be extremely efficient for introducing new additional mutations in strains that are already carrying several engineered alleles, such as some mouse models of lung and pancreatic cancer that are used at the CNIO. We have also used the system to generate knockin alleles (point mutations) and tag insertions with efficiencies close to 20% directly in zygotes. The characteristics of the CRISPR system – efficient, fast and easy to implement – make it extremely useful for creating constitutive mutations in the mouse and to test certain biological questions before embarking on the creation of conditional or more sophisticated alleles. For these types of alleles, gene targeting in ES cells may still be the method of choice and we are currently optimising the use of CRISPR in ES cells to increase the efficiency of this technology. ■

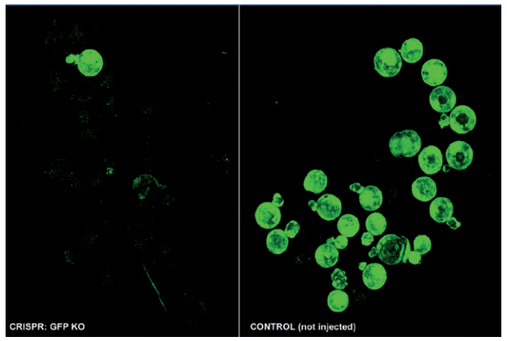

Figure Efficiency of GFP knockout via CRISPR in mouse embryos. Embryos are collected from B6.CBA females, crossed with 129Gt(ROSA)26Sortm(CAG-EGFP) Luo (KI/KI) males, at E0.5. Embryos are injected with gRNA_GFP97 (50ng/µl) and commercial Cas9 protein (100ng/µl) in the cytoplasm at the zygote stage and cultured *in vitro* for 3 days up to the blastocyst stage. Confocal images (maximal projection) of GFP fluorescence.

PUBLICATIONS

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- Varela E, Muñoz-Lorente MA, Tejera AM, Ortega S, Blasco MA (2016). Generation of mice with longer and better preserved telomeres in the absence of genetic manipulations. *Nat Commun* 7, 11739.
- Calvete O, Varro A, Pritchard DM, Barroso A, Oteo M, Morcillo MÁ, Vargiu P, Dodd S, Garcia M, Reyes J, Ortega S, Benitez J (2016). A knockin mouse model for human ATP4aR703C mutation identified in familial gastric neuroendocrine tumors recapitulates the premalignant condition of the human disease and suggests new therapeutic strategies. *Dis Model Mech* 9, 975-984.
- Martínez-Corral I, Stanczuk L, Frye M, Ulvmar MH, Diéguez-Hurtado R, Olmeda D, Makinen T, Ortega S (2016). Vegfr3-CreER (T2) mouse, a new genetic tool for targeting the lymphatic system. *Angiogenesis* 19, 433-445.
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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Álvaro García (PEJ)*, Sherezade Jiménez, Lorena Maestre (TS)**, Ana I. Reyes

**Plan de Empleo Joven* (Youth Employment Plan)
***Titulado Superior* (Advanced Degree)

OVERVIEW

The development of monoclonal antibody (mAb) technology has led to the generation of large panels of highly specific reagents that have had a tremendous impact on basic and applied research over the last four decades. MAbs have become indispensable tools for many of the laboratory techniques that are used to answer essential questions in biomedical research. Their outstanding specificity makes them excellent tools for enabling researchers to better understand biological processes; particularly in the investigation of new approaches for the diagnosis, prevention and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an *à la carte* generation of mAbs. We are highly specialised in the production of mouse and rat mAbs. The Unit also offers mAb production in gene-inactivated mice, mAb characterisation and validation, medium-scale mAb production, and a service of *Mycoplasma* testing for the cell culture facility.

"The Unit produces novel and high quality mAbs for use in basic research in order to gain new insights into the human cancer development process. We are also highly specialised in mAb characterisation, thereby providing CNIO researchers with reliable and well-validated reagents that give an added value to their research projects."

RESEARCH HIGHLIGHTS

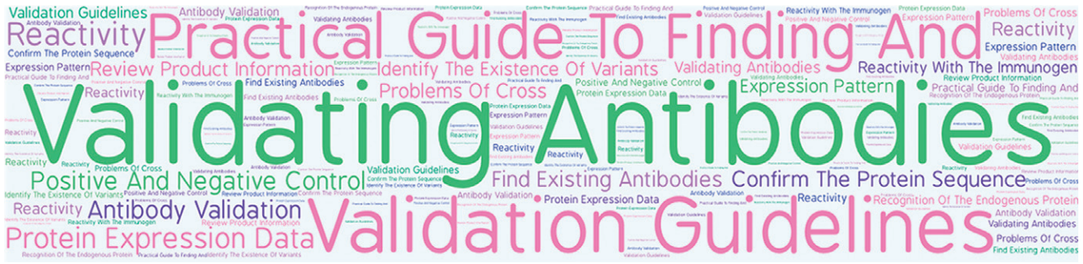

Figure Antibody validation cloud.

During the last 16 years, the Monoclonal Antibodies Unit has generated a large number of mAbs (against more than 130 different antigens), mostly targeting molecules for which mAbs are not commercially available. Many of those mAbs have been licensed to external companies, generating in turn royalties that represent an important source of revenue for the CNIO.

Each year we prepare and update a detailed CNIO mAbs catalogue, which contains the datasheets of more than 78 thoroughly validated high-quality mAbs (accessible at http:// www. cnio. es/ing/servicios/anticuerpos/default.aspx).

This year, in collaboration with the Custom Antibodies Service (CAbS) of the Institute for Advanced Chemistry of Catalonia of the Spanish Council for Scientific Research (IQAC-CSIC), we have successfully generated several mAbs against small molecules, compounds with low molecular weight such as vitamins, chemicals, hormones, etc., thus expanding our portfolio of reagents in this new field. We also established collaborations with several big pharmaceutical companies (e.g. Merck, Lilly) for the production of mAbs against molecules, of their interest, involved in cancer development.

EuroMAbNet and its commitment with Ab validation

In 2008, in collaboration with Oxford University, we founded EuroMAbNet (www.euromabnet.com), a non-profit organisation that includes internationally distinguished multidisciplinary academic laboratories specialised in antibody technologies. Their wealth of expertise ranges from the identification of new targets to the production of fully validated Abs and their use as research tools, clinically relevant diagnostic/prognostic reagents, and novel therapeutics.

The use of poorly characterised antibodies is of major concern to the scientific community, resulting in wasted time and valuable research funds, as well as in the publication and perpetuation of erroneous research results, which ultimately compromise the advancement of science. To address this problem, EuroMAbNet has published a position paper (Roncador *et al.*, 2016) and some easy to follow guidelines (http://www.euromabnet.com/guidelines) that provide a set of criteria and recommendations to help researchers select the most effective mAbs from those available in the market, and provide the strategic guidance needed to perform antibody validation.

EuroMAbNet also has a strong commitment to improving the education and training of junior scientists in Ab validation. With that in mind, we have started organising annual Antibody Validation Workshops (www.euromabnet.com) to provide practical guidelines about the principles underlying antibody validation, including the verification of Ab specificity, selectivity, sensitivity and reproducibility. These workshops outline the problems generated by the use of poorly validated reagents and educate researchers to minimise the purchase of ineffective Abs. ■

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Elena Andrés (until July), Guillermo Garaulet (since April) (TS)*, Lucía García (September- December), Silvia Leal, Cristina Penalba (PEJ-L)**,
Elka Jesarela San Martín, Gloria Visdominé

Titulado Superior (Advanced Degree)
***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

Molecular imaging involves specialised instrumentation, used alone or in combination with targeted imaging agents, to visualise tissue characteristics and/or biochemical markers. The data generated from molecular imaging studies can be used to help understand biological phenomena, identify regions of pathology, and provide insight regarding the mechanisms of disease. At the Molecular Imaging Unit, we offer state-of-the-art techniques such as Positron Emission Tomography (PET), Computed Tomography (CT), Ultrasounds (US) and Densitometry (DeXa).

"Molecular Imaging, especially PET, goes beyond the role of tumour detection and has also taken on the role of tumour characterisation."

RESEARCH HIGHLIGHTS

The main objectives of the Unit are to provide CNIO researches with state-of-the-art molecular imaging equipment and human resources in order to: guarantee the highest quality studies, develop and update protocols and techniques to optimise visualisation of tumours in both preclinical and clinical fields, as well as assess and advise researchers on the best-suited imaging modality for their research projects.

With the Immuno-PET strategy, the high specificity of the antibody is coupled with the high sensitivity of PET imaging to obtain a strong, non-invasive, tool for glioblastoma (GBM) and pancreatic carcinoma diagnosis and follow-up. In 2016, we published the results of our collaboration with the Seve-Ballesteros Foundation Brain Tumour Group and the Crystallography and Protein Engineering Unit at the CNIO. We reported the development of a new tracer (^{89}Z-LEM2/15) for the efficient detection of MT1-MMP in preclinical GBM models.

We have also provided imaging support in clinical trials conducted under CNIO's Clinical Research Programme. With the Breast Cancer Clinical Research Unit, we published the ^{18}F-FMISO-PET imaging results from a clinical trial aimed at selecting patients who will benefit from treatment with angiomodulators knowing the degree of tumour hypoxia by using this PET biomarker (FIGURE).

Furthermore, we continued our active participation in the international consortium focused on imaging, 'M+Visión' led by the Massachusetts Institute of Technology (MIT). ■

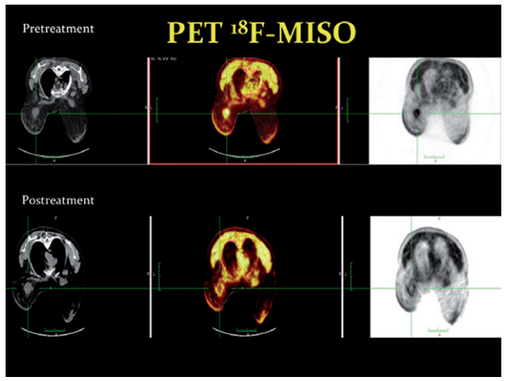

Figure PET-CT imaging with radiolabelled ^{18}F-MISO in patients with breast carcinoma. MISO uptake before treatment (upper panel) and after treatment (lower panel). We observe the reduction in uptake intensity and the change in the shape of the hypoxic volume after treatment.

PUBLICATIONS

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AWARDS AND RECOGNITION

- Scientific Advisory Board Chair and Faculty, the Madrid-MIT *M+Visión* Consortium, Spain.

FLOW CYTOMETRY CORE UNIT

Lola Martinez
Core Unit Head

Technicians
Ultan P. Cronin (TS)*, Elena Garrido (TS)*, Tania López (PEJ-L)**, Miguel Ángel Sánchez (TS)*

**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

Flow cytometry is a very useful tool in the oncology field. It enables multiparametric analysis for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other.

Our aim is to provide CNIO Groups with the necessary technical and scientific advice regarding the use of flow cytometric technologies, collaborating with them for the design, acquisition, data analysis and interpretation.

With our 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, we can cater to all our users' needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters. Analysers are available to users upon appropriate training and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations at a time, as well as allow for single cell cloning. We can accept human samples to sort under Biosafety regulations.

"*In vivo* LacZ detection has always been a challenge. We have optimised a protocol for the identification and isolation of LacZ expressing cells from haematopoietic and lung tissues."

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques of their interest. Some of the applications that have been developed and validated by our Unit include:

- → Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- → Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
- → Multicolour Immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
- → Functional Assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- → Microvesicle detection.

We have further developed our multicolour panels for the characterisation of the immune response by incorporating the new generation of Brilliant UV dyes from samples such as haematopoietic tissues, pancreas, skin, liver, and lung. Modifications in our analytical and cell sorters have also been applied to allow for this. Moreover, these panels could still be combined with the detection of proliferation and cell death. In terms of our cell sorting capabilities we included, at the end of the year, a MoFlo ASTRIOS in our portfolio of cell sorters. This cell sorter is equipped with 4 laser lines and 15 fluorescent detectors, which enable the isolation of up to 6 different populations simultaneously. The optical configuration in the ASTRIOS will allow for the use of the new generation of Brilliant UV dyes. ■

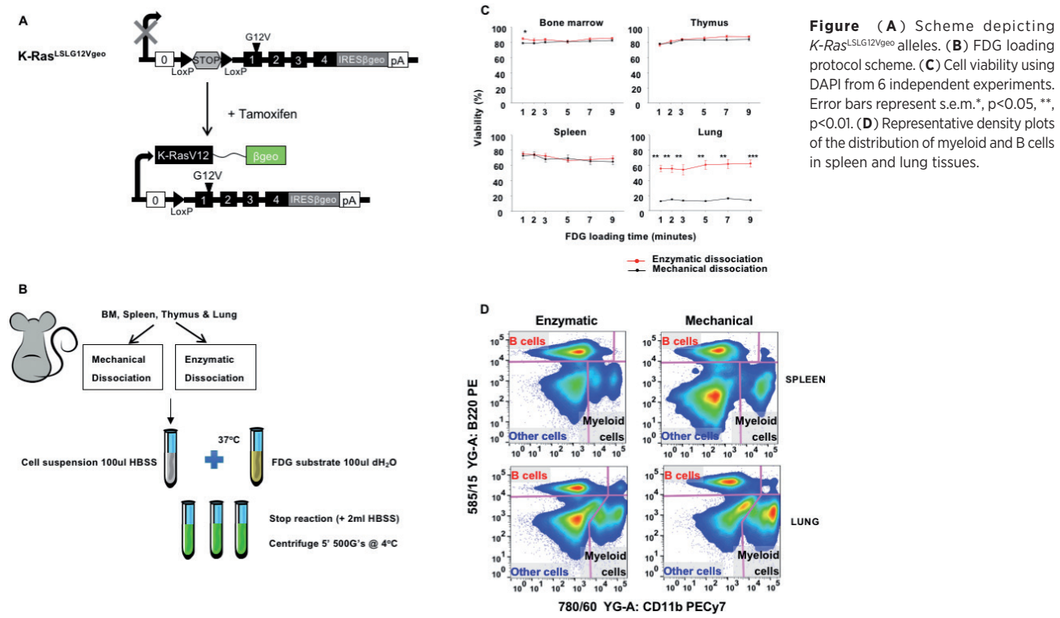

Figure (**A**) Scheme depicting *K-Ras*^{LSLG12Vgeo} alleles. (**B**) FDG loading protocol scheme. (**C**) Cell viability using DAPI from 6 independent experiments. Error bars represent s.e.m.*, p<0.05, **, p<0.01. (**D**) Representative density plots of the distribution of myeloid and B cells in spleen and lung tissues.

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CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Technicians
Jesús Gómez (PEJ-L)*, Manuel Pérez (TS)**, Joaquim Soriano (TS)**

**Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)
***Titulado Superior* (Advanced Degree)

OVERVIEW

Optical microscopy has traditionally been an indispensable tool in cell biology studies. In fact, one of the main challenges in oncology research is the study of specific markers, expression patterns or individual cells in the tumour environment.

The Confocal Microscopy Unit provides CNIO Research Groups with all the standard methodologies and the latest advances in microscopy. We offer access to state-of-the-art equipment and software packages related to confocal microscopy, including technical and scientific advice and support to the CNIO scientists. The Unit is also actively involved in developing, testing and implementing new microscopy technologies, tools and imaging applications that could be of interest to the Research Groups. Training activities are also an essential component of our mission.

"The Confocal Microscopy Unit is fully committed to disseminating advanced microscopy methodologies that are useful for cancer research in order to benefit society, always with the aim of increasing our understanding of the cell biology and the disorders of cells that cause cancer."

RESEARCH HIGHLIGHTS

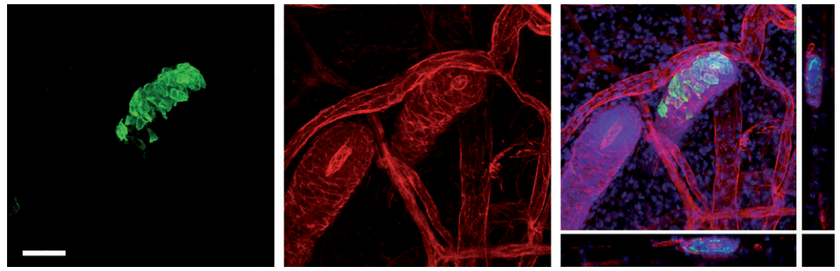

Figure Whole-mount of ear hair follicles. A global double-fluorescent reporter mouse (GFP/Tomato) was used for lineage tracing of epidermal cells.

The Confocal Microscopy Unit is equipped with 3 laser scanning confocal systems (Leica SP2 and SP5) that incorporate UV and multiphoton excitation, a white light laser and a Hybrid Detector, as well as 2 wide-field systems (a Deltavision 4D deconvolution station and a Leica DMRI6000 system, equipped with microinjection). All the microscopes are automated and equipped with incubators for live cell imaging.

In addition, the Unit has implemented the use of high-throughput technologies applied to confocal microscopy using 2 different systems:

- → An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows running HCS experiments on fixed and live cells in multi-well plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.
- → A Matrix Screening Application integrated into the SP5 confocal systems, allowing high-throughput feeding of the instrument, not only in multi-well plates, but also in tissue sections.

These advances enable us to increase the level of information obtained from a sample as well as carry out the automated screening of cell behaviour under different treatments.

During 2016, the Confocal Microscopy Unit contributed to the microscopy field in several aspects. It improved the intelligent screening technique with new algorithms for image acquisition, thereby creating new applications in both confocal and conventional fluorescence microscopy. The use of microfluidics with live-cell assays in perfusion chambers has also experienced a significant increase in performance and demand. In addition, the Unit patented a new device for improving hardware autofocus that will be of great relevance in high-resolution automated image acquisition. Moreover, the Confocal Microscopy Unit continues to dedicate a significant effort towards the development and implantation of High-Content Screening technology at the CNIO; for example, in 2016, we provided support for the running of screening assays for compounds that could modify mitotic checkpoints, integrity of nucleoli, DNA Damage, BrdU, cell proliferation, etc.

Last but not least, in the field of intravital microscopy, we already have several ongoing projects that are focused on metastasis and skin alteration studies. ■

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PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Graduate Student
Ana Martínez

OVERVIEW

Proteins catalyse and control almost all cellular processes in a living cell. The levels of protein abundance, together with their modification states and interactions, adapt dynamically to external or internal (genetic) stimuli and thus define the cell's functional state and determine its phenotype. Recent developments in sample preparation, liquid chromatography, mass spectrometry and data analysis have enabled researchers to investigate diverse proteomic facets in a systematic high-throughput manner, currently comparable to next-generation sequencing platforms. As a result, proteomics is positioned as one of the most powerful technologies to study, at the protein level, complex cellular processes. This vast amount of data is providing new insights into the molecular mechanisms underlying diverse human pathologies such as cancer.

"Mass spectrometry-based technologies enable probing the composition, structure, function and regulation of the proteome, providing new insights into the underlying mechanisms of cancer."

Technicians
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**Titulado Superior* (Advanced Degree)
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Student in Practice
Julia Beltran (since October)

RESEARCH HIGHLIGHTS

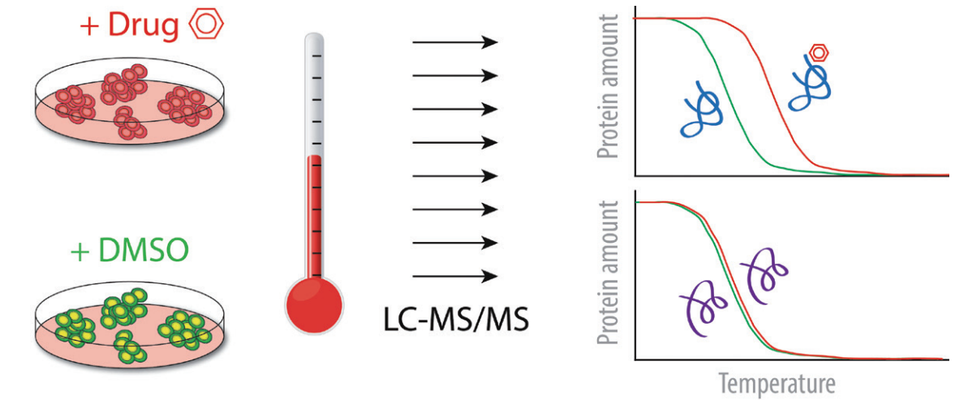

Figure Thermal proteome profiling (TPP). Cells are treated either with the compound of interest or the vehicle. Cells are subjected to increasing temperatures, and denatured proteins are discarded by centrifugation. Supernatants are analysed by LC-MS/MS enabling the reconstruction of the melting curves for all identified proteins. Proteins showing a difference in their melting point between drug and vehicle might be potential targets.

Throughout 2016, the Unit continued its mission of implementing and optimising quantitative proteomic strategies. More specifically, we have introduced a new fractionation method using high pH reverse phase micro columns, which minimises sample loss and thus is highly suitable for low amounts of material. We used this approach to post-fractionate samples enriched in phosphopeptides, substantially increasing the number of identifications. This workflow was used to determine phosphorylation dynamics upon activation of WT and kinase-mutant platelets (in collaboration with the Cell Division and Cancer Group), as well as to identify potential substrates of CDK8 involved in the establishment of ground state pluripotency (in collaboration with the CNIO Tumour Suppression Group). More recently, in collaboration with the Metabolism and Cell Signalling Group at the CNIO, we also used phosphoproteomics to better understand the molecular mechanism of the mTOR pathway. Together with the CNIO Genomic Instability Group, we are using a recent approach, named Thermal Proteome Profiling (Savitski *et al.*, see FIGURE), to identify protein targets of certain inhibitors (e.g. target deconvolution). We have also performed several AP-MS/MS experiments for different proteins (STAG1, STAG2, PDS5A, PDS5B) belonging to the cohesion complex (with CNIO's Chromosome Dynamics Group). Likewise, we have identified a large protein network (more than 300 proteins) that interacts with the RNA pol II complex (in collaboration with the Tumour Suppression Group). Over the last few years, the analysis of the protein content of exosomes has received great interest in the context of metastasis and the pre-metastatic niche. Along this line, we are conducting several proteomic analyses of exosomes from different origins in collaboration with CNIO's Microenvironment and Metastasis Group, the Gastrointestinal Cancer Clinical Research Unit and the Melanoma Group. ■

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HISTOPATHOLOGY CORE UNIT

Alba De Martino
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**Plan de Empleo Joven* (Youth Employment Plan)

"The Histopathology Core Unit participates in several External Quality Assessment Schemes, such as NordiQC and UKNEQAS, which independently evaluate the quality of the techniques performed at the Unit. In 2016, several protocols developed by the Unit were incorporated into the Best Methods section by the UKNEQAS."

OVERVIEW

Pathology is devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs that underlie disease. By using molecular, immunological and morphological techniques, pathology serves as the bridge between the basic sciences and clinical medicine.

The Histopathology Core Unit offers knowledge and expertise through a full range of services encompassing paraffin embedding and cutting, as well as the construction of tissue microarrays (TMAs). We also provide our users with histochemical stains upon request, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, and *in situ* hybridisation (ISH) (ALU sequences for mouse xenograft characterisation). Furthermore, the Unit offers other services, such as laser-capture microdissection; slide digitalisation for brightfield, polarisation light and fluorescence; image analysis; and quantification. The Unit collaborates with researchers at any stage of their career in the histological characterisation of phenotypically relevant animal models of disease, thus providing them with the Pathology expertise required for the success of their research projects.

RESEARCH HIGHLIGHTS

In 2016, the Unit beat previous records in the Histopathology database with more than 15,000 new entries. This corresponded to about 40,000 requests processed with approximately 37,000 paraffin-embedded blocks; 40,000 histochemical techniques performed; 13,000 routine immunohistochemistry techniques performed (not counting optimisation tests); 7,000 scanning requests for histological slide scanning and image analysis; and 70 requests for laser microdissection.

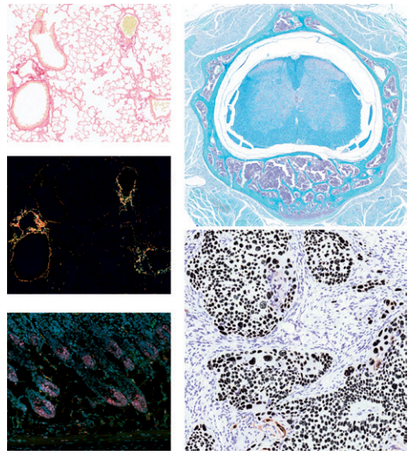

Figure Techniques developed at the Histopathology Core Unit and routinely used by CNIO researchers. Sirius Red, Lung. Brightfield (top left) and Sirius Red, lung. Polarised light (middle left). Immunofluorescence, skin (bottom left). Luxol Fast Blue staining, decalcified spinal cord (top right). Improved ALU II *in situ* hybridisation for mouse xenograft detection (bottom right).

All the developed techniques follow a standardised validation process. In 2016, the Unit added several new antibodies to its portfolio, which includes more than 3,000 tested and 1,000 currently available antibodies that have been optimised for both human and mouse tissue samples. The antibody validation process follows rigorous testing in order to achieve the best possible results and to demonstrate reproducibility between assay runs and between batches. This represents a highly valuable resource for CNIO researchers as well as for the external clinical and research community.

In respect of the importance that our researchers place on quality and reproducibility, our Unit participates in several External Quality Assessment Schemes, such as NordiQC and UK NEQAS, which evaluate the quality of the staining techniques performed at the Unit and in which more than 800 laboratories participate worldwide. In 2016, our Unit scored very high in the evaluated techniques, and several protocols developed by the Unit were incorporated into the 'Best Methods section' of the UKNEQAS Cellular Pathology Technique website (PAS staining and Haematoxylin-Eosin, among others). ■

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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services

"The accreditation of our animal research programme by the AAALAC reflects CNIO's compromise and high level of excellence with respect to the care and use of animal models, which are essential in cancer research."

The CNIO has a state-of-the-art Animal Facility, managed by Vivotecnia Management & Services. The Animal Facility's primary responsibility is the supply, husbandry and quality control of laboratory animals used by the Research Programmes in their experimental protocols. The strict compliance to national, EU and international recommendations regarding the use and care of animals in research is of paramount importance to the CNIO.

The high standards achieved by the CNIO with regards to the use and care of animals for experimentation have been recognised by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC); they granted us full accreditation in October 2016. AAALAC International is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. More than 950 companies, universities, hospitals, government agencies and other research institutions across 41 countries have earned AAALAC accreditation, which is considered one of the top international recognitions in this field.

The CNIO Animal Facility was established to assist researchers in the development and analysis of *in vivo* models. We are currently collaborating with as many as 25 CNIO Research Groups, Sections and Units from different Research Programmes.

Our Animal Facility has the capacity to house 19,000 type IIL cages. Our mouse lines are maintained and bred in the Facility's barrier area, which assures Specific Pathogen Free (SPF) health status through a comprehensive health surveillance programme. Microbiological and environmental parameters in the animal areas are constantly monitored. All mouse strains housed in the barrier are either generated within the barrier or introduced by rederivation. We also have an additional area with a capacity for 1,800 type II cages dedicated for the use of non-replicative strains of adenovirus, lentivirus and retrovirus, as well as for xenograft models. In this area, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are always manipulated in Type II biosafety cabins.

Daily operations and husbandry procedures are highly automated in order to safe-guard our personnel from any associated risks; robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing/filling of cages and bottles, etc. These automated systems maximize the productivity and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility has harboured more than 2,500 genetically modified mouse lines; currently, there are more than 700 genetically modified lines and more than 45,000 live mice. The Facility also provides access to more than 80 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, Tet transactivator strains and others.

The Animal Facility offers the possibility of running a broad number of experimental procedures in the premises, including the use of gamma irradiation, UV light and volatile carcinogenic agents, as well as surgical procedures, some behavioural studies, a non-invasive blood pressure system, and a lab animal monitoring system (Oxylet) that allows measuring a number of physiological parameters for metabolic profiling and phenotyping of mouse models.

Additionally, the monitoring of the mouse models through non-invasive imaging technologies is provided by the Molecular Imaging Core Unit, which has integrated all its image acquisition instruments within the Animal Facility. Likewise, the work of the Transgenic Mice Unit is performed in a laboratory inside the SPF barrier. Finally, the necropsy laboratory is equipped with instruments for the haematological and biochemical analysis of blood and urine, which complement the pathology and clinical diagnostics.

All the work carried out by the Animal Facility complies with both national and EU legislation –Spanish Royal Decree RD53/2013 and EU Directive 2010/63/UE– for the protection of animals used for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed and evaluated by the Research Ethics and Animal Welfare Committee of the *Instituto de Salud Carlos III*, as well as by the Institutional Animal Care and Use Committee (IACUC).

The *Orden* ECC/566/2015 stipulates that all animal procedures are to be carried out by qualified people in the possession of the corresponding accreditation as issued by the competent authority. Currently, the Animal Facility Service is performed by more than 35 qualified persons between care workers, technicians, supervisors and veterinarians involved in the breeding and care of animals, ensuring the welfare of the animals. The Animal Facility offers CNIO's new staff a short course focused on the training of personnel performing work with laboratory animals; this is complementary to the online courses that are a requisite to gain access to the facility.

In line with our commitment to maintain the highest possible standards in regards to animal research issues, the CNIO has joined the Agreement on Openness on Animal Research promoted by the Federation of Scientific Societies in Spain (COSCE), in collaboration with the European Animal Research Association (EARA), which was launched on September 2016. An institutional statement on the use of animals in research can be consulted in the CNIO website. ■

PUBLICATION

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EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

"With a proven track record in the development of advanced compounds for cancer therapy, The Experimental Therapeutics Programme (ETP) is now aligning its capabilities with CNIO's growing research on phenotypic drug discovery. Consequently, we are contributing with state-of-the-art approaches to Target Deconvolution activities."

The current ETP-CNIO pipeline encompasses targeted and phenotypic projects. The following highlights summarise some of our achievement during 2016.

Our most advanced targeted project is dedicated to CDK8 inhibitors. We have selected our first lead, ETP-27, which has yielded positive results in PK-PD studies and has shown early signs of efficacy in a MOLM13 xenograft model after oral administration. Currently, we have embarked on the fine optimisation of the *in vivo* exposure within this series.

Additional targeted projects, focused on Mastl and Haspin inhibitors, are undertaken in collaboration with Marcos Malumbres (CNIO Cell Division and Cancer Group). The Mastl project started with a cell-based screening of several ETP-libraries, where a few 'high-micromolar' hits emerged as potential Mastl inhibitors. However, a direct target engagement experiment to unequivocally identify Mastl as their molecular target was not possible due to the unavailability of isolated active Mastl protein. ETP's Biology Team has been able to set up highly efficient conditions for the purification, isolation and production of 'full length active Mastl kinase'. This important achievement has enabled, for the first time, the biochemical profiling of the identified hits and an additional targeted biochemical screening of ETP-libraries. As a result of these activities, we have identified several families of compounds as biochemical Mastl inhibitors, including ETP-750 with an IC_{50} of around 300 nM. These results shall pave the way for the discovery and development of advanced Mastl inhibitors in the near future. It is worth mentioning that the production of the protein was carried out by the CNIO Crystallography and Protein Engineering Unit.

Using the same cell-based assay, Malumbres' Group identified compounds that efficiently produce 'mitotic cell death', a new avenue for cancer therapy. The knowledge of the biochemical profile of these hits obtained by ETP has contributed to the identification of Haspin kinase as the target responsible for the observed phenotype. Currently, ETP is working on the discovery of Haspin inhibitors where we have already identified highly potent compounds in the low-nanomolar range.

We are also collaborating with P. Carmeliet (VIB-KU Leuven, Belgium) for the discovery of novel inhibitors of a particular enzyme in the field of vascular normalisation. We have carried out a screening campaign and we are now working on hit generation activities to identify catalytic inhibitors of this enzyme and their associated intellectual property.

Phenotypic screenings have proven to be advantageous for the discovery of innovative molecular targets and modulators, as well as to establish their link with disease. Nevertheless, the molecular target responsible for a desired phenotype needs to be identified and this deconvolution phase implies an extra step of complexity in the process. As mentioned above, we are working on several phenotypic projects:

ETP collaborates with Manuel Serrano (CNIO Tumour Suppression Group) in a project dedicated to the discovery of novel targets and modulators against Cancer Stem Cells (CSCs). We have been focusing on target deconvolution activities around previously identified hits that have the ability to selectively kill CSCs and to inhibit the tumour-initiating capacity of pancreatic CSCs. Our Medicinal Chemistry Team has designed and synthesised chemical probes around those molecules by using 'minimalist linkers'. These chemical fragments bear a photoactivatable group to achieve crosslinking of the modified hits with targeted proteins, and a special chemical group to perform 'click chemistry' that is useful to attach the 'cross-linked complex' to a reporter tag for imaging and/or pull-down experiments. The treatment of cells and cell lysates with these molecules has enabled the identification of a target candidate, which is currently under additional validation studies.

ETP is collaborating with Maria A. Blasco (CNIO Telomeres and Telomerase Group) in the discovery of TRF1 inhibitors, a project that also requires a 'target deconvolution' phase. During this year, we focused our attention on a series of TRF1 inhibitors with an unknown and perhaps innovative mechanism of TRF1 modulation. We have profiled the main hit, ETP-946, against large panels of enzymes and receptors representing more than 600 targets. Among them, we have retrieved 4 potential candidates, which are currently undergoing validation studies. The chemical modification of ETP-946 with 'minimalist linkers' has yielded potential affinity probes that are currently under evaluation. Last but not least, ETP has set up a 'thermal stability assay' to study the stabilisation of hTRF1 overexpressed in HEK293 cells in the presence of ETP-946 and analogues. These experiments will inform us about direct interactions of those compounds with TRF1.

Finally, I'd like to mention that other 'phenotypic exploratory projects' are also currently at the screening phase; these are undertaken in collaboration with the CNIO Researchers Manuel Valiente, Óscar Fernández-Capetillo and Massimo Squatrito.

MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

Staff Scientists
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Graduate Student
Francisco J. García

Technicians
Ivan Arribas (PEJ-L)*, Carmen Fernández (PEJ-L)*, Virginia Rivero (until February) (TS)**, Sandra Sanz (PEJ)***

Plan de Empleo Joven-Licenciado (Youth Employment Plan-Graduate)
***Titulado Superior* (Advanced Degree)
****Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

The Medicinal Chemistry Section is part of the Experimental Therapeutics Interdisciplinary Programme that is dedicated to early Drug Discovery activities in the oncology field. Our mission is to discover and to develop new anticancer agents based on novel hypotheses and targets generated by CNIO's Basic Research Groups, and to do so in close collaboration with these groups. Medicinal chemists are responsible for the design, the preparation and the optimisation of compounds for biological evaluation, as well as for the generation of Structure Activity Relationships (SARs) and the development of novel lead compounds with *in vivo* activity in appropriate animal models.

"We have successfully designed and synthesised affinity-based chemical probes used in imaging and pull-down/LC-MS/MS analysis experiments in order to discover novel targets/ inhibitors of CSC proliferation, in collaboration with the CNIO Tumour Suppression Group."

Recently, and due to the increasing number of projects related to the cell phenotype, our Programme is entering the chemical biology field in order to contribute to the deciphering of the precise protein target or mechanisms of action responsible for the observed phenotypes. In this regard, we chemically modify the active molecules into affinity probes – compounds with appropriate linkers that form either reversible or irreversible complexes with their targets inside living cells and/or cell lysates – that enable us to directly identify the target/s by extracting them via pull-down experiments, followed by mass spectrometry resolution and cellular localisation experiments using imaging techniques.

RESEARCH HIGHLIGHTS

During 2016, our Section was involved in several projects at different phases of the drug discovery process, among them:

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

In this funded project (grant no. SAF2013-44267-R), we have identified lead compound ETP-27, which has served to demonstrate in vivo Proof of Concept (PoC) for CDK8 inhibition in cancer with positive preliminary results. This chemical series was protected by a patent application that reached PCT level in August 2016 and has been exemplified during 2016. Additionally, crystallographic studies with ETP-27 and some analogues from this chemical series have been performed by the CNIO Crystallography and Protein Engineering Unit confirming the expected binding mode of the molecules in the catalytic site.

Currently, we are dedicated to the fine optimisation of the lead compound, trying to increase the oral exposure levels *vs* time. Modifications in the molecule to reduce *Clearance* or to increase the *Volume of Distribution* by introducing basicity, for example, are being considered. The final objective of the project is to obtain an advanced product that is ready for preclinical regulatory development and further clinical studies.

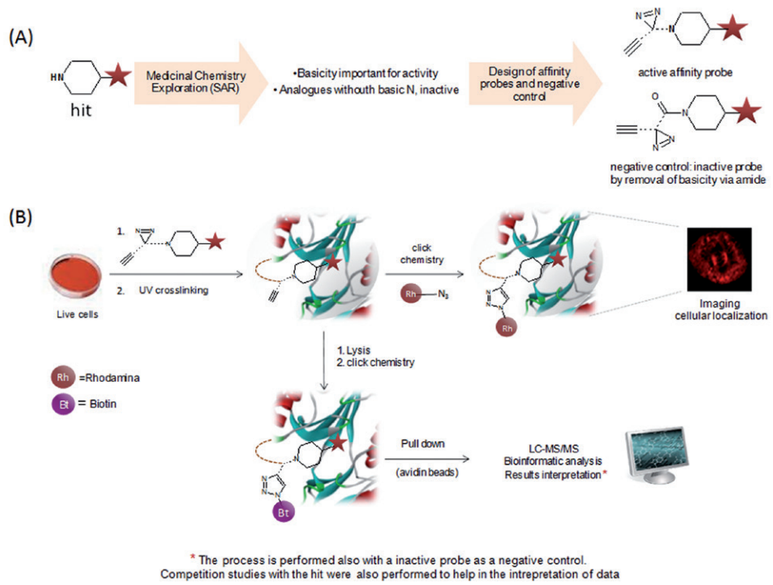

Figure 1 (**A**) Affinity probes (ETP) for target identification of inhibitors of CSC proliferation. After SAR we identified a particular basic fragment that is essential for activity. Affinity probes were synthesised; they included this fragment and a minimalist linker in their design. As a negative control, we synthesised the inactive probe, in which the basicity was removed. (**B**) Overall workflow (performed by Tim Cash, from the Tumour Suppression Group). Incubation of the affinity probe in cell culture (living cells and/or cell lysates) followed by photoirradiation to capture close proteins. Subsequent click chemistry with Rhodamine-N_3 and/or Avidin Beads allows for imaging and pull-down experiments.

Microtubule-associated serine/threonine protein kinase-like (MASTL) inhibitors

A chemical exploration around the hit identified in the biochemical High-Throughput Screening (HTS) with active full length human MASTL protein has been set up; the aim is to define the pharmacophore required for Mastl activity and to increase the activity of current hits, in order to obtain more potent inhibitors that can be used in biological assays as tool compounds. We will then use this information for the design of novel Mastl inhibitors, including *Intellectual Property* in their structures.

HASPIN inhibitors

Haspin inhibitors that produce a rapid and efficient mitotic cell death have been identified. We have started a chemical programme in order to explore the current hits and also to generate novel compounds. We are exploring 2 chemical series with haspin inhibitory activity in the low nanomolar range, but also with several main off-targets. Synthesis of analogues has allowed us to learn how selectivity can be achieved without affecting haspin activity. Crystallographic studies of 2 hits from different chemical series have been performed by the CNIO Crystallography and Protein Engineering Unit.

Kinase X* inhibitors

We finalised the Hit-to-Lead phase in collaboration with VIB (Belgium) and have obtained a novel chemical series, in which we identified a potent compound with controlled selectivity and oral bioavailability. The compound has been delivered to VIB to be characterised in different *in vivo* studies.

Kinase Y* inhibitors

We are collaborating with VIB for the generation of novel inhibitors of a particular kinase. Several reference compounds and analogues have been synthesised in order to help with the validation studies. After analysis of the hits from an HTS campaign, we have concluded that they were not good enough as starting points for further exploration. Therefore, a hit generation plan has been designed and is currently ongoing.

Inhibition of Cancer Stem Cell (CSC) proliferation

In a collaborative project with the CNIO Tumour Suppression Group, we identified several hits that are able to modulate CSC proliferation, stemness and, at sublethal doses, inhibit the tumour

*confidential

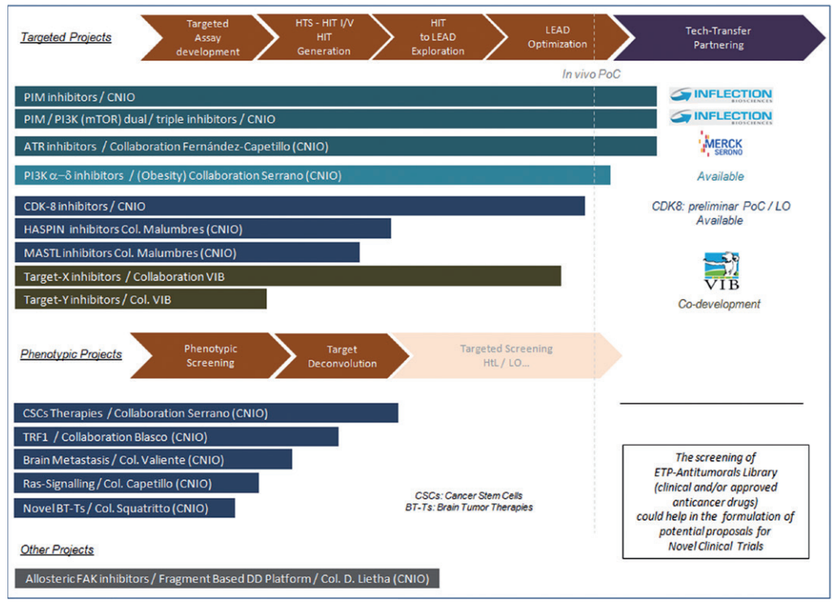

Figure 2 Experimental Therapeutics Programme Pipeline.

initiating capacity of pancreatic CSCs. In order to decipher the target behind the observed phenotype, we have performed chemotype searches linking the structure with potential targets and profiling in broad panels of enzymes and receptors. As a result of the chemical exploration, we have found that the presence of basicity in these molecules is essential for activity. Based on this discovery, we have been able to successfully synthesise different affinity probes by adding a 'minimalist linker' to the basic centre, retaining the required cellular activity. These modified molecules, after treatment with cells or cell lysates and photo-irradiation, can covalently capture their binding proteins in a distance-dependent manner. The subsequent click chemistry reaction of the terminal alkyne group of the linker with different reporters (i.e. rhodamine-N_3 or biotin-N_3) enables, via pull-down experiments, the identification of potential cellular protein targets of the drug, as well as imaging-based determination of their cellular localisation. Because these experiments require the use of appropriate controls, we have also been able to synthesise inactive analogues by removing the basicity of the hit and have performed similar pull down and imaging experiments. The already identified candidate targets are going through a validation process.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group. After a screening campaign, using a cell-based assay to measure the removal of TRF1 from telomeres, we have identified several hits, among them ETP946. Our main objective during 2016 has been both the deconvolution of the molecular target behind the observed effect using this hit, as well as the chemical exploration to increase SAR knowledge within this chemical series. In the deconvolution studies, we carried out several chemotype searches and an extensive profiling of the compound against a broad range of enzyme and receptor panels. From these studies we have identified 4 candidate targets that are currently under study. Also, we have generated the first affinity probes by the introduction of appropriate linkers in the molecule that will allow for reversible or irreversible interactions with their molecular targets. Currently, we are testing these probes to address their TRF1 modulation in cells. If they show activity they will be very useful for further imaging localisation and pull-down experiments in order to identify the responsible molecular targets for TRF1 modulation, including TRF1 itself. ■

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- Aragoneses-Fenoll L, Montes-Casado M, Ojeda G, Acosta YY, Herranz J, Martínez S, Blanco-Aparicio C, Criado G, Pastor J, Dianzani U, Portolés P, Rojo JM (2016). ETP-46321, a dual p110α/δ class IA phosphoinositide 3-kinase inhibitor modulates T lymphocyte activation and collagen-induced arthritis. *Biochem Pharmacol* 106, 56-59.

PATENT

- Pastor J. *et al.* (2016). New Compounds. *PCT/GB2016/052641.*

BIOLOGY SECTION

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**Titulado Superior* (Advanced Degree)

***Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

In the Experimental Therapeutics Programme, we are working on both targeted and phenotypic-based drug discovery projects. In the targeted projects, the Biology Section is devoted to the biochemical, cellular, and *in vitro*/*in vivo* pharmacological characterisation of the compounds synthesised within the Programme. Our aim is to obtain novel anticancer agents with optimised profiles that are able to demonstrate *in vivo* proof of concept in animal models of cancer.

In phenotypic-based projects, we perform target engagement and target deconvolution tasks using label-free techniques and chemical probes, respectively. The cellular thermal shift assay is a label-free technology that enables drug binding studies to target proteins in their relevant cellular contexts. Alternatively, we use engineered chemical probes to deconvolute the possible targets of our molecules. For that purpose, we first have to confirm that they behave similarly to the parent compound in the phenotypic screening.

These molecules bear a linker with functionalities that permit their crosslinking with targeted proteins and the attachment of reporter tags for imaging and pull down experiments in cells and cell lysates. Thus, through immunofluorescence assays, we can determine the cellular localisation of the complex of the chemical probe with its targets and, by pull down experiments, using active and inactive chemical probes also in competition with the parent compound followed by mass spectrometry analysis, we can identify candidate targets for the observed phenotype.

Furthermore, in exploratory screening projects carried out in collaboration with other CNIO Groups, we provide support by preparing customised compound assay plates from our ETP-libraries, adding the compounds using automated liquid-handling instruments that allow rapid, accurate, and reproducible compound dispensing and assay plate setup. All this instrumentation is integrated in a platform that allows sample tracking and recording.

"Upon production of active human full length MASTL protein, with the support of the CNIO Crystallography and Protein Engineering Unit, we have set up a biochemical assay that has allowed us to perform both target engagement validation with the hits obtained from a MASTL phenotypic screening, and a High-Throughput screening where we have identified a MASTL inhibitor with an IC_{50} of 300nM among other hits."

RESEARCH HIGHLIGHTS

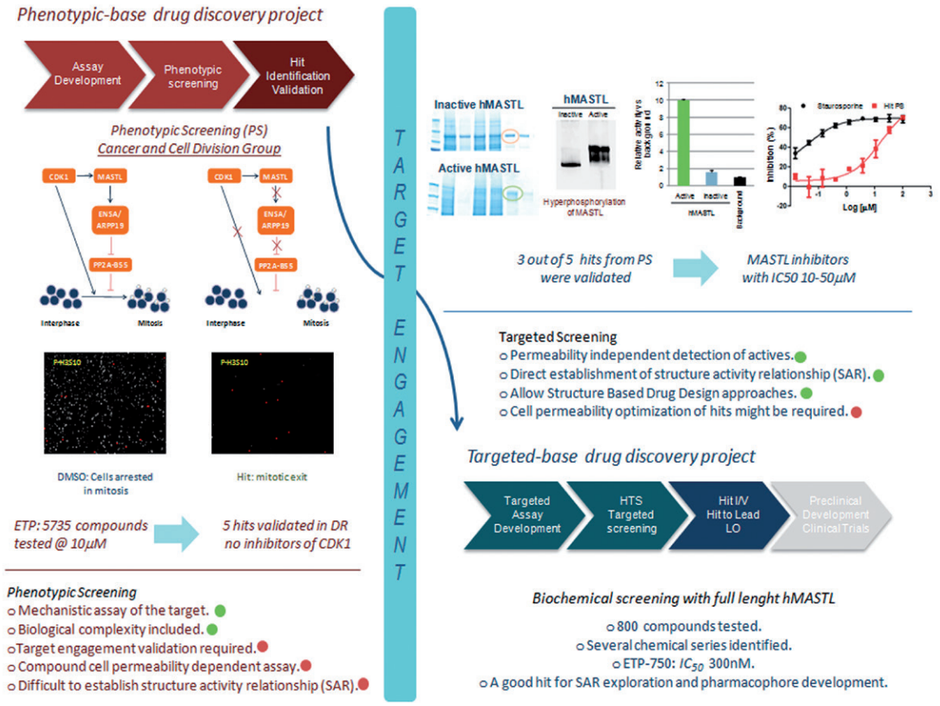

Figure From phenotypic- to target-based drug discovery; the MASTL case project. An HTS screening, together with a phenotypic screening designed to identify compounds that allow exit from mitosis of mitotically arrested cells, was used to identify potential MASTL inhibitors. Five hits were identified. To perform target engagement we produced and purified hMASTL full length protein and set up a biochemical assay. We confirmed 3 hits as MASTL inhibitors with an IC_{50} between 10 and 50 μM. We performed a screening of 800 compounds with the biochemical targeted assay identifying a MASTL inhibitor with an IC_{50} of 300nM, which is a good starting point for SAR exploration and pharmacophore development.

During 2016, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

This is a funded project (grant no. SAF2013-44267-R). We have identified ETP-27, a highly selective picomolar CDK8/CDK19 inhibitor with picomolar cellular inhibition of P-STAT1-S727. The main off-target identified is Haspin; ETP-27 demonstrates a cellular selectivity of > 30 fold for CDK8 vs. Haspin. This compound shows good solubility, permeability, metabolic stability in human and rat microsomes that is moderate in the mouse, and no alerts in terms of CYP-P450 inhibition and hERG binding, as well as important toxicity related parameters. Moreover, the compound is orally bioavailable as observed in pharmacokinetic (PK) studies in mice; in mechanistic PK-PD studies, modulation of biomarker PSTAT1-S727 was observed between 1 and 4 h after oral treatment. ETP-27 has shown promising results in an efficacy study in MOLM13 xenografts, where it demonstrated a 50% tumour growth inhibition after 10 mg/kg twice-a-day treatment over 16 days. Our next steps will focus on th*e in vivo* testing of new compounds resulting from the fine optimisation of ET-27 in order to achieve an even higher *in vivo* exposure.

Microtubule-associated serine/threonine protein kinase-like (MASTL)

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We have previously reported the production of active human full length MASTL protein to run biochemical assays. We used it to validate the hits coming from the phenotypic screening and to perform a biochemical screening of our ETP-640 library. In a single point screening assay, with the cut-off value set at 40% inhibition, we achieved a hit rate of 0.02. Hits were confirmed in a dose response assay and a number of analogues were tested. We have identified a 300nM hit, which is a good starting point for the exploration of Structure-Activity-Relationships (SAR) and for pharmacophore development. This information will be used later on for hit generation and subsequent hit-to-lead (HtL) exploration of novel inhibitors.

Kinase X

This project is a collaborative undertaking with VIB (Belgium). We have identified a lead compound with nanomolar inhibition activity for the target, good solubility, permeability and high selectivity against a panel of 456 kinases. Moreover, the compound is orally bioavailable, well tolerated in mice and, in a distribution study, was detected in several tissues for up to 8 hours. This lead compound is being used at VIB for *in vivo* validation studies.

Kinase Y

In this second project, also in collaboration with VIB, we have characterised at the biochemical level, 30 compounds synthesised in hit generation activities.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres has been used to test 72 compounds that include analogues of a second hit, ETP-946, identified in the initial screening; chemical probes derived from it have been generated. Moreover, we have set up a cellular thermal shift assay with over-expressed human TRF1 to validate if this series of compounds interacts directly with TRF1, and with potential to serve as a platform for identifying compounds that directly interact with TRF1.

Cancer stem cells (CSC) and gluconeogenesis

These projects are carried out in collaboration with the CNIO Tumour Suppression Group. For the CSC project, we collaborated in the deconvolution studies of the identified hits, evaluating their activity in kinase and receptor panels, as well as providing technical support for the assays using chemical probes. For the gluconeogeneis project, we have performed pharmacokinetic profiles of the hits obtained in the screening; the most promising compound has been tested *in vivo* by our collaborator.

Brain metastasis screening

The CNIO Brain Metastasis Group has developed an *ex vivo* assay to search for drugs that kill human brain metastasis in mice. ETP-Biology has provided support in running the experiments and also to validate the *in vitro* screening results obtained with the approved or in clinical trial ETP-antitumour library. Two classes of drugs are under further characterisation.

Focal adhesion kinase (FAK)

This project is undertaken in collaboration with the CNIO Cell Signalling and Adhesion Group. We have set up a biochemical assay with purified protein produced by our collaborator, using both the catalytic domain alone and the full length protein. We have tested 80 compounds coming from a virtual screening analysis; the hit rate obtained was of 0.21 with a cut off value set at 90% inhibition. Five of the compounds with an IC_{50} below 500nM have been selected for crystallisation studies.

Support to other CNIO Groups

We have given support to Manuel Serrano's Group by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), in tumour and host-mouse plasma samples, the levels of a standard-of-care-drug administered in nanoparticles in order to improve the delivery of chemotherapeutics to their site of action. ■

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PATENT

- Pastor J. *et al.* (2016). New Compounds. *PCT/GB2016/052641.*

CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

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**Titulado Superior* (Advanced Degree)

SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer immunometabolism. Our Section is funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules that regulate the metabolism of malignant cells, with the objective of killing them, either directly, acting synergistically with other anti-tumour agents, or activating the anti-tumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of the metabolic reprogramming regulated by oncogenes like *RAS*, as well as the characterisation of the metabolic status of tumours (Cerezo A. *et al*, February 2016; Keystone symposium meeting on 'New Frontiers in Understanding Tumor Metabolism' in Banff, Canada). For this purpose, we have developed a series of biochemical and cell-based assays exploiting advanced techniques such as extracellular flux analysis (Seahorse technology), NMR and metabolomics. Finally, each target goes through an *in vivo* validation process using xenografts, allografts and mouse models developed at the CNIO; this process includes the use of non-invasive *in vivo* imaging technologies, as well as the immunohistochemical characterisation of tumours for different metabolic, immune and tumour markers.

SCIENTIFIC CONTEXT

The observation of an altered metabolic state in cancer cells dates back to the early 20th century when Otto Warburg observed that cancer cells preferentially utilise glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels (Warburg 1956), a phenomenon known as the 'Warburg effect'. Warburg argued that, 'this altered metabolic state was the underlying cause for cancer'.

The molecular mechanisms driving an altered tumour metabolism have only recently begun to be understood as a result of large-scale genomic sequencing as well as advances in metabolomic profiling technologies. Recent studies have shown that many oncogenes, including Myc and Ras, impart an altered metabolic phenotype in cancer cells through the regulation of genes involved in central metabolic pathways such as glycolysis, fatty acid metabolism, oxidative phosphorylation, and the one carbon pool.

Cellular metabolism is a fine tuned process; tumours may rely heavily on specific metabolic pathways to obtain their energy while using other pathways to grow in order to give tumour cells a growth advantage. This situation may leave tumour cells in a frail position under certain treatments or circumstances, while normal cells may be able to compensate and survive (FIGURE, C). Furthermore, the high requirements of nutrients and other soluble factors, and the release of metabolites with immunosuppressive properties, together with the hypoxic conditions found in tumours, creates a 'non-friendly' microenvironment for an anti-tumour immune surveillance, while facilitating the growth of other tumour-promoting cells such as the stroma and myeloid cells (FIGURE A,B). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes involved in this process. Checkpoint-blockade immunotherapy has been one of the most exciting advances made in cancer treatment in recent years. Metabolic interplay in the local microenvironment can mediate T cell differentiation and function. 'Checkpoint-blockade' antibodies can also influence cellular metabolism. Finally, recent clinical trials have shown that combination immunotherapy based on immune checkpoints blockade, provides even higher response rates than either approach alone. ■

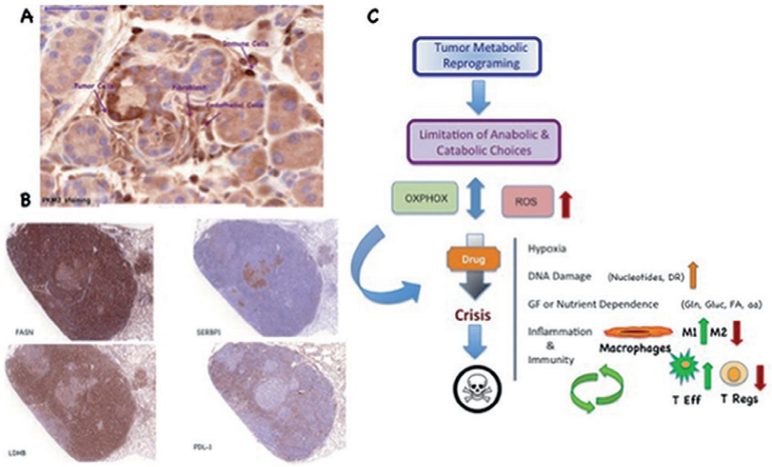

Figure (**A**) Immunohistochemical analysis of metabolic enzyme PKM2, showing the tumour microenvironment in a PANIN-3 from a Pancreatic Adenocarcinoma model *Elas-tTA/tetO-Cre;K-Ras(+/LSLG12Vgeo);P53(lox/lox):* PDAC (Carmen Guerra & Mariano Barbacid). In addition to the tumour cells, there are fibroblasts, endothelial, myeloid cells and immune cells that may be facilitating tumour growth, while the immune cells involved in the anti-tumour immune response are absent or inhibited. (**B**) Immunohistochemical analysis showing that the expression of the immune checkpoint ligand PDL1 correlates with the expression of specific tumour profiling markers (SERBP1, Ambrogio *et al.*, 2016) and certain metabolic markers (LDHB and FASN) in a lung adenocarcinoma derived from a *KrasLSLG12Vgeo;Trp53lox/lox* (Chiara Ambrogio & Mariano Barbacid). (**C**) Cartoon depicting a strategy to control tumour growth through the regulation of specific metabolic targets. Tumours may rely heavily on specific metabolic pathways to grow and evade immune surveillance, as well as to obtain energy. This rapid growth also results in increased DNA damage, either through an increase in the production of ROS or due to replication stress. This situation leaves tumour cells more vulnerable to certain metabolic interventions as well as increasing the anti-tumour response of the immune system.

CNIO - LILLY EPIGENETICS SECTION

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**Titulado Superior* (Advanced Degree)

"Our goal is to identify epigenetic events that contribute to tumourigenesis and that might be susceptible to modulation by therapeutic agents."

SCOPE OF THE CNIO - ELI LILLY PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer epigenetics. Our Section is funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules that are able to modulate the epigenome of malignant cells, and ultimately block the growth and spread of tumours. Potential targets (FIGURE) are being validated *in vitro* and *in vivo* using animal models developed at the CNIO. Furthermore, we are currently setting up biochemical and cell-based assays with the aim of understanding the mechanism of action of such targets at the molecular level.

SCIENTIFIC CONTEXT

Recent studies have shown that the alterations that take place in cancer cells not only occur at the DNA sequence but also at the level of the epigenome. Eukaryotic DNA is wrapped around histone proteins to constitute chromatin, which plays fundamental structural and regulatory roles. The epigenome consists of chemical changes in both DNA and histones that can be inherited through cell division and are controlled by the action of a large set of epigenetic regulators that possess enzymatic activity. Ultimately, DNA and histone modifications control the level of chromatin condensation, which in turn regulates the accessibility of transcription factors to the chromatin and, therefore, gene expression.

During the past few years several studies, including our own, have suggested that the deregulation of the chromatin-modifying machineries can lead to aberrant gene expression causing cancer and other human diseases. The epigenome is regulated in a highly dynamic fashion by the coordinated action of regulators that are able to write, erase and read histone and DNA modifications (FIGURE). Thus, contrary to genetic mutations, epigenetic aberrations can be reversed by targeting the appropriate epigenetic regulators. Indeed, drugs targeting DNA methyltransferases and histone deacetylases have successfully demonstrated anticancer properties and are currently used in the clinic. Therefore, identifying the molecular function of critical epigenetic regulators and their complex relationship with the cancer epigenome, as well as the development of small molecular inhibitors of their activities, hold great promise for cancer therapy (FIGURE). ■

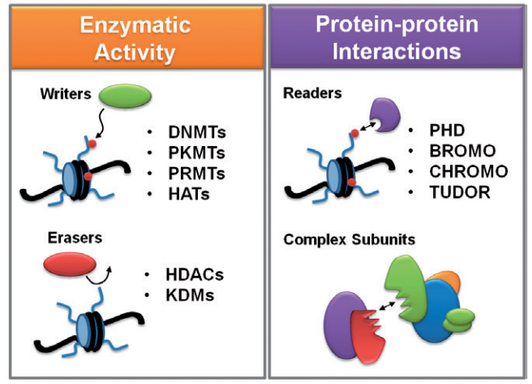

Figure Strategies for targeting epigenetic regulators. The enzymatic activities of DNA methyltransferases (DNMTs), protein lysine methyltransferases (PKMTs), protein arginine methyltransferases (PRMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), or lysine demethylases (KDMs), are amenable to inhibition by small molecules. Additionally, molecular probes can be used to block the interactions of readers containing PHD, Bromo, Chromo or Tudor domains with modified histones, or to disrupt the interaction between critical core components of chromatin-related complexes.

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TECHNOLOGY TRANSFER AND VALORISATION OFFICE

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Technician Rocío Manzano (Pej-L)*
**Plan de Empleo Joven- Licenciado* (Youth Employment Plan-Graduate)

The CNIO's Technology Transfer and Valorisation Office at CNIO (TTVO) acts as a bridge that connects the research results generated by CNIO's scientists to the Centre's commercial partners, thereby helping to ensure that new products are developed for the public interest. Without this bridge the public, and in particular cancer patients, would not benefit from the full potential of the discoveries made by CNIO scientist. Close alignment of the technology transfer expertise with CNIO's innovation strategy priorities serves to optimise the transfer of the research materials and novel scientific discoveries to the health sector. The TTVO performs an all-round management and follow-up of all aspects, including relationships with stakeholders, in order to ensure the appropriate intellectual property protection and commercial viability of the research results generated by CNIO's scientist. Additionally, the Office proactively follows up on the progress of scientific activity at the CNIO in order to identify projects with high transfer potential; it plays a managerial and advisory role in the entire process in order to ensure the efficient use of the patent system, identify appropriate commercial partners for a timely development of technologies, negotiate licenses, monitor the activities of licensees regarding the achievement of milestones, and the payment of royalty fees.

In this context, the TTVO Office managed the execution of 182 agreements related to the CNIO's intellectual property in 2016. About 70% of these were of international nature, which is a reflection of the international relevance of the scientific research at the CNIO. Many pertain to the exchange of research materials and data with the external scientific community and health based companies. Other involved Research Collaboration Agreements (RCAs). RCAs are an important mechanism used by CNIO for successful cooperation with industry; they ensure that the risks and benefits related to research results and intellectual property are shared. In 2016, the industry investment secured via RCAs totalled 3.8 Mio euros, nearly 10% of CNIO's yearly budget.

Public-private partnerships are potent tools for the valorisation of research results whereby scientific knowledge gets converted into diagnostic and therapeutic products and services. Valorisation of CNIO's research results through alliances with industry is not just about 'money', but also about the impact that can be created for the public benefit and, in particular, for cancer patients. In 2016, a project based on the findings of CNIO scientists that boosting levels with nicotinamide riboside (NR) prevented and abolished aggressive tumour formation received nearly 1 Mio in funding from the MINECO *Retos-Colaboración* programme. The project was focussed on the development of a new NR-based therapy that could be used for Hepatocellular Carcinoma and other tumours.

"Valorisation of CNIO's research results through alliances with industry is not just about 'money', but also about the impact that can be created for the public benefit and, in particular, for cancer patients."

The inventions of CNIO scientists that have the potential to be transferred to the market are protected through patents. The CNIO's patent portfolio is composed of 26 families. Licensed patents are managed by our licensees and the rest is managed by the TTVO Office. Patents and unpatented research tools are licensed to increase their availability to the scientific community, as well as to create opportunities for our business partners and to provide a financial return on public investment. The royalty fees collected from licenses in 2016 exceeded 650,000 euros. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 44 inventors, about 10% of CNIO's researchers, have contributed and benefited from this achievement.

Fostering an innovation culture among our scientists is one of our priorities. With the support of *Fundación Banco Santander*, we uphold our agreement with the prestigious IE Business School, through which many of our investigators – 3 new in 2016 – have already obtained training in market-oriented innovation strategies. Finally, these achievements stand testament to the excellence and hard work of the CNIO scientists and to CNIO's unwavering encouragement of innovation and technology transfer activities.

PRIVATE SPONSORS

"We take this opportunity to express our thanks and appreciation to all our sponsors and donors for the generous support that we received from them in 2016. They play an inherent role in our present and future successes."

One of the ***Fundación "la Caixa's"*** main goals is to support an innovative programme aimed at fostering international fellowships in order to attract the most outstanding students from the international arena to obtain their doctoral degrees at accredited *'Severo Ochoa'* Centres of Excellence. This acclaimed *"la Caixa"*-Severo Ochoa International PhD Programme assures highly competitive standards by guiding exceptional students towards a career in oncology research; a basic principle is that the selection process is not to be limited to Spanish students only but also includes international students. During 2016, 2 pre-doctoral students received one of these internationally recognised fellowships. The ***Fundación "la Caixa"*** also helps finance our most prominent international conferences, the CNIO-"la Caixa" Foundation Frontiers Meetings.

Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. *Fundación CRIS* generously supports 4 research groups at the CNIO: the Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos; the Breast Cancer CRU, headed by Miguel Quintela; and the H12O-CNIO Haematological Malignancies CRU, led by Joaquín Martínez-López. These Groups focus on the translation of advances in cancer research into improvements in patient care.

The ***Fundación Seve Ballesteros*** is a private not-for-profit institution focused on securing, financing and promoting research projects centred on brain tumours. *Fundación Seve Ballesteros* supports the Seve Ballesteros Foundation – CNIO Brain Tumour Group, headed by Massimo Squatrito, since 2012. This Group focuses on the identification of markers for brain tumours as its principal activity.

The ***Fundación Banco Santander*** funds the *Banco Santander* Foundation – CNIO Fellowships for Young Researchers. These fellowships are aimed to support highly talented and motivated young scientists who trained in the UK/USA and wish to pursue their postdoctoral training at the CNIO. One young scientist, Ana Ortega, who came from the Sloan Kettering Institute for Cancer Research in New York, joined the CNIO thanks to a *Banco Santander* Foundation-CNIO Fellowship in 2016. Additionally, thanks to the support of the *Fundación Banco Santander*, a group of 3 young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.

The ***Fundación Marcelino Botín*** and the ***Banco Santander*** are committed to supporting scientific research and knowledge transfer from academia to the market through science programmes; this transfer is regarded as one of the main driving forces for Spain's economic and social development. These 2 well-recognised organisations collaborate with the CNIO in this regard by supporting the research groups led by Manuel Serrano, Maria A. Blasco and Óscar Fernández-Capetillo.

The ***Fundación Jesús Serra-Catalana Occidente*** continues to fund the Visiting Scientists Programme that was established to support prestigious international professors for short stays at the CNIO. The beneficiary of the *Jesús Serra* Foundation's Visiting Scientist Award in 2016 was Patrick Sung, Professor of Molecular Biophysics and Biochemistry and of Therapeutic Radiology, Yale University School of Medicine in New Haven (USA).

AXA Research Fund (ARF), a global initiative of scientific philanthropy run by the insurance group AXA, awarded an AXA-CNIO Endowed Permanent Chair in Molecular Oncology to Mariano Barbacid as part of its 2011 call.

OTHER SPONSORS

The Centre also benefits from generous support from private institutions, companies and foundations, as well as via external fundraising from local associations that are equally dedicated to the battle against cancer: ***Fundación Juegaterapia, Fundación Investigación Biomédica Hospital Universitario 12 de Octubre, Fundación Banco Sabadell, Asociación Española Afectados por Sarcoma (AEAS), Fressia Group, Compañia Logística de Hidrocarburos, Asociación para la Investigación y Formación Neoplásica (ASIFEN), ASISA Vida, French Embassy , AVON Cosmetics S.A.***

And last but not least, we would also like to extend our heartfelt thanks to all **'CNIO Friends'** donors, sponsors and benefactors who – with their generous donations to support cancer research at the CNIO – have ensured the continuation of our research endeavours throughout 2016.

Communication

COMMUNICATIONS

NURIA NORIEGA Head Of Communications

Communications Officer (until April)
Vanessa Pombo

Communications Officer **Cristina de Martos (since April)**

This year, yet again, CNIO's discoveries and activities were prominently featured in various domestic and international media outlets. In the printed and digital press the CNIO exceeded 2,600 media mentions; it is clear that the CNIO is being brought closer to the public eye, thereby increasing awareness of cancer research and slowly interweaving it into our national culture.

"At the CNIO, we want to give science and research the strong voice they deserve as they are our true means of coping with cancer."

One of the most widely commented articles in 2016, published in the prestigious *Science* journal in November, concerned cell reprogramming; it was published under the title *Tissue Damage and Senescence Provide Critical Signals for Cellular Reprogramming in vivo*, and was authored by the researchers Manuel Serrano and Lluc Mosteiro, from the CNIO Tumour Suppression Group. The discovery, which places the focus on tissue damage as a tool for cellular reprogramming and for the potential regeneration of tissue, was featured in major media outlets, including the front page of the daily *El Mundo*, as well as receiving radio and TV coverage.

A major milestone in 2016 was the agreement that CNIO established with the Spanish Radio and Television Corporation (RTVE) on the occasion of the World Cancer Day, which took place on 4 February. Thanks to this collaboration, the Spanish public TV and radio broadcasting stations featured the voices of CNIO researchers and the latest developments in the oncology field.

As one of the top leading Cancer Research Centres in the world, the CNIO also hit the headlines beyond our borders. In 2016, the CNIO submitted 26 press releases to the global news service, EurekAlert! Throughout the year, these stories received nearly 83,000 hits from around the world and were taken up by prestigious international media such as the BBC, The Guardian, The Scientist or Scientific American.

Our social networks are consolidating their communities. By December 2016, our Twitter channel had over 10,842 followers, with whom we keep an ongoing and valuable dialogue via the platform. The 'CNIO Friends' social media has also become more consolidated; in December 2016, our Facebook page dedicated to this philanthropic initiative reached over 33,800 followers, highlighting, once again, the solidarity of our society and its growing interest in cancer research and the advances that can help fight the battle against cancer.

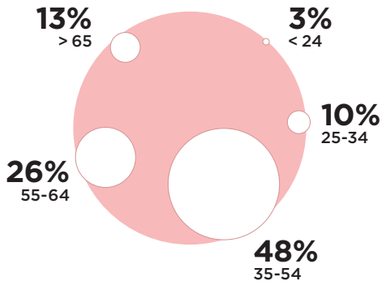
It's another joy for us to mention that in May, the 'CNIO Friends' Facebook page received the Internet Day Award in its category. A distinction that, within the short period of the initiative's existence, has served to boost its wider public dissemination.

SOCIAL MEDIA PLATFORMS

CNIO FRIENDS

FACEBOOK 33,837 FOLLOWERS

AGE DISTRIBUTION

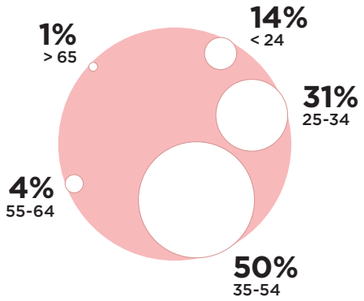


GENDER DISTRIBUTION

FEMALE	83%	
MALE	17%	

TWITTER 567 FOLLOWERS

AGE DISTRIBUTION



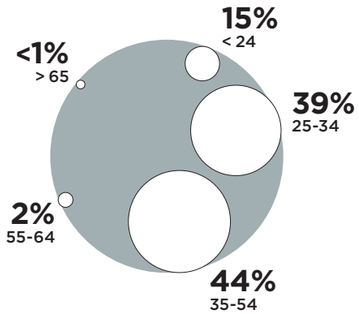
GENDER DISTRIBUTION

FEMALE	62%	
MALE	38%	

CNIO

TWITTER 10,842 FOLLOWERS

AGE DISTRIBUTION

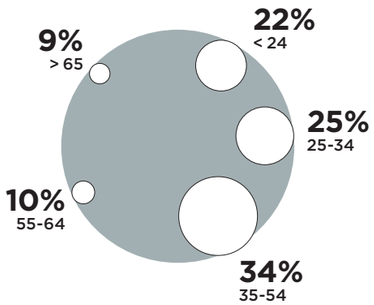


GENDER DISTRIBUTION

FEMALE	54%	
MALE	46%	

YOUTUBE 12,370 VIEWS

AGE DISTRIBUTION



GENDER DISTRIBUTION

FEMALE	42%	
MALE	58%	

PRESS CLIPPINGS

1 *ABC*, January 23, 2016
2 *Expansión* (front page), February 11, 2016
3 *El Ojo Clínico*, La 2, March 13, 2016
4 *BBC*, March 16, 2016
5 *SINC*, March 16, 2016
6 *La Razón*, March 27, 2016
7 *Diario Médico*, April 11, 2016
8 *La Sexta Noticias*, April 17, 2016

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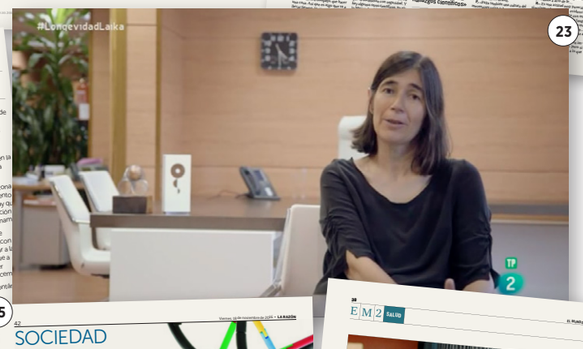

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24

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9 *Lab24*, 24h, May 10, 2016
10 *La Razón*, June 3, 2016
11 *Correo Gallego*, June 10, 2016
12 *El Diario Vasco*, June 13, 2016
13 *Diario Médico* (front page), July 11, 2016
14 *ABC*, July 16, 2016
15 *Gaceta Médica*, July 18, 2016
16 *La Voz de Galicia*, July 29, 2016
17 *Diario Médico*, September 14, 2016
18 *ABC*, September 24, 2016
19 *QUO*, September 27, 2016
20 *El Pais Semanal*, October 9, 2016
21 *El Mundo*, October 15, 2016
22 *La Razón*, October 19, 2016
23 *Orbita Laika*, October 19, 2016
24 *En Intermedio*, La Sexta, November 2, 2016
25 *La Razón*, November 18, 2016
26 *El Mundo* (front page), November 25, 2016

INVITED GUEST SPEAKERS (Distinguished Seminar Series)

Edurne Pasaban, March 8, 2016

Diane Simeone, June 27, 2016

Francisco J. Martínez Mojica, September 16, 2016

Francisco J. Ayala, October 14, 2016

Charles Brenner, October 28, 2016

Celeste Simon, December 2, 2016

SOCIAL EVENTS

CNIO's Director, Maria A. Blasco (left), and the President of the CLH Group, José Luis López de Silanes (right), signed an agreement to foster collaboration between both entities. Through this collaboration, the company joined 'CNIO Friends' in order to support research and training of research personnel, with the goal of advancing the development of innovative and specific therapies against cancer. March 18, 2016.

The first distinction received by the 'CNIO Friends' initiative came from social media. Indeed, its Facebook page rose to first place in its category in the Internet Day Awards, sponsored by Google, *Fénix Directo*, *Telefónica*, Facebook, *El Corte Inglés*, and HP. May 12, 2016.

In 2016, the CNIO once again, participated in the European Researchers' Night; an event funded by the EU Framework Programme. Over 200 participants visited the CNIO that opened its doors to the public as part of its commitment to scientific dissemination and education. The event provided guests with the opportunity to meet researchers in an interactive and entertaining way. September 30, 2016.

The CNIO hosted an exhibition entitled 'Marie Sklodowska-Curie. A Pole in Paris', which revisited the personal and professional life of this key woman of the 20th century. This initiative of the CNIO Women in Science Office (WISE) stands testament to CNIO's commitment to promoting and upholding the work of women scientists. November 2016.

International Affairs

INTERNATIONAL AFFAIRS

CAROLINA POLA Director of International Affairs

The year 2016 marks the creation of the Department of International Affairs (IAs) at the CNIO; its setup underscores the Centre's commitment with increasing its international impact to continue its growth in scientific and professional excellence. The overarching goal of the Department is to provide a strategic global vision for the CNIO to facilitate the coordination of efforts from the different departments with the aim of substantially strengthening our international reach with the research community, policy makers and society. The lines of work initiated this year will enable us to reinforce four of CNIO's fundamental concepts: knowledge sharing, value creation, research and innovation advancement, and professional and academic development.

"Broadening our reach and scientific impact through international collaborations is crucial for the CNIO investigators. We will continue consolidating our presence abroad with new initiatives."

As an integral part of the CNIO, we strive to consolidate our Centre's institutional leadership and reputation abroad, as well as to establish new robust partnerships and participate in European and International projects. This vision is now framed in the concept of 'Responsible Research & Innovation (RRI)' that will help us to further increase our impact on society by contributing to scientific advances and by endorsing innovative science policies.

The department focuses on the continuation of existing CNIO initiatives and on propelling novel projects abroad. One of our key activities for the upcoming year is the co-organisation of an Innovative Medicine Initiative (IMI) Oncology Workshop that will be hosted at the CNIO and spearheaded by the CDTI; its mission is to coordinate national efforts in upcoming cancer calls of this European public-private initiative. In this same vein, we have established a strategic workflow to coordinate CNIO's efforts, keep investigators informed and facilitate their participation and leadership in H2020 projects—an effort to help CNIO researchers to reinforce their scientific potential and strengths in order to become even more competitive in the international scene.

We have already sown the seeds for exciting alliances with international research centres of excellence that will pan out throughout 2017, not only in Europe but also in other countries where we can complement and synergise in regards to science, innovation and training. The CNIO will be formulating new ways of collaboration to continue attracting international talent and to provide our investigators with the most exciting, diverse and global setting for their professional and scientific growth.

CNIO Offices

DEAN'S OFFICE

MARÍA S. SOENGAS
Dean for Academic Affairs

Participants
Mónica Álvarez, Ana F. Batalha, Hugo Bernard, Jasminka Boskovic, Bruna Calsina, Daniela Cerezo, Almudena Chaves, Guillermo de Cárcer, Lucía T. Díez, Silvia Janeiro, Eleonora Lapi, Ana Losada, Jorge Martínez, Raúl Martínez, David Olmeda, Laura Remacha, Maria Rigau, Federica Schiavioni

The CNIO is recognised for the relevance and international projection of our Scientific Programmes. Key to this success is a solid core of undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. In fact, personnel in training constitute over 60% of the workforce in our institute. As such, the CNIO dedicates particular emphasis to career development, supported in part by highly competitive PhD and Postdoctoral Programmes. Agreements are also in place with multiple universities and medical centres, to ultimately bridge the gap between academic and clinical environments. Also very successful are our undergraduate summer internships, as well as diverse exchange and visitor programmes. Ultimately, our mission is to nurture and foster the development of our scientists-in-training, in order to maximise their chances of success.

The CNIO Student Association (CNIOSA) and Postdoc Association (CNIOPDA) are the driving forces behind an inspiring series of seminars and workshops that we hold throughout the year. In this context, scientific reasoning, grant writing, manuscript organisation and CV preparation are just some of the many topics covered in our curriculum. Likewise, we acknowledge that career options extend beyond the bench and we therefore pay special attention to the areas of public communication, management of intellectual property, and the creation of *start-ups* or *spin offs*. These activities are performed in concert with CNIO's Training Programmes and the Innovation and Communication Offices that are both deeply committed to providing the best environment for our personnel. We are most grateful to the *Fundación Jesús Serra*, for their continuous support to strengthen career development programmes at the CNIO.

We believe that an informed society is better prepared to understand (and if needed, face) the diseases that constitute human cancer. Therefore, we are actively involved in knowledge dissemination. Members of CNIOSA and CNIOPDA have participated in various school visits and Open Doors activities such as the *Semana de la Ciencia* or the *European Researchers' Night*; all these events were highly attended, attracting over 250 participants of all ages.

A particularly inspirational event this year was our Annual CNIO Lab Day. We were fortunate to host **Simon Gifford,** co-founder of *Mashauri Limited*, Director of *Genesis Management Consulting* and Professor at the prestigious *Instituto de Empresa*. Gifford gave an inspiring talk on how MBAs and entrepreneurs think and behave differently. He also spoke about his personal experience in setting up various consulting companies, emphasising the value of risk-taking and independent thinking. We also had seven outstanding talks given by CNIO trainees that covered exciting discoveries in the fields of epidemiology, epigenetics, proteomics, metastasis and drug development. Progress made in other basic and translational aspects of cancer were discussed in over sixty posters, which together emphasised the breadth of research covered by our different Scientific Programmes.

Another main highlight of the Lab Day was the announcement of the recipients of our '*Director's List Awards*'. These are recognitions of outstanding contributions made by our personnel in 3 categories: (1) predoctoral fellows with publications of the highest scientific impact; (2) excellence in research by postdoctoral and staff investigators; and (3) altruistic volunteering to further the mission of the Centre related to training, scientific divulgation and outreach.

1. Awards for Excellence in Research by Predoctoral Fellows

Early in the year, the family Agüera-Nieto contacted the CNIO about giving a donation to support research conducted by young scientists. This led to the inauguration of the '*Antonia Nieto Award*', in honour of their mother, to recognise the PhD student authoring the article with the highest impact. In 2016, the award went to Lluc Mosteiro for her impressive work published in *Science* on the mechanisms of cellular reprogramming *in vivo*. We are still moved by the kindness and commitment of the Agüera-Nieto family, who came to the CNIO to hand over this award and to share their multiple ideas and suggestions for the *Amigos del CNIO* initiative. Their energy is yet an additional inspiration for us to further strive to make significant contributions towards the advancement of cancer research.

"At the CNIO we aim high: to carry out the most innovative and competitive basic and translational research, and to best prepare our trainees for the future, so that they can fulfil their potential as influential leaders."

Additional awards in the PhD category were for Ana Teijeiro (*Cancer Cell*), Cristina Mayor-Ruiz (*Mol Cell*), Juan J. Montero (*Nat Commun*) and Eva Pérez-Guijarro (*Nat Commun*).

2. Award for Excellence in Research by Postdoctoral/ Staff Investigators

The awardee was Matilde Murga, for her outstanding contributions to the fields of DNA replication and genomic instability (*Mol Cell* and additional coauthored papers in *Nat Struct and Mol Biol, Oncotarget* and *Sci Signaling*).

3. Outstanding Contribution to Outreach and Awareness

This year's recipient was Guillermo de Cárcer, for his tireless efforts in the organisation of the European Researcher's Night. This is an event for which he coordinated over 50 volunteers, resulting in a flawless and exciting open-doors activity. The award was presented by Marcos Argumosa, who is himself an utterly impressive example of altruism, having run 10 consecutive marathons in 2015 to support the *Amigos del CNIO* initiative.

In summary, we are as proud as ever of the achievements accomplished by our vibrant community of young investigators at the CNIO. We thank all those public and private contributors that have helped to support and fuel their efforts, and will make sure that the next years will be even more successful in moving the cancer field forward in a meaningful manner for the patients.

CNIO WOMEN IN SCIENCE OFFICE

Lola Martínez
Coordinator

Members
Marinela Méndez: *Work-Life Balance Coordinator,* Francisca Mulero: *Seminars and Events Coordinator,*

The CNIO Women in Science Office (WISE) was created at the end of 2012 with the aim to give visibility to women, to promote institutional awareness on gender equality, and to try to promote and support women in their professional careers. The WISE Office is composed of CNIO volunteers from different areas of the Centre, people who believe there is still a real need for action to be undertaken to ensure gender equality in the research career. Recent studies from different organisations in Spain and the European Union still display the typical 'scissors' graphic regarding the distribution of gender along the career ladder, together with an approximate 20% salary gap between men and women. Furthermore, women are still underrepresented as recipients of prestigious scientific awards; as an example, another year has gone by without a single Nobel Prize, in any scientific discipline, being awarded to a woman. The issue does not seem to be that women are not present in academia; the latest data show that 55% of women pursue university studies. Although it is worrying to see the lack of female students in the so-called 'STEM' careers, they are still well represented at the pre- and post-doctoral stages. However, this representation drops to a meagre 25% of women at the Principal Investigator level, and it is even lower at the levels of Department Directors and beyond.

When experts analyse the data and try to identify the causes, one factor that keeps coming up repeatedly is the existence of cultural stereotypes between men and women. Since those stereotypes need to be challenged at an early age, the Office has decided to, from 2016 onwards, open up its seminars to schools and high schools with 2 main purposes: to make teenagers aware of those gender issues, and to promote equality and hopefully vocations among young girls to enter the scientific career.

We are certain that within the CNIO community, we all need to continue working together, with the other CNIO administrative offices as well as the Works Council, in order to reach our common goal, namely, to maintain the CNIO's high level of scientific productivity and to ultimately make it an outstanding centre to work where gender barriers are completely eliminated and there is a sensible balance between work and life.

Albanderi Alfraidi, Maria A. Blasco, Nicole Dölker, Raquel García-Medina, Francesc Madriles, Alba de Martino, Cristina de Martos (since November), Diego Megías, Fernando Peláez, Carolina Pola (since November), Alejandra Tavera

The Office consists of two working groups:

- → Work/Life Balance – aimed to promote and support initiatives to help improve the delicate balance between professional and personal life at the CNIO.
- → Seminars and Events – aimed to raise awareness of gender issues, and provide networking opportunities to all CNIO researchers.

"Within the CNIO community, we continue to work together towards the elimination of gender barriers, the empowerment of women in Science and society and the promotion of scientific vocations among young girls. Scientific Excellence can only be fully achieved through gender equality."

In 2016, the WISE Office was again able to invite and welcome several top female leaders from different areas to tell us about their experience with gender issues, giving our young scientists ideas and advice on how to best overcome some of the hurdles that they may face during their careers, while also giving CNIO researchers the opportunity to expand their networks.

Some of the seminars organised by the WISE Office during 2016 are:

- → EXPEDITION TO SUCCESS: Achieving goals and overcoming difficulties. Edurne Pasaban. Mountaineer, Tolosa, Spain. March 8th.
- → *Recuerdos y Olvidos Feministas*. María Teresa Fernández de la Vega. President of the Women for Africa Foundation, former Vice President of Spain. May 10th.
- → *Las Sinsombrero, sin ellas la historia no está completa*. Tánia Balló. Documentalist. Barcelona, Spain. June 28th.
- → Marie Skłodowska-Curie: Medical Physics pioneer and inspiration to female scientists. Dr Guadalupe Martín Martín. Medical Physicist. Fuenlabrada University Hospital. Madrid, Spain. November 7th.

Also, in November, we hosted the itinerant exhibition on the life and work of two-time Nobel Laureate, Marie Sklodowska-Curie, who is still an inspiration to women scientists. We put together six organised visits to the exhibition combined with a tour to different CNIO laboratories for students and the general public; these were a great success.

Since 1901, the year when Alfred Nobel established the awards bearing his name, only 17 women have been honoured with the recognition in the scientific field and unfortunately, many were left out despite their now widely recognised merits. We wish to contribute towards changing those numbers, and so we are working on developing different mentorship and leadership programmes for CNIO researchers.

Facts & Figures

SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director

Raquel Ares, Sonia Cerdá, Almudena del Codo (since March), M. Dolores Liébanes, Victoria López, Ana Merino, Juan Ramón Molina, Mercedes Moro, Leyre Vergés (since December)

The Scientific Management Department at the CNIO is committed to assisting with the facilitation of all those key areas that help our scientists to better focus their efforts on their research. The Department encompasses various Offices: Projects and Consortia, Education and Training Programmes, Scientific Events, Scientific Publishing, and Library & Archives.

"Every day, we work towards building a strong and flexible framework to support our scientists and to help them achieve excellence."

The mission of the Projects' Office is to guide the CNIO scientists through all stages related to the application and management processes of externally-funded projects, whether they be financed through either public and/or private institutions, or stem from either national or international funding bodies. The Office coordinates the internal call alerts, manages the ethical certification for projects involving animal experimentation or human samples, supports scientists with the preparation of the project proposals, manages the ongoing projects, and contacts the funding agencies to resolve any issues or deal with questions.

The Training Office is the central point for training at the CNIO; it aids the recruitment process, serves as an advocate for all fellows, provides administrative support, and creates educational and learning opportunities. It is responsible for helping PhD students, postdoctoral scientists and post-resident MDs by announcing call alerts and providing the relevant key information; helping foreign students with their paperwork at the foreign office; organising the summer training call; and, in general, in collaboration with the Personnel Department, managing student's grants.

The Events Office organises CNIO meetings, such as the CNIO Frontiers Meetings, the Distinguished Seminars series, the external Scientific Advisory Board (SAB) meeting, CNIO Progress Reports, as well as Faculty retreats, among others. The Office also helps scientists by providing advice for the organisation of specific events, including scientific divulgation events, and arranges the CNIO guided visits.

The Publications Office is responsible for the preparation of institutional scientific publications, including the CNIO Annual Report, booklets of the Scientific Advisory Board meeting and those of other symposia, as well as scientific dissemination leaflets. The Office also provides support for the scientific editing of press notes and other publications of scientific divulgation to a non-specialised audience.

The Library administers the electronic subscriptions of over 300 scientific journals at the CNIO and manages journal article requests for journals that the CNIO is not subscribed to. The Library also provides information regarding reference management software.

COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies. In 2016, researchers at the CNIO were involved in 148 projects that received extramural funding.

CNIO actively participates 64 collaborative projects in total: 30 were international collaborative projects (4 of which are coordinated by the CNIO) and 34 collaborative projects at the national level (12 of them coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7 Framework Programme and Horizon 2020, the Interreg SUDOE Programme, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the Melanoma Research Alliance, the Paradifference Foundation, the Worldwide Cancer Research and the Volkswagen foundation

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2016, 21 of these projects received international funds while 63 of them received national funding (mainly the Spanish Ministry of Economy, Industry and Competitiveness and the Institute of Health Carlos III). The international individual projects are funded by the European Commission (European Research Council (ERC) grants and the Marie Curie Actions), the Worldwide Cancer Research (WCR), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN COMMISSION

7TH FRAMEWORK PROGRAMME (2007-2013)

COST ACTION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (coordinator)	COST Action EU Pancreas: An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions for a rare disease (Ref.: COST BM1204)

EURATOM

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Serrano, Manuel	RISK-IR: Risk, Stem Cells and Tissue Kinetics-Ionising Radiation (Ref.: 323267)

INNOVATIVE MEDICINES INITIATIVE JOINT UNDERTAKING (IMI JU)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	e-TOX: Integrating bioinformatics and chemoinformatics approaches for the development of expert systems allowing the *in silico* prediction of toxicities (Ref.: 115002)
Valencia, Alfonso	Open PHACTS: An open, integrated and sustainable chemistry, biology and pharmacology knowledge resource for drug discovery (Ref.: 115191-2)

INTEGRATED PROJECT (IP)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	BLUEPRINT: A BLUEPRINT of haematopoietic epigenomes (Ref.: 282510)
Valencia, Alfonso	ASSET: Analysing and striking the sensitivities of embryonal tumours (Ref.: 259348)
Valencia, Alfonso	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research (Ref.: 305444)

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	ITN aDDRess: Joint training and research network on chromatin dynamics and the DNA damage response (Ref.: 316390)

NETWORKS OF EXCELLENCE (NOE)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	EUROCANPLATFORM: A European platform for translational cancer research (Ref.: 260791)

SMALL OR MEDIUM-SCALE FOCUSED RESEARCH PROJECTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	TransBioBC: Translation of novel Biomarkers for Bladder Cancer for clinical outcome prediction (Ref.: 601933)
Robledo, Mercedes	ENS@T- CANCER: European network for the study of adrenal tumours-structuring clinical research on adrenal cancers in adults. (Ref.: 259735)

ERA-NET ON TRANSLATIONAL CANCER RESEARCH (TRANSCAN)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	Bio-PaC: Biomarkers of tumor recurrence in pancreatic cancer (financed by ISCIII, Ref.: AC14/00025)

ERA NET NEURON II: NETWORK OF EUROPEAN FUNDING FOR NEUROSCIENCE RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human (Financed by MEIC Ref.: PCIN-2015-007)

HORIZON 2020 (2014-2020)

RESEARCH INFRASTRUCTURES, INCLUDING E-INFRASTRUCTURES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	ELIXIR-EXCELERATE: Fast-track ELIXIR implementation and drive early user exploitation across the life-sciences (Ref.: 676559)
Valencia, Alfonso	OpenMinTeD: Mining INfrastructure for TExt and Data (Ref.: 654021)

MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	ITN IMMUTRAIN: Training network for the immunotherapy of cancer (Ref.: 641549)

SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	BRIDGES: Breast cancer risk after diagnostic gene sequencing (Ref.: 634935)

INDUSTRIAL TECHNOLOGIES: ADVANCED MATERIALS AND NANOTECHNOLOGIES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Hidalgo, Manuel	NoCanTher: Nanomedicine upscaling for early clinical phases of multimodal cancer therapy (Ref. :685795)

INTERREG SUDOE PROGRAMME[1]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	European Network for Translational Research and Innovation in Oncology /Réseau Européen de Recherche translationnelle et d'Innovation en oncologie (Ref.: SOE1/P1/F0082)

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor; Soengas, María S. (coordinator)	Imaging and therapeutic targeting of lymphangiogenesis in melanoma (Ref.: 269626)
Soengas, María S. (coordinator)	Imaging and targeting dormant and pro-metastatic melanoma lesions *in vivo* (Ref.: 401181)

THE PARADIFFERENCE FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Robledo, Mercedes	SDHB-related metastatic paraganglioma: search for the cure

US CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/US DEPARTMENT OF DEFENSE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Radiolabeled exosomes for the early detection of metastases and to predict breast cancer premetastatic niche (Ref.: W81XWH-13-1-0249)
Peinado, Héctor	Organ-tropic metastatic secretomes and exosomes in breast cancer (Ref.: W81XWH-13-1-0427)
Peinado, Héctor	Exosomes in Development and Therapy of Malignant Mesothelioma (Ref.: W81XWH-14-1-0199)

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Characterization and functional analysis of breast cancer secreted exosomes in malignant progression (Ref.: U01CA169538)
Peinado, Héctor	Exosome-mediated transfer of c-MET to bone marrow progenitors promotes metastasis (Ref.: R01CA169416)
Valencia, Alfonso	GENCODE 2: Integrated human genome annotation: generation of a reference gene set (Ref.: HG007234-01)

VOLKSWAGEN FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Lietha, Daniel	Nanoapertures loaded with individual molecules (Ref.: 86416-1)

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (coordinator)	Oral microbiotic profiles and its association with risk of pancreatic ductal adenocarcinoma (Ref.: 15-0391)

1. This Programme is cofunded by the European Regional Development Fund (ERDF)

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION

7TH FRAMEWORK PROGRAMME (2007-2013)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health (Ref.: 617840)

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	PERSMEDOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy (Ref.: 334361)
Peinado, Héctor	WHRI COFUND ADIPOMET: Analyzing the crosstalk of tumor and adipose tissue during metastasis (Ref.: 608765)
Ramón, Santiago; Moreno, María	WHRI COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target (Ref.: 608765)
Squatrito, Massimo	GLIDD: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas (Ref.: 618751)
Wagner, Erwin F.; Gago, Nuria	WHRI COFUND STEM-PSO: Unraveling the contribution of Epidermal and Non-Epidermal Progenitor (Ref.: 608765)

HORIZON 2020 (2014-2020)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	ERC Starting Grant NutrientSensingVivo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)
Hidalgo, Manuel	ERC Advanced Grant AVATAR: Integrating Genomics and Avatar Mouse Models to Personalize Pancreatic Cancer Treatment (Ref.: 670582)
Serrano, Manuel	ERC Advanced Grant CELLPASTICITY: New Frontiers in Cellular Reprogramming: Exploiting Cellular Plasticity (Ref.: 669622)

EUROPEAN FOUNDATION FOR THE STUDY OF DIABETES (EFSD)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Growth factors and nutrients in type 2 diabetes: role of URI in β cell plasticity and glucose homeostasis

HOWARD HUGHES MEDICAL INSTITUTE (HHMI)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	Exploring the role of replicative stress in cancer and ageing (Ref.: 55007417)

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)

PROSTATE CANCER FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Targeting telomeres in cancer (Ref.: 16-1177)
Lietha, Daniel	Targeting regulatory mechanisms for allosteric cancer drug discovery (Ref.: 15-1177)
Malumbres, Marcos	New therapeutic strategies by inhibiting Mastl in breast tumors (Ref.: 15-0278)
Peinado, Héctor	Evaluation of obesity as a novel risk factor in metastasis (Ref.: 16-1244)
Pérez Moreno, Mirna A.	Defining the role of macrophage-derived Wnts in squamous cell carcinoma (Ref.: 15-1219)
Soengas, María S.	Harnessing endo/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system (Ref.: 15-1374
Wagner, Erwin F.	Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis (Ref.: 13-0216)

US CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/US DEPARTMENT OF DEFENSE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Role of exosomes and Endoglin in Neurofibromatosis Progression (Ref.: W81XWH-16-1-0131)

NATIONAL GRANTS COLLABORATIVE PROJECTS

COMMUNITY OF MADRID / *COMUNIDAD AUTÓNOMA DE MADRID*[2]

Comunidad de Madrid

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano; Malumbres, Marcos (coordinator)	*Programa ONCOCYCLE: El ciclo celular y los microRNAs en la autorenovación y diferenciación de células progenitoras (Ref.:* S2011/BMD-2470)
Blasco, Maria A.; Serrano, Manuel (coordinator)	*Programa ReCaRe: Reprogramación en cáncer y regeneración* (Ref.: S2011/BMD-2303)
Campos-Olivas, Ramón; Lietha, Daniel	*Programa BIPEDD 2: Plataforma integrada de bioinformática para el descubrimiento de nuevos fármacos basado en la estructura del receptor* (Ref.: S2011/BMD-2457)
González-Neira, Anna	*Programa VISIONANIMAL: Modelos animales para el estudio de enfermedades de la visión* (Ref.: S2011/BMD-2439)
Martínez, Jorge L.	*Programa ANGIOBODIES 2: Desarrollo de anticuerpos recombinantes para uso terapéutico y diagnostico en angiogénesis patológica y para la identificación de nuevos marcadores angiogénicos* (Ref.: S2011/BMD-2312)
Montoya, Guillermo	*Programa INTERACTOMICS: Interactomics del centrosoma* (Ref.: S2011/BMD-2305)
Robledo, Mercedes	*Programa TIRONET: Fisiopatología tiroidea: Mecanismos implicados en cáncer, autoinmunidad y mecanismo de acción de hormonas tiroideas* (Ref.: S2011/BMO-2328)
Soengas, María S.	*Programa NANODENMED: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas* (Ref.: S2011/BMD-2351)
Real, Francisco X.	*Programa CEL-DD: Linajes y competición celular en el desarrollo y la enfermedad (Ref.: P2010/BMD-2315)*

INSTITUTE OF HEALTH CARLOS III / *INSTITUTO DE SALUD CARLOS III (ISCIII)*

SUB-PROGRAMME OF COOPERATIVE HEALTH RESEARCH THEMATIC NETWORKS/*SUBPROGRAMA DE REDES TEMÁTICAS DE INVESTIGACIÓN COOPERATIVA (RETICS)*[3]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cigudosa, Juan C.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)* (Group RD12/0036/0037)
Malats, Núria	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)* (Group RD12/0036/0050)
Real, Francisco X.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)* (Group RD12/0036/0034)

SUB-PROGRAMME OF GRANTS FOR RESEARCH SUPPORT PLATFORMS IN HEALTH SCIENCES AND TECHNOLOGY/ *SUBPROGRAMA DE AYUDAS PARA PLATAFORMAS DE APOYO A LA INVESTIGACIÓN EN CIENCIAS Y TECNOLOGÍAS DE LA SALUD*[4]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB*[2] (PT13/0001/0005)
Morente, Manuel M. (coordinator)	*Plataforma de Biobancos (Coordination node and group* (PT13/0010/0001)
Muñoz, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB*[2] (Group PT13/001/0010)
Valencia, Alfonso	*Plataforma de recursos biomoleculares y bioinformáticos, PRB*[2] (Group PT13/0001/0030)

RESEARCH PROJECTS IN HEALTH[5]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Cellular aging in first episode early-onset psychosis. Collaboration with Gregorio Marañón Hospital (Ref.: PI14/00397)
Blasco, Maria A.	Safety and efficacy of gene therapy with telomerase in acute myocardial infarction. Impact on ventricular remodeling in an experimental porcine model. Collaboration with Gregorio Marañón Hospital (Ref. DTS15/00095)

MINISTRY OF HEALTH, SOCIAL SERVICES AND EQUALITY / *MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD (MSSSI)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Hidalgo, Manuel (coordinator)	Chemosensitivity profiles for the personalized therapy of advanced colorectal cancer (Ref.: EC11-017)
Hidalgo, Manuel (coordinator)	Personalized treatment for pancreatic cancer patients (Ref.: EC10-278)
Hidalgo, Manuel (coordinator)	Personalized treatment for pancreatic cancer patients II (Ref.: EC11-005)

MINISTRY OF ECONOMY, INDUSTRY AND COMPETITIVENESS / *MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD (MEIC)*

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016)

EXCELLENCE NETWORKS / *REDES DE EXCELENCIA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (coordinator); Blasco, Maria A.; Fernández-Capetillo, Óscar; Malumbres, Marcos; Real, Francisco X.; Serrano, Manuel	OncoBIO: Cancer biology (Ref.: SAF2014-57791-REDC)
Malumbres, Marcos (coordinator)	CellSYS: Functional and Systems Biology of Cell Proliferation (Ref.: BFU2014-52125-REDT)
Serrano, Manuel (coordinator)	SENESTHERAPY: Cell senescence in cancer therapy (Ref.: SAF2014-56720-REDT)

CHALLENGES-COLLABORATION/*RETOS-COLABORACIÓN*[6]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	*NRCANCER- Desarrollo de nueva terapia antitumoral basada en nicotinamida-ribosido* (Ref.: RTC-2016-5431-1)
Soengas, María S.	*Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores* (Ref.: RTC-2014-2442-1)

2. This Programme is cofunded by European Structural and Development Funds (ERDF and ESF) European Union, European Structural and Investment Funds

3. This Programme is cofunded by the European Regional Development Fund (ERDF) European Union, European Regional Development Fund

4. This Programme is cofunded by the European Regional Development Fund (ERDF) European Union, European Regional Development Fund

5. This Programme is cofunded by the European Regional Development Fund (ERDF) European Union, European Regional Development Fund

SPANISH ASSOCIATION AGAINST CANCER / *ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (coordinator)	A multifaceted approach to target pancreatic cancer (Ref.: GC16173694)
Benítez, Javier	Cancer and immunodeficiency in children (Ref.: CEI14142070)
Malats, Núria; Real, Francisco X. (coordinator)	Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293)
González Pisano, David; Peinado, Héctor; Soengas, María S. (coordinator)	Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets (Ref.: GCB15152978)

LA MARATÓ TV3 FOUNDATION / *FUNDACIÓN LA MARATÓ TV3*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	Molecular analysis of Capicua, a novel tumor suppressor involved in RTK signaling and transcriptional repression (Ref.: 20131730/31)
Fernández-Capetillo, Óscar	Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment (Ref.: 20134130/31)
Soengas, María S.	Role of RNA binding proteins in melanoma progression: searching for new diagnostic markers and therapeutic targets (Ref.: 20131430/31)

MADRI+D FOUNDATION / *FUNDACIÓN PARA EL CONOCIMIENTO* MADRI+D

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Dean's Office for Academic Affairs; Soengas, María S.	European Researchers' Night 2014, organized by Madri+d Foundation and founded by European Comission on the framework of H2020 Programme

6. This Programme is cofunded by the European Regional Development Fund (ERDF)

NATIONAL GRANTS INDIVIDUAL PROJECTS

INSTITUTE OF HEALTH CARLOS III / *INSTITUTO DE SALUD CARLOS III (ISCIII)*

RESEARCH PROJECTS IN HEALTH[7]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	Biologic and genetic bases of telomere shortening in hereditary breast cancer. Searching for new high susceptibility genes in *BRCAX* families with short telomeres (Ref.: PI12/00070)
Cascón, Alberto	Exome sequencing of trios, mother-father-proband, in pediatric patients with multiple pheochromocytomas/paragangliomas (Ref.: PI12/00236)
Cascón, Alberto	Next generaton sequencing of genes directly and indirectly involved in the Krebs cycle, applied to pheochromocytomas/paragangliomas with hypermethylated phenotype (Ref.: PI15/00783)
Cigudosa, Juan C.	Genetic diagnostics by next-generation-sequencing in myeloid neoplasias: step towards its clinical use and characterization studies on the mutation genomic and functional pathological effects (Ref.: PI12/00425)
García, María José	Definition of novel ovarian cancer susceptibility genes using next-generation sequencing technology and a LOH-candidate region approach in high-risk non-BRCA1/BRCA2 patients (Ref.: PI12/01319)
González-Neira, Anna	Personalizing breast cancer treatment: prediction model construction for taxanes and anthracyclines efficacy thought the integration of different genomic approaches (Ref.: PI12/00226)
Hidalgo, Manuel	Targeting Pancreatic Cancer Stroma (Ref.: PI13/00230)
Malats, Núria	Aetiology of pancreas cancer: Application of "omics" technologies in the assessment of risk factors (Ref.: PI12/00815)
Malats, Núria	Building and validation of risk prediction models for pancreas cancer. The application of a multi-omics approach (Ref.: PI15/01573)
Molina, María Esther	Dietary patterns, antioxidants and biomarkers of oxidant-antioxidant status in the EPIC-Granada and EPIC-Gipuzkoa (European Prospective Investigation into Cancer and Nutrition) cohort (Ref.: PI12/00002)
Pérez de Castro, Ignacio	An integrative Study of Chromosomal Instability and Cancer: looking for prognostic markers and therapeutic opportunities (Ref.: PI14/00227)
Olmos, David	Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer (Ref.: PI13/01287)
Quintela, Miguel Ángel	From systems biology to clinical trials: high-throughput studies and definition of predictive factors and resistance mechanisms against breast cancer drugs (Ref.: PI13/00430)
Robledo, Mercedes	Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility (Ref.: PI14/00240)
Rodríguez, Sandra	Ewing Sarcoma Model: induction of the t(11;22) translocation in human mesenchymal stem and iPS cells by the CRISPR-Cas9 system and study of the cellular context and other secondary events role (Ref.: PI14/01884)
Squatrito, Massimo	Investigating the role of Fra1 and Fra2 in glioma tumor formation and treatment response (Ref.: PI13/01028)
Urioste, Miguel	PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene (Ref.: PI14/00459)

7. This Programme is cofunded by the European Regional Development Fund (ERDF)

MINISTRY OF ECONOMY, INDUSTRY AND COMPETITIVENESS / *MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD (MEIC)*

NATIONAL R&D&I PLAN 2008-2011

SUB-PROGRAMME FOR NON-TARGETED FUNDAMENTAL RESEARCH PROJECTS / *SUBPROGRAMA DE PROYECTOS DE INVESTIGACIÓN FUNDAMENTAL NO ORIENTADA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
UluÇkan, Özge	PsorTACEmiR21: Investigating the role of microRNA21/ TIMP-3/TACE in psoriasis - evaluating the potential therapeutic implications (Ref.: SAF2012-39670)
Valencia, Alfonso	Development of biocomputing systems and subjacent computational methods for the analysis of oncologic personalised therapies (Ref.: BIO2012-40205)
Wagner, Erwin F.	HepAP-1: From liver physiology to hepatitis and hepatocellular carcinoma (HCC): role of AP-1 (Fos/Jun) proteins (Ref.: BFU2012-40230)

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016)

CENTRES AND UNITS OF EXCELLENCE "SEVERO OCHOA" SUB-PROGRAMME / *SUBPROGRAMA DE APOYO A CENTROS Y UNIDADES DE EXCELENCIA 'SEVERO OCHOA'*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Center of Excellence "Severo Ochoa" (Ref.: SEV-2015-0510)

R&D EXCELLENCE PROJECTS / *PROYECTOS DE I+D EXCELENCIA*[8]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Méndez, Juan	REPLICON: Molecular mechanisms that control eukaryotic DNA replication (Ref.: BFU2013-49153-P)
Ramón, Santiago	CADstructure: Structural determination of the architecture of CAD, an antitumoral target that controls the biosynthesis of pyrimidines (Ref.: BFU2013-48365-P)
Ruiz, Sergio	RSHIPS: Replicative stress during somatic cell reprogramming (Ref.: SAF2013-49147-P)

CHALLENGES-RESEARCH /*RETOS-INVESTIGACIÓN*[9]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	PANTHER: A three prong strategy to fight pancreatic ductal adenocarcinoma (Ref.: SAF2014-59864-R)
Blasco, Maria A.	TeloHealth: Telomeres, telomerase and disease (Ref.: SAF2013-45111-R)
Djouder, Nabil	MILC: Metabolic inflammation in liver cancer (Ref.: SAF2013-46089-R)
Efeyan, Alejo	NUTRIENTOR: Physiology of nutrient sensing and signaling by the mTOR complex 1 (Ref.: SAF2015-67538-R)
Fernández-Capetillo, Óscar	BREAKINGRAD: Exploring the limits of radioresistance in mammals (Ref.: SAF2014-59498-R)
Losada, Ana	COHESIN: Cohesin function and regulation: a multidisciplinary approach (Ref.: BFU2013-48481-R)
Malumbres, Marcos	Cyclexit: Physiological and therapeutic relevance of mitotic kinases and phosphatases (Ref.: SAF2015-69920-R)
Muñoz, Daniel	REMODEL: Cellular senescence as an active player in tissue remodeling (Ref.: BFU2014-60020-R)
Muñoz, Javier	steMS: Understanding ground state pluripotency of embryonic stem cells through mass spectrometry-based proteomics (Ref.: SAF2013-45504-R)
Ortega, Sagrario	HaploEScancer: Haploid ES cells for cancer research (Ref.: SAF2013-44866-R)
Osorio, Ana	IPAGEN: Exploring the mechanism of action of PARP inhibitors in breast and ovarian cancer patients. Identification of new genetic predictors of response (Ref.: SAF2014-57680-R)
Pastor, Joaquín	CDK8eDD: CDK8 a novel target in cancer therapy. Relevance of CDK8 kinase activity, discovery and optimization of selective orally bioavailable CDK8 inhibitor (Ref.: SAF2013-44267-R)
Peinado, Héctor	METASTAXOMEs: Role of tumor-secreted exosomes in lymph node microenvironment reprogramming during metastasis (Ref.: SAF2014-54541-R)
Pérez Moreno, Mirna A.	ESSENCE: Extrinsic control of the skin stem cell niche in homeostasis and cancer (Ref.: BFU2015-71376-R)
Real, Francisco X.	TRANS-PDAC: Transcriptional control of pancreatic cancer development (Ref.: SAF2015-70553-R)
Rodríguez, Cristina	PREDICT: Identification of genetic markers and physiopathologic factors predictive of the peripheral neuropathy of paclitaxel and of other oncologic drugs: massive sequencing of candidate genes (Ref.: SAF2015-64850-R)
Serrano, Manuel	CANCERAGE: Cancer and ageing-associated diseases: new frontiers and new strategies (Ref.: SAF2013-48256-R)
Soengas, María S.	MEL-STOP: Vesicular trafficking in melanoma progression and treatment response (Ref.: SAF2014-56868-R)
Valiente, Manuel	ReACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis (Ref.: SAF2014-57243-R)
Valencia, Alfonso	EPIC: Expression Patterns of Inverse Comorbidity (Ref.: BFU2015-71241-R)
Wagner, Erwin F.	CANPSOR: Investigating Cancer Risk in Psoriasis (Ref.: SAF2015-70857-R)

EXCELLENCE-EUROPE / *EUROPA EXCELENCIA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Cristina	ANGIOMARKER: Predicting antiangiogenic drug response in cancer: markers and mechanisms (Ref.: SAF2015-70820-ERC)
Valiente, Manuel	BrainMET: Deconstructing metastatic disease in the brain (Ref.: SAF2015-62547-ERC)

RESEARCH-EUROPE / *EUROPA INVESTIGACIÓN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	CancerCureAdvisor: An open bioinformatics platform for personalized treatment of cancer (Ref.: EUIN2015-62887)

NETWORKS AND SCIENTIFIC MANAGERS-EUROPE / *EUROPA REDES Y GESTORES*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	CNIO in Horizon 2020: support for proposal preparation and project management (Ref.: EUC2014-51617)

8. This Programme is cofunded by the European Regional Development Fund (ERDF)

9. This Programme is cofunded by the European Regional Development Fund (ERDF)

YOUNG RESEARCHERS PROGRAM / *PROGRAMA JÓVENES INVESTIGADORES*[10]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez, Mónica	GPGenCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer (Ref.: SAF2014-60442-JIN)
Lecona, Emilio	UBQREP: Modulation of DNA Replication by ubiquitination of chromatin proteins (Ref.: BFU2014-55168-JIN)

SCIENTIFIC INFRASTRUCTURES / *INFRAESTRUCTURSA CIENTÍFICO-TECNOLÓGICAS*[11]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Javier	*Sistema cromatográfico UHLPC acoplado a Espectrómetro de Masas de alta resolución para estudios de proteómica avanzada* (Ref.: CNIO15-EE-2855)
Pisano, David G.	Clúster SMP de Análisis HPC (Ref.: CNIO15-EE-3845)

ASTRAZENECA FOUNDATION / *FUNDACIÓN ASTRAZENECA*

AstraZeneca

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	*Cáncer de próstata familiar y esporádico asociado a alteraciones genéticas, germinales y/o somáticas, en genes de la reparación del DNA*
Quintela, Miguel Ángel	*Reprogramación inmune en cáncer de mama preexpuesto a antiangiogénicos inductores de apoxia*

FERO FOUNDATION / *FUNDACIÓN FERO*

fero Fundación de Investigación Oncológica

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Liquid biopsy by nanoplasmonic detection of exosomes: predicting response to (immuno- and radio)-therapy
Valiente, Manuel	Predictive biomarkers for brain metastasis in small cell lung cancer

SPANISH SOCIETY OF MEDICAL ONCOLOGY / *SOCIEDAD ESPAÑOLA DE ONCOLOGÍA MÉDICA (SEOM)*

SEOM Sociedad Española de Oncología Médica

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	*Validación de una firma de expresión con utilidad pronóstica en cáncer de próstata resistente a la castración en una cohorte multi-institucional de pacientes tratados con docetaxel*

ATRESMEDIA CORPORATION / *ATRESMEDIA CORPORACIÓN*

constantes y vitales

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	*Premio Constantes y Vitales en la categoría "Joven talento en investigación biomédica" 2015* (A3M 2015)

10. This Programme is cofunded by the European Regional Development Fund (ERDF) EUROPEAN UNION European Regional Development Fund

11. This Programme is cofunded by the European Regional Development Fund (ERDF) EUROPEAN UNION European Regional Development Fund

OLGA TORRES FOUNDATION / *FUNDACIÓN OLGA TORRES*

TOT

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Understanding the role of growth factors and nutrients in inflammatory bowell disease and colon cancer

FUNDACIÓN PROYECTO NEUROFIBROMATOSIS

FUNDACIÓN PROYECTO NEUROFIBROMATOSIS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	*Uso de exosomas circulantes como marcadores de progresión en neurofibromatosis y para la determinación de nuevas estrategias terapéuticas*

BBVA FOUNDATION / FUNDACIÓN BBVA

Fundación BBVA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	PerMed: Precision Medicine from Big Data to Cognitive Computing CNIO (Ref.: 76/2016)

FUNDACIÓN PFIZER

fundación Pfizer

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Hector	Tumour exosome integrins determine organotropic metastasis

EDUCATION AND TRAINING PROGRAMMES

One of the principal goals of the CNIO is to increase its training capacity in order to give students and professionals the opportunity to advance their careers in the healthcare sector. During 2016, the CNIO signed new agreements with several Spanish Universities and other institutions, namely with the *Universidad Autónoma de Barcelona*, UNED, the International School of Protocol, University Claude Bernard Lyon, *Universidad Francisco de Vitoria, Universidad de Navarra, Universidad de Córdoba, Universidad CEU San Pablo, Fundación Jesús Serra, Fundación "la Caixa"*, and the ISFPS Claudio Galeno of Madrid.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2012	2013	2014	2015	2016
Training of PhD students	121	116	108	105	**110**
Post-doctoral training	81	67	55	48	**51**
Training for MDs	16	21	14	25	**17**
Laboratory training for MSc/BSc students	42	36	73	80	**95**
Laboratory training for technicians	26	19	21	27	**26**
Master's Degree in Molecular Oncology (graduated)	37	37	34	29	**25**

TRAINING OF BSC/MSC STUDENTS

The CNIO is committed to training junior scientists at the onset of their careers. To this end, the Centre has established a programme that offers BSc and MSc students the opportunity to obtain hands-on practical laboratory experience by working on ongoing research projects in one of the CNIO groups. The CNIO offers 2 types of short-term laboratory training:

- → An annual Summer Training Programme for undergraduate students, from any country, who are in their last years of study in the biomedical field. The Programme encompasses 8 weeks of full-time laboratory training (285 hours). During this time, the students actively participate in research projects in one of the CNIO groups. During 2016, 6 students from 3 different countries participated in this programme.
- → Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 95 students participated in these programmes, of which 6 ended up joining the CNIO as pre-doctoral students.

TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, in 2016, 10 students obtained their PhD degrees and 25 more joined the CNIO. More than 25% of the 110 students working at the CNIO in 2016 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the *Fundación "la Caixa"* offers international fellowships to PhD students to enable them to carry out their thesis projects in biomedical research in Spanish centres of excellence. The CNIO was chosen, as one of 4 such centres, to launch a programme for outstanding young pre-doctoral students from all over the world who have an interest in pursuing an ambitious PhD project. Since 2013, the Ministry of Economy, Industry and Competitiveness has undertaken efforts to link the *"la Caixa"*/CNIO International PhD Programme to distinguished research centres accredited as "Severo Ochoa Centres of Excellence". The third call of this new *"la Caixa"*-Severo Ochoa International PhD Programme was very successful, attracting around 130 eligible applications from undergraduates from 32 different countries. During 2016, 2 pre-doctoral students received one of these internationally recognised fellowships.

The distribution of students across the CNIO's Research Programmes in 2016 was as follows: 54.5% of the students worked in the Molecular Oncology Programme, 11.8% in the Cancer Cell Biology Programme, 8.2% in the Structural Biology and Biocomputing Programme, 13.6% in the Human Cancer Genetics Programme, 9.1% in the Clinical Research Programme, 1.8% in the Biotechnology Programme and the remaining 0.9% in the Experimental Therapeutics Programme.

FUNDING OF PHD TRAINING	NO.
SPANISH ENTITIES	86
Ministry of Economy, Industry and Competitiveness / *Ministerio de Economía, Industria y Competitividad (MEIC)* (Predoctoral fellowships)	36
Ministry of Economy, Industry and Competitiveness / *Ministerio de Economía, Industria y Competitividad (MEIC)* (I+D Projects)	7
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte (MECD)* (Predoctoral fellowships)	4
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (I+D Projects)	6
"la Caixa" Banking Foundation / *Fundación Bancaria "la Caixa"* (Predoctoral fellowships)	23
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC* (I+D Projects)	1
ATresMedia Foundation / *Fundación ATresMedia*	1
Cris Foundation / *Fundación Cris*	4
CIBERER	2
CNIO	2
INTERNATIONAL ENTITIES	24
European Commission Framework Programme / H2020	2
Marie Skłodowska-Curie actions of the European Commission	4
European Research Council	5
Portuguese Foundation for Science and Technology (FCT)	1
China Scholarship Council	1
European Foundation for the Study of Diabetes	1
Melanoma Research Alliance	2
Boehringer Ingelheim Fonds	1
Boehringer Ingelheim International GMBH	1
Hoffmann-La Roche	1
Pfizer	3
Prostate Cancer Foundation Young Investigator Award	1
Volkswagen Foundation	1
TOTAL	**110**

POST-DOCTORAL TRAINING

One of the CNIO's prime objectives is to attract young researchers who have recently obtained their PhD or MD degrees and to offer them highly interesting research projects at the forefront of cancer research.

In 2016, 51 postdoctoral fellows worked at the CNIO. Notably, more than one third of these fellows were from outside of Spain, many coming from very prestigious international institutions.

For yet another year, the *Fundación Banco Santander* upheld its agreement with the CNIO in 2016 and we continued the highly competitive fellowship programme aimed to support outstanding young scientists who have been trained in the UK or in the USA, and who wish to start or continue their postdoctoral training at the CNIO. One young scientist, who came from the Sloan Kettering Institute for Cancer Research (New York), joined the CNIO thanks to a Santander Foundation-CNIO Fellowship in 2016. Another scientist, coming from the City University of New York, was awarded one of these fellowships in late 2016.

Thanks to the donations received through the "CNIO Friends" platform we launched the inaugural Postdoctoral Contract "CNIO Friends" Programme in 2016, thereby enabling us to recruit 2 scientists for a period of 2 years each.

Furthermore, in 2016, as a result of an agreement signed with the *Juegaterapia* Foundation, we were able to create a third Postdoctoral Contract, "Juegaterapia-CNIO Friends", which enabled us to contract a scientist to carry out a project on paediatric oncology.

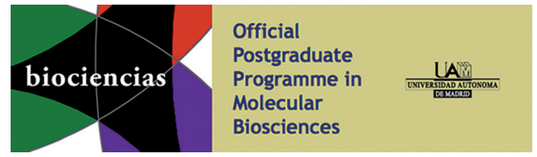
FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS	NO.
SPANISH ENTITIES	31
Ministry of Economy, Industry and Competitiveness / *Ministerio de Economía, Industria y Competitividad (MEIC)* (Postdoctoral fellowships)	5
Ministry of Economy, Industry and Competitiveness / *Ministerio de Economía, Industria y Competitividad (MEIC)* (I+D Projects)	4
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (I+D Projects)	2
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (Postdoctoral fellowship)	1
Madrid-MIT *M+Visión* (Postdoctoral fellowship)	1
Spanish Association Against Cancer *(AECC)* / *Fundación Científica de la AECC* (Postdoctoral fellowships)	4
Botín Foundation / *Fundación Botín*	1
Banco Santander Foundation / *Fundación Banco Santander*	1
Cris Foundation / *Fundación Cris*	2
La Marató TV3 Foundation / *Fundació La Marató TV3*	2
HM Hospitals / *HM Hospitales*	1
CIBERER	1
CNIO	6
INTERNATIONAL ENTITIES	20
European Commission Framework Programme / H2020	2
European Research Council	2
Association for International Cancer Research	5
Melanoma Research Alliance	1
Worldwide Cancer Research UK	1
US Department of Defense	1
Fulbright Commission	1
Boehringer Ingelheim International GMBH	2
Celgene	1
Daiichi Sankyo Agreement	1
Pfizer	3
TOTAL	**51**

POSTGRADUATE PROGRAMMES

In addition, the CNIO — in collaboration with several academic institutions in Spain — provides access to a variety of postgraduate programmes that cover the areas of Cellular & Molecular Biology, Molecular Biomedicine, Biotechnology, Biocomputing & Computational Biology, Clinical & Applied Cancer Research, Therapeutic Targets, and Molecular Oncology.

Official Postgraduate Programmes in Biosciences

The majority of the international postgraduate trainings offered at the CNIO are developed in collaboration with the Faculty of Medicine and Faculty of Sciences at the Autonomous University of Madrid (UAM) through 4 Official Postgraduate Programmes, namely the Doctorate in Biosciences, Masters in Molecular and Cell Biology, Masters in Molecular Biomedicine, and Masters in Biotechnology.



Master's Degree in Biocomputing and Computational Biology

The Master in *Bioinformática y Biología Computacional* — directed by Alfonso Valencia, Director of CNIO's Structural Biology and Biocomputing Programme — is organised together with the National School of Health of the National Institute of Health Carlos III (*Escuela Nacional de Sanidad del Instituto de Salud Carlos III, ENS-ISCiii*), and the Madrid Science Park (*Parque Científico de Madrid*, PCM).

Official Master's Degree in Clinical and Applied Cancer Research

CNIO and the CEU-San Pablo University in Madrid (USP-CEU) jointly organise a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.

Official Master's Degree in Therapeutic Targets, Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department at the University *of Alcalá de Henares* (*UAH*) for the *Máster Oficial en Dianas Terapéuticas, Investigación y Desarrollo.*

Master's Degree in Molecular Oncology

The main objective of this Master's degree, organised in collaboration with the Centre for Biomedical Studies (*Centro de Estudios Biosanitarios, CEB*), is to offer training in molecular oncology with emphasis on the latest findings in translational research that are essential for state-of-the art oncological clinical practice. Upon successful completion of the 500 hours of training, a certificate for a Master's degree in Molecular Oncology — recognised by the European School of Oncology (ESO) — is awarded.

LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology and is organised through agreements with 18 institutions that provide secondary education for laboratory technicians in Spain. It provides students with hands-on knowledge in cellular and molecular biology techniques. The programme consists of 20 weeks (707-712 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student of Analytical Assays and Quality Control for 12 weeks (370 hours), and to 3 students of Clinical Diagnosis for 12 weeks (380 hours). Of the 26 students who participated in this programme in 2016, 5 were hired by the CNIO.

TRAINING FOR MDS

In line with CNIO's commitment to bridge the gap between bench and bedside, the Centre offers 3 programmes providing excellent training opportunities to MDs and other health care professionals. Training usually consists of a 3-month period during residency. In 2016, 17 medical residents from 11 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2016, the *Ramón y Cajal* Programme supported 8 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (now Spanish Ministry of Economy, Industry and Competitiveness) aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected on the basis of their potential capacity to lead independent projects and groups, or to contribute successfully to the ongoing research in the existing groups. Ten other scientists were funded by similar programmes, including the *Juan de la Cierva* programme (5 contracts); the *ISCIII Miguel Servet* (1 contract) and *SEOM-Río Hortega* (contract funded by the Spanish Society of Medical Oncology, 1 contract) programmes; and the Spanish Association Against Cancer (*AECC*, 3 contracts).

VISITING RESEARCHER PROGRAMME

The Jesús Serra Foundation, part of the Catalana Occidente Group, aims to help eminent international specialists work together with CNIO researchers for a few months in order to expand their knowledge in areas of common interest. During 2016, Patrick Sung from the Yale University in New Haven (USA) was beneficiary of the Jesús Serra Foundation's Visiting Researcher Programme.

Fundación Jesús Serra
Catalana Occidente

"SCIENCE BY WOMEN" PROGRAMME

Thanks to this Programme, launched by the Spanish "*Fundación Mujeres por África*", Dorcas Osei Safo from the University of Ghana, Legon, stayed at the CNIO as a visiting scientist in the Experimental Therapeutics Programme, from January to July 2016.

FUNDACIÓN IMA MUJERES POR ÁFRICA

SCIENTIFIC EVENTS

CNIO-"LA CAIXA" FOUNDATION FRONTIERS MEETINGS

The CNIO-"la Caixa" Foundation Frontiers Meetings are the main international conferences that are jointly organised by the CNIO and the "la Caixa" Foundation. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate of scientific ideas. The invited speakers – 20 internationally renowned leaders in oncology – present their latest findings during 2 and a half days. Up to 100 additional participants are selected, via a widely publicised call for applications, based on their potential to make relevant contributions to the conference by presenting hot topics as posters or short talks.

CANCEROMATICS III-TUMOR HETEROGENEITY
13-16 NOVEMBER 2016

ORGANISERS

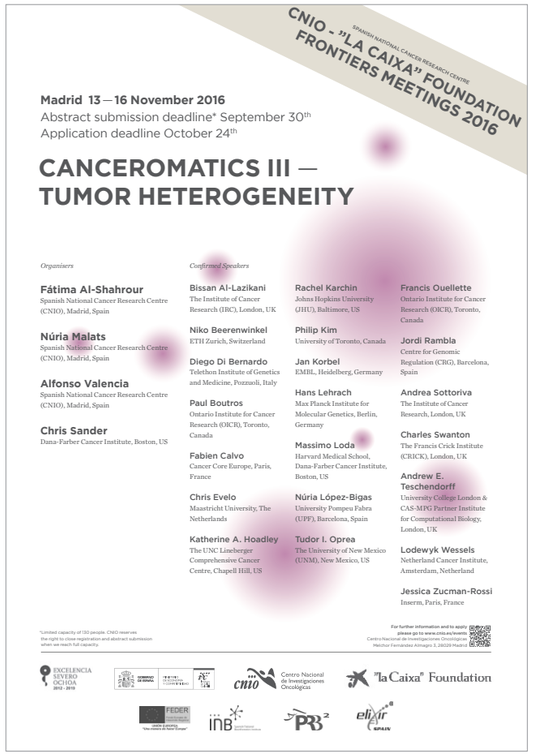
- **Fátima Al-Shahrour**, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain
- **Núria Malats**, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain
- **Chris Sander**, Dana-Farber Cancer Institute, Boston, US
- **Alfonso Valencia**, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain

SESSIONS

- Pan-Cancer analysis
- Analysis of mutations and functional
- Consequences of mutations on pathway and network
- Tumor heterogeneity
- Tumor heterogeneity (part II)
- Drug prediction and repurposing
- Drug prediction and repurposing (part II)
- Translational Genomics
- Translational Genomics (part II)

SPEAKERS

- **Bissan Al-Lazikani,** The Institute of Cancer Research (IRC), London, UK
- **Niko Beerenwinkel**, ETH Zurich, Switzerland
- **Diego Di Bernardo**, Telethon Institute of Genetics and Medicine, Pozzuoli, Italy
- **Paul Boutros**, Ontario Institute for Cancer Research (OICR), Toronto, Canada
- **Fabien Calvo,** Cancer Core Europe, Paris, France
- **Chris Evelo**, Maastricht University, The Netherlands



- **Katherine A. Hoadley**, The UNC Lineberger Comprehensive Cancer Centre, Chapell Hill, US
- **Rachel Karchin**, Johns Hopkins University (JHU), Baltimore, US
- **Philip Kim**, University of Toronto, Canada
- **Jan Korbel**, EMBL, Heidelberg, Germany
- **Hans Lehrach**, Max Planck Institute for Molecular Genetics, Berlin, Germany
- **Massimo Loda**, Harvard Medical School, Dana-Farber Cancer Institute, Boston, US
- **Núria López-Bigas**, University Pompeu Fabra (UPF), Barcelona, Spain
- **Tudor I. Oprea**, The University of New Mexico, (UNM), New Mexico, US
- **Francis Ouellette**, Ontario Institute for Cancer Research (OICR), Toronto, Canada
- **Jordi Rambla**, Centre for Genomic Regulation (CRG), Barcelona, Spain
- **Andrea Sottoriva**, The Institute of Cancer Research, London, UK
- **Charles Swanton**, The Francis Crick Institute (CRICK), London, UK
- **Andrew E. Teschendorff**, University College London & CAS-MPG Partner Institute for Computational Biology, London, UK
- **Lodewyk Wessels**, Netherland Cancer Institute, Amsterdam, Netherland
- **Jessica Zucman-Rossi**, Inserm, Paris, France

In addition, 6 short talks were selected among participants' contributions and 19 posters were presented.

OTHER MEETINGS & CONFERENCES

The CNIO annually hosts various international meetings and conferences. Within this category are the 5 international events held in 2016.

PANCREOS KICK-OFF MEETING
17 MARCH 2016

Participants by country and supporters of the PancreOS are presented:

- Pancreatic Cancer Europe – **Alfredo Carrato**
- EUPancreas COST - **Nuria Malats**
- TTD Spanish Cooperative Group - **Inma Ruiz and Juan José García**
- ENCR/JCR/EU - **Carmen Martos**

BLUEPRINT, ROADMAP, ENCODE AND 1000 GENOMES: BROWSE THROUGH THEM WITH ENSEMBL
4-5 APRIL 2016

ORGANISER

- CNIO Bioinformatics

SPEAKER

- **Denise Carvalho-Silva**, Ensembl Team, European Bionformatics Institute (EBI).

MAKING ACCESS TO PERSONALISED MEDICINE A REALITY FOR PATIENTS
15 SEPTEMBER 2016

SPEAKERS

- **Nuria Malats,** Spanish National Cancer Research Centre (CNIO)
- **Denis Horgan,** EAPM
- **Antoni Andreu,** Ministry of Health
- **Ramon Gonzalez Carvajal,** Regional Ministry of Health of Andalusia
- **Maria Blasco,** Spanish National Cancer Research Centre (CNIO)
- **Natacha Bolaños,** Spanish Group for Cancer Patients, GEPAC
- **Emilia Sanchez Chamorro,** Madrid Health Ministry
- **Ruth Vera,** Spanish Society of Medical Oncology (SEOM)
- **Ivo Gut,** Centro Nacional de Analisis Genomico CNAG-CRG
- **Pablo del Pino,** Celgene
- **Federico Plaza,** Roche Pharma / Roche Institute

VII CONGRESO NACIONAL DE BIOBANCOS Y EL I CONGRESO LATINOAMERICANO DE BIOBANCOS
16-17-18 NOVEMBER 2016

ORGANISERS

- *Biobanco IDIS*
- *Complejo Hospitalario Universitario de Santiago-CHUS*
- *Fundación Ramón Domínguez*
- *Plataforma Red Nacional de Biobancos*
- CNIO

SPEAKERS

- **Maimuna Mendy**, *Agencia Internacional de Investigación en Cáncer (IARC). Biobanco IARC*
- **Gustavo Stefanoff**, *Biobanco del Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA)*
- **Balwir Matharoo-Ball**, Nottingham University Hospital NHS Trust
- **Gonzalo Héctor Ardao**, Hospital Central de las Fuerzas Armadas (HCFFAA)
- **Hugo Campos**, *Biobanco del* A. C. Camargo Cancer Center
- **Luz María Ruíz Godoy**, *Biobanco del Instituto Nacional de Cancerología (INCan)*
- **Liliana Virginia Siede**, *Ministerio de Ciencia, Técnica e Innovación Productiva de la Nación Argentina*
- **Pilar Nicolás**, *Universidad de Deusto*, Bilbao
- **Máximo Fraga**, *Biobanco CHUS/IDIS*
- **Jorge Pombo Otero**, *Servicio de Anatomía Patológica CHUAC*
- **Rocío Aguilar**, *Biobanco del Sistema Sanitario Público de Andalucía*
- **Ana Caroline Neuber**, Biobank, Barretos Cancer Hospital, Barretos, SP
- **Cristina Villena Portella**, *Centro Investigación Biomédica en Red, Enfermedades Respiratorias (CIBERES)*
- **Diego Santos**, *Proyecto Coppadis*
- **Susana Teijeira**, *Banco de Cerebros. Biobanco Vigo. (IISGS)*

- **Silvia Sánchez**, *Instituto de Investigación Sanitaria Hospital La Fe*
- **Gema Huesa**, BarcelonaBeta Brain Research Center, *Fundación Pasqual Maragall*
- **Manuel Rodríguez Castro**, *AGAELA*
- **Carmen López Rodríguez**, *FEGEREC*
- **Anna Bosch-Comas**, Biobanc HCB-IDIBAPS
- **Ángela González Ferro**, *Hospital Universitario Lucus Augusti*
- **Nuria Ajenjo**, *Centro Nacional de Investigaciones Oncológicas, CNIO*
- **Natalia Cal Purriños**, *Fundación Profesor Novoa Santos - Instituto Investigación Biomédica A Coruña (INIBIC)*
- **Roberto Bilbao**, *Biobanco Vasco*
- **Pilar Nicolás**, *Universidad de Deusto*, Bilbao
- **Rodrigo Dienstmann**, *Instituto de Oncología del Hospital Vall d'Hebron*
- **Juan Cruz Cigudosa**, *Centro Nacional de Investigaciones Oncológicas (CNIO)*
- **Abel González**, *Universitat Pompeu Fabra*
- **Manuel M Morente**, *Plataforma Red Nacional de Biobancos (ISCIII)*
- **Inés Aroca Siendones**, *Biobanco Sistema Sanitario Público de Andalucia*
- **Verónica Valdivieso Gómez**, *Biobanco SSPA*
- **Ana Garcia Díaz**, *Centro de Diagnóstico de Enfermedades Moleculares*
- **Raquel Bermudo**, *Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)*
- **Rosa María Pinto Labajo**, *Banco Nacional de ADN Carlos III, Instituto de Investigación Biomédica de Salamanca*
- **Mara Ortega Gómez,** *Biobanco HUP. Instituto de Investigación sanitaria Hospital Universitario de la Princesa*
- **Joan Ramón Gómez Cortijo**, *Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana*
- **Raquel Amigo**, *Hospital Universitari i Politècnic La Fe (IIS-La Fe)*
- **Gustavo Stefanoff**, *Instituto Nacional de Cáncer (INCA)/ Ministerio de Salud*
- **Carol Aristimuño**, *Biobanco Vasco: Nodo Hospital Universitario Araba*
- **Joan Amoedo Cibeira**, *GoodGut SL*
- **Andrés García Montero,** *Banco Nacional de ADN Carlos III*
- **Máximo Fraga**, *Biobanco CHUS/IDIS*
- **Teresa Escamez**, *Biobanco en Red de la Región de Murcia (BIOBANC-MUR)*

SENESCENCE & CANCER
2ND ANNUAL MEETING OF THE SPANISH NETWORK OF CELLULAR SENESCENCE
25 NOVEMBER 2016

GROUPS OF THE SPANISH NETWORK:

- **Joaquín Arribas** (Vall d'Hebron Institute of Oncology, Barcelona, Spain) and members from his laboratory
- **Manuel Collado** (*Instituto de Investigación Sanitaria – Complejo Hospitalario Universitario de Santiago de Compostela*, Spain) and members from his laboratory
- **Bill Keyes,** (previously at the Centre of Genomic Regulation, Barcelona, and currently at the IGBMC, Strasbourg, France) and members from his laboratory
- **Ramón Martínez** (*Univ. Politécnica de Valencia*, Valencia, Spain) and members from his laboratory
- **Ignacio Palmero** (*Instituto de Investigaciones Biomédicas* "Alberto Sols", Madrid, Spain) and members from his laboratory
- **Manuel Serrano** (CNIO, Madrid, Spain) and members from his laboratory
- **Anxo Vidal** (*Universidad de Santiago de Compostela*, Spain) and members from his laboratory

EXTERNAL INVITED SPEAKERS:

- **Juan Carlos Acosta**, IGMM, Edinburgh, UK
- **Marco Demaria,** ERIBA, Groningen, The Netherlands
- **Valery Krizhanovsky**, Weizmann Institute, Rehovot, Israel

TRAINING COURSES AND WORKSHOPS

The CNIO is committed to disseminating the results of state-of-the-art cancer research to the wider community, including medical professional and junior scientists, thereby enabling them to stay abreast of recent developments in specialised techniques. This is achieved through training courses and hands-on workshops organised by CNIO scientists and and technologists.

FLOW CYTOMETRY SEMINAR SERIES
25-26-27 JANUARY 2016

SPEAKERS/ORGANISERS

- **Rui Gardner**, Head of the Flow Cytometry Lab. *Instituto Gulbenkian de Ciência, Oeiras*, Portugal
- **Lola Martinez**, Head of the Flow Cytometry Unit, CNIO, Madrid, Spain

ADVANCED CELL SORTING COURSE
28-29 JANUARY 2016

SPEAKERS/ORGANISERS

- **Rui Gardner**, Head of the Flow Cytometry Lab. Instituto Gulbenkian de Ciência, Oeiras, Portugal
- **Lola Martinez**, Head of the Flow Cytometry Unit, CNIO, Madrid, Spain

FORMACIÓN CONTINUADA PARA LA REALIZACIÓN DE LAS FUNCIONES DEL RD/2013: TÉCNICAS DE IMAGEN EN INVESTIGACIÓN PRECLÍNICA
25 FEBRUARY 2016

SPEAKER

- **Francisca Mulero**, Molecular Imaging Core Unit, Biotechnology Programme, CNIO

WORKSHOP REDIEX EXO-IMAGING CNIO
26-28 JULY 2016

SPEAKERS

- **Hernando del Portillo**
- **Héctor Peinado**
- **Isabel Guerrero**
- **Diego Megias**
- **Juan Monteagudo**
- **Susana Garcia Silva**
- **Ana Amor**
- **Marta Hergueta**
- **Maria Yañez-Mo**
- **Antonio Marcilla**
- **Francesc E. Borrás**
- **Juan Manuel Falcón-Pérez**
- **Antonio Osuna**
- **Lucía Robado**

URO-ONCOLOGICAL PATHOLOGY TUTORIAL: A 3-DAY "MEET THE EXPERT"
24-25-26 NOVEMBER 2016

ORGANISERS

Núria Malats and **Francisco X. Real**, CNIO, Madrid, Spain

TALLER CEGEN-PRB2: ESTUDIOS DE ASOCIACION: DISEÑO Y ANÁLISIS DE DATOS
29 NOVEMBER 2016

ORGANISER

- CEGEN

SPEAKERS

- **Javier Benítez**, Director del Nodo del CeGen*ISCIII en Madrid. Director del Programa de Genética Humana, Centro Nacional de Investigaciones Oncológicas*, CNIO
- **Anna Gonzalez –Neira**, *Jefe de Unidad de Genotipado Humano-CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas*, CNIO
- **Guillermo Pita,** *Unidad de Genotipado Humano-CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas*, CNIO
- **Rosario Alonso**, *Unidad de Genotipado Humano- CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas*, CNIO
- **Pablo Fernández**, *Jefe de Área de Epidemiología Ambiental y Cáncer, Centro Nacional de Epidemiología ISCIII, Madrid. CIBER en Epidemideología y Salud Pública -CIBERESP*
- **Ana Osorio**, *Grupo de Genética Humana, Centro Nacional Investigaciones Oncológicas, CNIO*
- **Agustín Fernández Fernández**, *Unidad de epigenética del Cáncer, Instituto Universitario de Oncología del Principado de Asturias (IUOPA)*
- **Sara Ruiz –Pinto**, *Unidad de Genotipado Humano-CeGen, Centro Nacional Investigaciones Oncológicas*, CNIO
- **Lola Alonso**, *Grupo de Epidemiología Genética, Centro Nacional Investigaciones Oncológicas*, CNIO

COURSE OF ANIMAL LABORATORY FROM C TO D
12-13-14-15-16 DECEMBER 2016

ORGANISERS

- CNIO
- *Animalaria Formación y Gestión*

SPEAKERS

- **Manuel Berdoy**, University of Oxford, UK
- **Ignasi Sahun**, PCB, Spain
- **José M. Orellana**, University of Alcala, Spain
- **Sagrario Ortega**, CNIO, Spain
- **Ignacio Álvarez**, UCM, Spain
- **Marlees Leenars**, Radbound UMC, Netherlands
- **Ángel Naranjo**, CNB, Spain
- **Javier Guillén**, AAALAC International, Spain
- **Pedro Pablo López**, CNIO, Spain
- **Violeta Solis**, Glaxo Smitkline, Spain
- **Alba de Martino**, CNIO, Spain
- **Belén Pintado**, CBM, Spain
- **Francisca Mulero**, CNIO, Spain
- **Isabel Blanco**, CNIO, Spain

CNIO DISTINGUISHED SEMINARS

The purpose of the Distinguished Seminars Series is to invite outstanding and internationally renowned scientists to give a seminar and to meet with researchers at the CNIO. Distinguished Seminars are recurrent events that are open to the general public and are held throughout the year, usually on Fridays at noon in the CNIO Auditorium. Each Distinguished Seminar series includes world-leading scientists who address topics that are of general interest to the CNIO faculty.

The purpose of this international seminar series is not limited to bringing outstanding cancer researchers to the CNIO, but also serves to annually invite 3 to 4 opinion leaders from other areas of science, technology, and literature; the overarching goal is to enable the CNIO to present its know-how as well as its vision on contemporary and future technological, societal and cultural challenges. These "out-of-the-box" seminars are sponsored by the "*Fundación Banco Sabadell*". The breadth of expertise and topics covered creates a multidisciplinary and intellectually challenging environment that goes far beyond the frontiers of cancer research.

In total, the CNIO hosted 23 distinguished speakers in 2016.

DATE	SPEAKER	ORGANISATION	TITLE	
JANUARY				
15/01/2016	**Giulio Draetta**	Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center, Houston, USA	Integrating functional genomics with drug discovery to overcome resistance to treatment in multiple cancer	
FEBRUARY				
05/02/2016	**Sarah Teichmann**	EMBL- European Bioinformatics Institute & Wellcome Trust Sanger Institute, Cambridge, UK	Understanding Cellular Heterogeneity	
12/02/2016	**Romain Quidant**	ICREA, ICFO- The Institute of Photonic Sciences, Barcelona, Spain	Applications to oncology of light and nanotechnology	Fundación BancoSabadell
19/02/2016	**Joseph Jonkers**	Netherlands Cancer Institute, Amsterdam	Genetic dissection of breast cancer development, therapy response and resistance in mouse models	
26/02/2016	**Cory Brayton**	Johns Hopkins University School of Medicine, Baltimore, USA	Research relevant immune variations in mice	
MARCH				
04/03/2016	**Michael Sieweke**	Center of Immunology Marseille-Luminy, France	Beyond stem cells: Dissecting lineage identity and self-renewal	
11/03/2016	**Nicholas Dyson**	James and Shirley Curvey MGH Research Scholar, Harvard Medical School, Boston, USA	The consequences of Rb inactivation	
APRIL				
15/04/2016	**Andras Nagy**	Mount Sinai Hospital Lunenfeld-Tanenbaum Research Institute, Toronto, Canada	Reprogramming Leads to Multiple States of Pluripotency in the Artificial Cell Space	
22/04/2016	**Herbert Waldmann**	Max-Planck Institute of Molecular Physiology, Dortmund, Germany	Chemical Biological Modulation of Ras-Signaling	
29/04/2016	**Navdeep S. Chandel**	Northwestern University, Feinberg School of Medicine, Chicago, USA	Mitochondria as signaling organelles	
MAY				
06/05/2016	**Andrés Moya**	University of Valencia, Spanish Evolutionary Biology Society (SESBE), Valencia, Spain	Man: Nature and Future	Fundación BancoSabadell
13/05/2016	**Anna M. Wu**	Crump Institute for Molecular Imaging, David Geffen School of Medicine at UCLA, Los Angeles, US	ImmunoPET: Engineered antibodies for noninvasive imaging of tumors and immune cell subsets	
20/05/2016	**Mathias Heikenwälder**	DKFZ - German Cancer Research Center, Heidelberg, Germany	How cells of the immune system control development of fatty liver disease, non-alcoholic steatohepatitis and subsequent	
JUNE				
03/06/2016	**Stephan Herzig**	Institute for Diabetes and Cancer IDC Helmholtz Center, Munich, Germany	Cancer and Metabolism: A bi-directional connection	

10/06/2016	**Adolfo Ferrando**	Columbia University Medical Center, New York, USA	Oncogenic circuitries and mechanisms of resistance in acute lymphoblastic leukemia
27/06/2016	**Diane Simeone**	University of Michigan Health System, Ann Arbor, USA	TRIM29: an Oncogenic Driver in Human Cancers
SEPTEMBER			
16/09/2016	**Francisco Juan Martinez Mojica**	University of Alicante, Spain	The history behind «the CRISPR craze»
OCTOBER			
14/10/2016	**Francisco J. Ayala**	University of California, Irvine, US	Genetic Engineering and Mankind's Future
21/10/2016	**Mike Hall**	Biozemtrum, University of Basel, Switzerland	mTOR signaling in growth and metabolism
28/10/2016	**Charles Brenner**	Roy J. Carver Chair & Head of Biochemistry, University of Iowa, US	Nicotinamide Riboside: From Discovery to Human Translation
NOVEMBER			
11/11/2016	**Stig E. Bojesen**	*Herlev and Gentofte Hosptial*, Copenhagen University Hospital Faculty of Health Sciences, University of Copenhagen, Denmark	DNA and cancer in the general population
DECEMBER			
02/12/2016	**Celeste Simon**	Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, USA	Metabolic adaptations during tumor progression
16/12/2016	**Hans-Guido Wendel**	Memorial Sloan-Kettering Cancer Center, New York, US	Restoring tumor suppression with CAR-T micro-pharmacies

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous ad-hoc seminars throughout the year. A total of 49 ad-hoc seminars were organised by CNIO researchers in 2016.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
08/01/2016	**Rosa María Señarís**	University of Santiago de Compostela, Spain	Metabolism of cancer cells: Lessons from astrocytic tumors
11/01/2016	**Michele de Palma**	The Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, Suiza	microRNA regulation of tumor-associated macrophages and response to immunotherapy
FEBRUARY			
11/02/2016	**Jeremy Graff**	Biothera Pharmaceutical, Inc., Minneapolis, US	Imprime PGG, a Pathogen Associated Molecular Pattern (PAMP), triggers a coordinated, anti-cancer immune respons

12/02/2016	**Arianna Bianchi**	Institute of Genetic Medicine, Newcastle University in Newcastle, UK	Development of a novel scaffold-free 3D spheroid model of the cornea
22/02/2016	**Eva Gonzalez Suarez**	Bellvitge Biomedical Research Institute (PEBC) -IDIBELL Duran i Reynals Hospital, Barcelona, Spain	Therapeutic opportunities for RANK pathway in cancer
MARCH			
01/03/2016	**Chris Thorne**	Horizon Discovery Ltd., Cambridge, United Kingdom	Lessons learned from high throughput CRISPR targeting in human cell lines
APRIL			
12/04/2016	**Marc A. Marti-Renom**	National Centre for Genomic Analysis (CNAG) - Center for Genomic Regulation (CRG), Barcelona, Spain	Structure determination of genomes and genomic domains by satisfaction of spatial restraints
12/04/2016	**Karl-Dimiter Bissig**	Baylor College of Medicine Center for Cell and Gene Therapy, Houston, US	From Cancer to metabolic disease - novel applications of human liver chimeric mice
13/04/2016	**Rafael De Cabo**	National Institute on Aging, Baltimore, US	Dietary Interventions for Healthy Aging
26/04/2016	**Mattia Pelizzola**	Center for Genomic Science of the IIT, Milan, Italy	Epitranscriptomic and regulatory determinants of transcriptional dynamics
JUNE			
02/06/2016	**Dana Branzei**	IFOM Foundation - The FIRC Institute of Molecular Oncology Foundation, Milan, Italy	DNA replication and recombination: tight connections
02/06/2016	**Maaike Pols**	Scientific Outreach Manager Faculty of 1000, London, UK	A new way of writing, discovering and sharing science
08/06/2016	**Damien P. Devos**	CABD; Pablo de Olavide University - CSIC, Sevilla, Spain	Deciphering the evolution of genome locus conformation from 4C data
13/06/2016	**Eusebio Manchado**	Novartis Institute for Biomedical Research, Basel, Switzerland	Identifying vulnerabilities in undrugabble cancers using RNAi multiplexed technology
16/06/2016	**Maria Soriano Carot**	Addgene, Cambridge, US	Addgene, an easy way to share plasmids
20/06/2016	**Sean Post and Miguel Gallardo**	MD Anderson (Prof. Department of Leukemia), Houston, US	Developing Personalized Therapies for a High-Risk Patient Population with Acute Myeloid Leukemia
22/06/2016	**Marek Wagner**	University of Bergen, Norway	The tumor microenvironment and its contribution to melanoma growth and progression
23/06/2016	**Alberto Gandarillas**	Marqués de Valdecilla Institute-IDIVAL, Santander, Spain	A novel differentiation-mitosis checkpoint
28/06/2016	**Randy Y.C. Poon**	Hong Kong University of Science and Technology, Kowloon, Hong Kong	Exit Strategies – the many fates of mitosis in cancer cells
30/06/2016	**Gabriel Victora**	Whitehead Institute for Biomedical Research, Cambridge, US	Clonal Dinamycs in the Antibody Response
JULY			
14/07/2016	**Pekka Katajisto**	Institute of Biotechnology, University of Helsinki, Finland	Age-selective segregation of organelles by stem-like cells
22/07/2016	**Kathleen Meyer**	Institute of Pharmacology and Toxicology, TU Munich, Munich, Germany	Essential role for premature senescence of myofibroblasts in myocardial fibrosis
26/07/2016	**Michael T. Heneka**	University of Bonn, Clinic und Polyclinic for Neurology, Bonn, Germany	Innate immune activation: a detrimental connection between inflammation and neurodegeneration

AUGUST			
08/08/2016	**Luis Arnes Pérez**	Biomedical Informatics, Columbia University, New York, US	Functional analysis of long non-coding RNAs associated with Pancreatic Ductal Adenocarcinoma
09/08/2016	**Mark A. Febbraio**	Garvan Institute of Medical Research, Sidney. Australia	Molecular mechanisms for the protective effects of physical exercise on cancer incidence
SEPTEMBER			
02/09/2016	**James DeGregori**	University of Colorado, Aurora, US	Connecting cancer to aging: An evolutionary approach using in silico and in vivo modeling
07/09/2016	**Alvaro Ingles Prieto**	Institute of Science and Technology, Klosterneuburg, Austria	Optogenetic control of Receptor Tyrosine Kinases with light-oxygen-voltage
13/09/2016	**Guillem Paniagua Soriano**	PhD student at Leiden University, Netherlands	Proteasome inhibitor acquired drug-resistance in Multiple Myeloma
14/09/2016	**Yann Cormerais**	Centre Scientifique de Monaco	Amino Acids and Cancer: LAT1 a transporter essential for mTORC1 activity and tumor growth
26/09/2016	**Ana Janic**	The Walter and Eliza Hall Institute, Melbourne, Australia	Identification of the critical p53 tumour suppression mechanisms in vivo
26/09/2016	**Iain Cheeseman**	Whitehead Institute for Biomedical Research, Cambridge, US	Dissecting the Mechanisms of Cell Division using CRISPR/Cas9
OCTOBER			
06/10/2016	**Ronald Koop**	PerkinElmer, Waltham, US	Advanced In Vivo Optical Imaging: Tomography, Spectral Unmixing and Co-Registration
13/10/2016	**Llucia Albertí Servera**	Basel University, Switzerland	Single-cell analysis of early haematopoietic development: multipotenitality or heterogeneity?
18/10/2016	**Masayuki Yamamoto**	Tohoku University Graduate School of Medicine, Sendai, Japan	The Keap1-Nrf2 System and Cancer Development
21/10/2016	**Senthil Muthuswamy**	Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, US	Tumor and Normal Pancreas Organoids: A live biobank platform for discovery and translation
27/10/2016	**Maria Sibilia**	Institute for Cancer Research. Medical University of Vienna, Austria	Immune regulation in inflammation and cancer
NOVEMBER			
03/11/2016	**Diana Pippig and Magnus Bauer**	Ludwig Maximilian University, Munich, Germany	Probing Protein Structure and Function by means of Atomic Force Microscopy
17/11/2016	**Alejandro Sweet- Cordero**	Stanford University School of Medicine, US	Genomic analysis of osteosarcoma patient-derived xenografts defines therapeutic opportunities and metastatic driver
22/11/2016	**Arkaitz Carracedo**	CIC bioGUNE, University of the Basque Country, Derio, Spain	Metabolic rewiring in prostate cancer
22/11/2016	**Marta Kovatcheva**	Koff Laboratory, Memorial Sloan-Kettering Cancer Center, NY, US	New roles for old proteins: MDM2, ATRX and HRAS drive the transition from quiescence to senescence
28/11/2016	**Eli Gilboa**	Miller School of Medicine, University of Miami, USA	Inducing neoantigens in therapeutic and prophylactic cancer immunotherapy
29/11/2016	**Sophie Vasseur**	CRCM. Cancer Research Center of Marseille, France	Decrypting the metabolic flexibility of pancreatic adenocarcinoma

DECEMBER			
05/12/2016	**Graham Robertson**	Centenary Institute, Camperdown, Australia	Skin inflammation, type 2 immunity and the atopic march
05/12/2016	**Amalio Telenti**	Human Longevity Inc., San Diego, US	Genomes are just data
12/12/2016	**Jorge Moscat**	Sanford Burnham Prebys Medical Discovery Institute, La Jolla, US	Stroma-epithelium signaling crosstalk by p62 in cancer
14/12/2016	**Alfredo Caro**	CIC bioGUNE	Center for Cooperative Research in Biosciences, Derio, Bilbao	Anti-cholesterolemic treatment promotes prostate cancer aggressiveness
15/12/2016	**Federico Pietrocola**	Cordeliers Research Center University of Paris Descartes, Paris, France	Caloric Restriction Mimetics: mechanisms and impact on anticancer therapy
16/12/2016	**Albert Jordan**	Molecular Biology Institute of Barcelona, Spain	Specificities and genomic distribution of somatic human histone H1 subtypes
21/12/2016	**Aranzazu Villasante**	Columbia University, New York City, US	Cancer Engineering: A Translation from One Lan guage to Another

CNIO-WOMEN IN SCIENCE (WISE) SEMINARS

These seminars are aimed to raise gender awareness via lectures given by gender experts and/or role models, and also to provide CNIO researchers with an opportunity to expand their networks.

12/01/2016	**María Concepción Ferreras**	You Tube, Head of Partnerships, Southern Europe and Russia	Woman and Technology: a positive story
19/01/2016	**Margery Resnick**	Massachusetts Institute of Technology, Cambridge, US and International Institute in Madrid, Spain	In Her Own Voice: MIT's Earliest Women Scientists
23/02/2016	**Pilar Garrido**	IRYCIS. Ramón y Cajal University Hospital, Madrid, Spain	Lung cancer in women: a different disease?
08/03/2016	**Edurne Pasaban**	Mountaineer, Tolosa, Spain	EXPEDITION TO SUCCESS: Achieving goals and overcoming difficulties
10/05/2016	**María Teresa Fernández de la Vega**	President of Women for Africa Foundation (*Fundación Mujeres por África*), Former Vicepresident of Spain	Recuerdos y olvidos feministas
28/06/2016	**Tánia Balló**	Documentalist and Film Director, Barcelona, Spain	Las Sinsombrero, sin ellas la historia no está completa
27/09/2016	**Ángeles González-Sinde**	Scriptwriter, Film Director. Former Spanish Minister of Culture	Out of focus: women and film (*Fuera de foco: mujeres y cine*)
07/11/2016	**Guadalupe Martín Martín**	Radiofísica Hospitalaria/Medical Physicist. *Servicio de Radiofísica* / Medical Physics Service. Fuenlabrada University Hospital, Madrid, Spain	Marie Sklodowska-Curie: Medical Phsycs pioneer and inspiration to female scientists
20/12/2016	**Christina Rosenvinge**	Spanish singer-songwriter, actress and producer	30 years of work: my view on the politics and procedures of the Spanish and global music scenes/*30 años de trabajo: mi visión en la política y procedimientos en la escena musical Española y global*

SCIENTIFIC DIVULGATION EVENTS

RESEARCHERS' NIGHT
30 SEPTEMBER 2016

This year, the CNIO participated in the Researchers' Night; an activity aimed at bringing researchers closer to the general public and concerned families in order to give them the opportunity to learn more about what researchers do for society. Each year, more than 300 European cities participate, in parallel, in what is a great night for science. During the activities – promoted by the European Commission and coordinated by the Madrid Regional Government and the madri+d Foundation – a total of 200 people came to the Spanish National Cancer Research Centre (CNIO) to attend Researchers' Night (September 30, 2016) and to learn about cancer research. The activities, which were entirely organised by voluntary contributions from 30 young researchers, provided guests the opportunity to meet researchers in an interactive and entertaining way. These included hands-on experiments, view of a virtual tour through the facilities thanks to a video project recorded by scientists from CNIO "CNIO for Kids", and a speed dating session with the researchers.

OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER
7-20 NOVEMBER 2016

The CNIO also dedicates considerable efforts to bringing science and society closer together; one of these endeavours is its collaboration with the madri+d research network for the organisation of the Madrid Science Week (XVI *Semana de la Ciencia*, 7-20 November, 2016). In 2016, 50 people participated in the guided visit to the Centre's facilities.

MARIE CURIE EXHIBITION
NOVEMBER 2016

The CNIO hosted an exhibition entitled "Marie Sklodowska-Curie. A Pole in Paris"; the exhibition revisited the personal and professional life of one of the key women of the 20th century. The exhibition opened on 7 November — the birthday of this Polish-French scientist — and could be visited until the 30th of November. This initiative of the CNIO's Women in Science Office (WISE) stands testament to our commitment to promoting and upholding the work of women scientists. The exhibition attracted 296 visitors.

BCNMOMENTS
14 JULY 2016

During this year, the company bcnmoments organised the "Leading Program Madrid"; a programme sponsored by the "la Caixa" Foundation that awards the 20 highest *selectividad* test scores within the Community of Madrid. The selected students had the opportunity to get to know different success stories in a broad range of companies and institutions, including the CNIO. During their "Business Experience" at the CNIO in July 2016, the students had the chance to visit the labs guided by young scientists.

GUIDED VISITS

Throughout the year, the CNIO provides tailor-made opportunities to visit its installations and to learn about the essentials of cancer research. During 2016, more than 471 people participated in such guided visits; most of them were ESO and *Bachillerato* student groups, but also professionals in the health sector.

ADMINISTRATION

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Minister of Economy, Industry and Competitiveness
Ministro de Economía, Industria y Competitividad

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Director of the National Institute of Health Carlos III
Director del Instituto de Salud Carlos III

→ **Appointed Members**

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Secretario General de Sanidad y Consumo del Ministerio de Sanidad, Servicios Sociales e Igualdad

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Directora de la Agencia Estatal de Investigación del Ministerio de Economía, Industria y Competitividad

· **Cristina Ysasi-Ysasmendi Pemán**
Director of the Department of National Affairs of the Cabinet of the Presidency of the Government
Directora del Departamento de Asuntos Nacionales del Gabinete de la Presidencia del Gobierno

· **Margarita Blázquez Herranz**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirectora General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III

· **Luis Gabilondo Pujol**
Director General of Health of the Health Department of the Government of Navarre
Director General de Salud de la Consejería de Salud del Gobierno de Navarra

· **Carlos Pesquera González**
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Jefe de Gabinete de la Consejera de Sanidad del Gobierno de Cantabria

· **Luis Ángel León Mateos**
Director of Health Research Planning and Promotion of the Galician Health Service (*SERGAS*)
Director del Área de Planificación y Promoción de la Investigación Sanitaria del Servicio Gallego de Salud de la Xunta de Galicia (SERGAS)

· **Sandra García Armesto**
Managing Director of the Aragon Institute of Health Sciences
Directora Gerente del Instituto Aragonés de Ciencias de la Salud

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· **Rafael Pardo Avellaneda**
Director, *BBVA* Foundation
Director de la Fundación BBVA

· **Jaume Giró Ribas**
CEO of *"la Caixa"* Banking Foundation *Caixa d' Estalvis i Pensions de Barcelona*
Director General de la Fundación Bancaria Caixa d' Estalvis i Pensions de Barcelona, "la Caixa"

· **Ignacio Polanco Moreno**
Chairman, *Grupo PRISA*
Presidente del Grupo PRISA

· ***Caja Madrid* Foundation** (until September 2016)
Fundación Caja Madrid

→ **Secretary**

· **Margarita Blázquez Herranz**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirectora General de Redes y Centros de Investigación Cooperativa, Instituto de Salud Carlos III

→ **Legal Advisor**

· **Fernando Arenas Escribano**
Chief State's Attorney of the Ministry of Health, Social Services and Equality
Abogado del Estado Jefe en el Ministerio de Sanidad, Servicios Sociales e Igualdad

* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:
- At the close of the financial year, the accumulated remuneration received by the Top Management of the Foundation – the CNIO's Director plus the Managing Director – has amounted to a total of 203,845 Euros. This amount was received as base salary, seniority, and position supplement.
- Members of the CNIO Board of Trustees are not remunerated.

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	PROJECTS & CONSORTIA	**Liébanes, M. Dolores** Head **Ares, Raquel**	**Merino, Ana** **Vergés, Leyre** (since December)
	EDUCATION & TRAINING PROGRAMMES	**Molina, Juan Ramón** Head	**Del Codo, Almudena** * (since March)
	SCIENTIFIC EVENTS	**Moro, Mercedes** Head	
	SCIENTIFIC PUBLISHING	**Cerdá, Sonia** Head	
	LIBRARY & ARCHIVES	**López, Victoria** Head	
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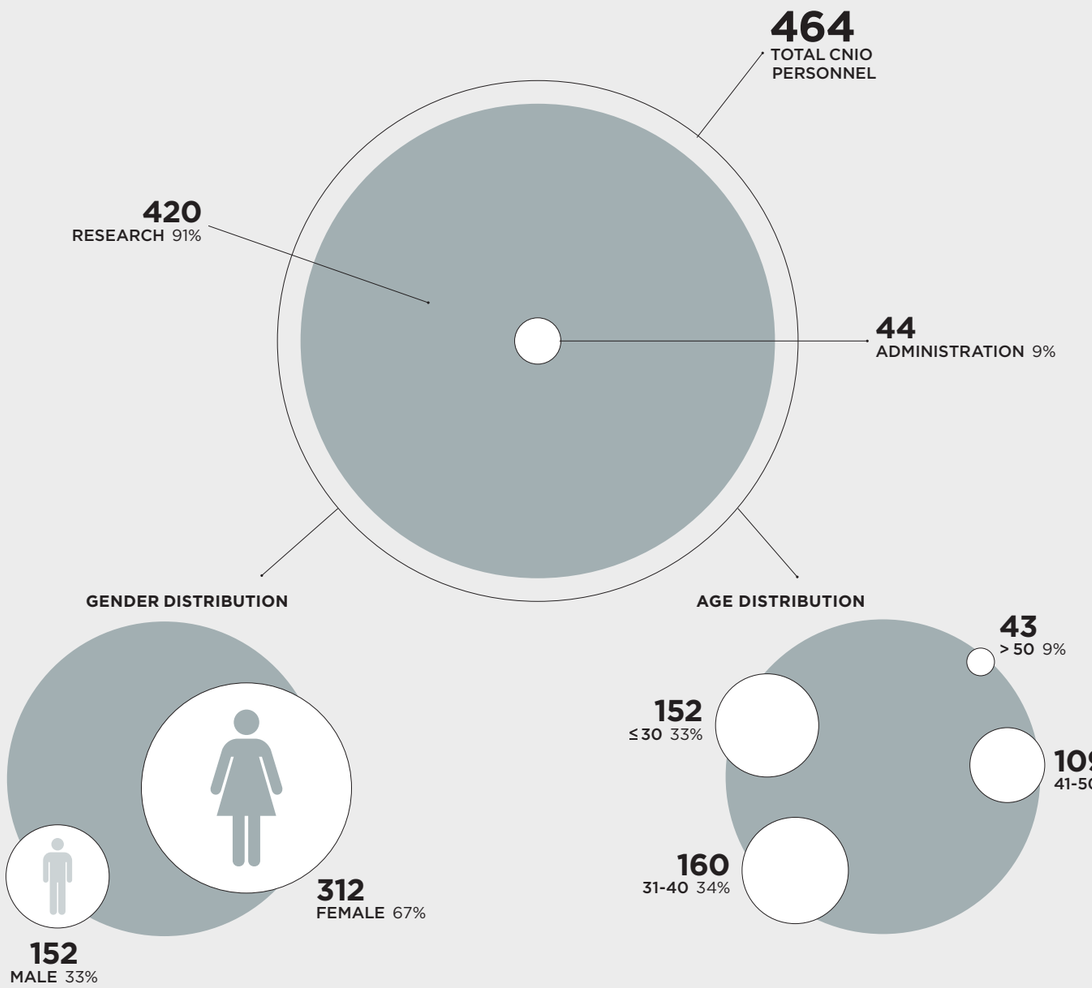
EXTRAMURAL CLINICAL RESEARCH	**López, Antonio** Director (until December)		

* *Plan de Empleo Joven* (Youth Employment Plan)

CNIO PERSONNEL 2016



GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

GROUP LEADERS, HEADS OF CLINICAL RESEARCH UNIT/SECTION	FEMALE	32%	**10**
	MALE	68%	**21**
HEADS OF UNIT/BIOBANK	FEMALE	53%	**10**
	MALE	47%	**9**
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	50%	**7**
	MALE	50%	**7**
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	33%	**4**
	MALE	67%	**8**

SCIENTIFIC PERSONNEL 2016

TOTAL SCIENTIFIC PERSONNEL **420**

DISTRIBUTION BY PROGRAMMES

STRUCTURAL BIOLOGY AND BIOCOMPUTING 10%	44
BIOTECHNOLOGY 11%	47
CANCER CELL BIOLOGY 10%	40
HUMAN CANCER GENETICS 12%	51
CLINICAL RESEARCH 18%	75
MOLECULAR ONCOLOGY 30%	127
EXPERIMENTAL THERAPEUTICS 9%	36

DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 10%	43
GRADUATE STUDENTS 25%	104
STAFF SCIENTISTS 17%	72
PRINCIPAL INVESTIGATORS 12%	50
TECHNICIANS 36%	151

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS	FEMALE	67%	**29**
	MALE	33%	**14**
GRADUATE STUDENTS	FEMALE	70%	**73**
	MALE	30%	**31**
STAFF SCIENTISTS	FEMALE	71%	**51**
	MALE	29%	**21**
PRINCIPAL INVESTIGATORS	FEMALE	38%	**19**
	MALE	62%	**31**
TECHNICIANS	FEMALE	74%	**111**
	MALE	26%	**40**
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**283**
	MALE		**137**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL **420**

POST-DOCTORAL FELLOWS 14%	29
GRADUATE STUDENTS 34%	71
STAFF SCIENTISTS 15%	32
PRINCIPAL INVESTIGATORS 11%	23
TECHNICIANS 26%	56
TOTAL 100%	211

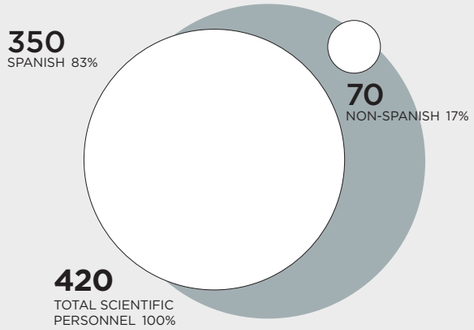
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

POST-DOCTORAL FELLOWS 10%	13
GRADUATE STUDENTS 24%	30
STAFF SCIENTISTS 22%	27
PRINCIPAL INVESTIGATORS 10%	13
TECHNICIANS 34%	43
TOTAL 100%	126

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

POST-DOCTORAL FELLOWS 1%	1
GRADUATE STUDENTS 3%	3
STAFF SCIENTISTS 16%	13
PRINCIPAL INVESTIGATORS 17%	14
TECHNICIANS 63%	52
TOTAL 100%	83

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

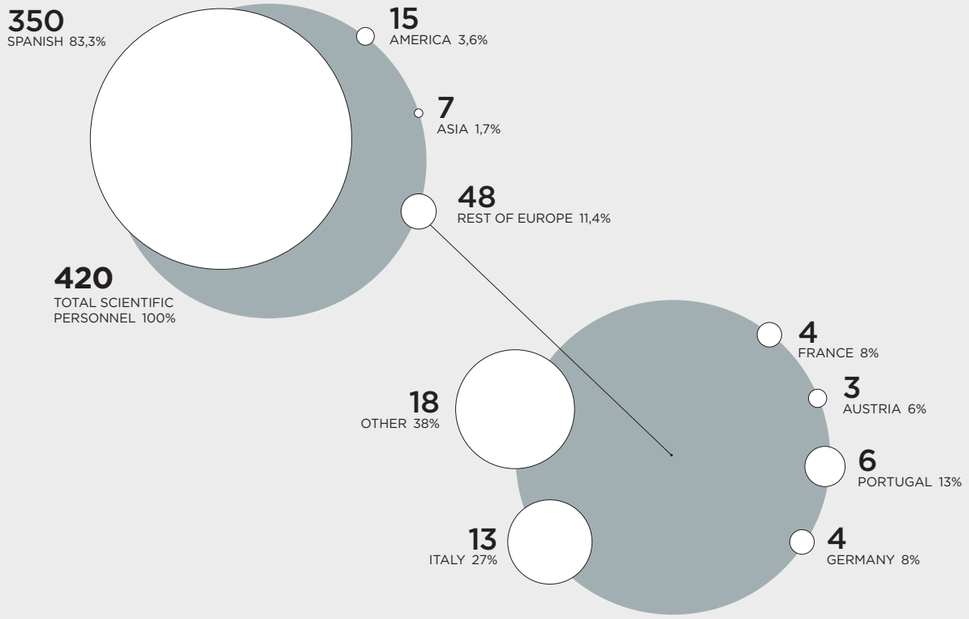


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 33%	14
GRADUATE STUDENTS 24%	25
STAFF SCIENTISTS 22%	16
PRINCIPAL INVESTIGATORS 12%	6
TECHNICIANS 6%	9

Total foreign scientific personnel 70
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



CNIO Friends

CNIO FRIENDS

The 'CNIO Friends' initiative celebrated its first two years of existence at the end of 2016, and it did so by looking towards the future with all the enthusiasm, commitment, and effort it received from its donors. Since its inception, the community of Friends has continuously increased and more and more people decided to renew their commitment, and thereby their loyalty and trust, to the Centre's scientific research. We closed 2016 with a community of about 800 Friends, in addition to inheritances and legacies from individuals who decided to support the CNIO's cutting-edge research; our challenge now is to get our message across more effectively with every announcement we make.

In 2016, thanks to our Friends, we launched the first three cancer research postdoctoral contracts after a competitive assessment process based on scientific excellence. The first two were allocated to sponsor the researchers Paulina Gómez, from the Genetic and Molecular Epidemiology Group, and Vera Pancaldi, from the Structural Computational Biology Group. The former will be working on an international project aimed at analysing the relationship between multiple risk factors and pancreatic cancer, one of the cancers with the highest mortality rates today; the latter will be studying how DNA structures affect tumour aggressiveness, among other factors.

Both researchers stressed "the crucial importance of philanthropy as a driving force in scientific research and their surprise about the difference between the Anglo-Saxon system - where philanthropy is fully integrated - and our country's system". Indeed, philanthropy is a sign of commitment to a cause, as well as a tool for individuals and society in general to support cancer research regardless of the economic circumstances.

The third contract came from the *Juegaterapia* Foundation and their famous 'baby pelones' toys, by which they underlined their commitment to childhood cancer research. Last June, the CNIO welcomed one of the most acclaimed popular music stars in our country, Alejandro Sanz, to commemorate the collaboration between the *Juegaterapia* Foundation and our Centre. Thanks to this grant, the CNIO will host a researcher in 2017 in the Epithelial Carcinogenesis Group who, together with the CNIO-*Hospital Niño Jesus* Clinical Trials Unit, will study neuroblastic tumours – the most common tumour type in the first 2 years of life – and tumours of the central nervous system, the most common solid tumours in children.

"Our Friends: a key partner in our mission of conquering cancer."

Businesses also joined our community of Friends in 2016. Indeed, the CNIO signed a collaborative agreement with *ASISA Vida* in relation to a new product launched by the insurance company that offers specific coverage for gynaecological cancer. Thanks to the insurance company's commitment to this cause, 5% of the premiums of this product will be donated to the 'CNIO Friends' initiative to fund cancer research. Another agreement was formalised with *Grupo CLH*, the leading company in Spain for the transportation and storage of oil products.

Our CNIO volunteers are another highly valuable asset. Dozens of them came out last November to meet new Friends at the *La Vaguada* Shopping Centre, where they installed a mini-laboratory to introduce the initiative to the residents and visitors of the popular *Barrio del Pilar* district in Madrid, where the centre is located. This initiative was one of many examples that helped raise funds for research: there was the event organised by the secondary school *IES La Encantá de Rojales*, Alicante, consisting of a charity run in May to raise funds for CNIO's research; the Edinburgh Marathon for which participants put on their running shoes in name of cancer research; and the charity *tuna* music festival at the Madrid Polytechnic University. Thanks to all of you for supporting our work!

The latest cancer survival figures are encouraging – some types of cancer, such as breast cancer, now exceed 80% – however, there is still a long and difficult road ahead. Our Friends are key in the effort of finding new solutions to fight this disease. Our heartfelt appreciation goes out to each and every one of them.

‘CNIO FRIENDS’ POSTDOCTORAL CONTRACTS

At the beginning of the year, the CNIO organised the first recruitment programme funded by donations from the ‘CNIO Friends’ philanthropic initiative. After a thorough selection process of the candidates, the first two grants were allocated to sponsor the researchers Paulina Gómez, from the Genetic and Molecular Epidemiology Unit, and Vera Pancaldi, from the Structural Computational Biology Group.

Paulina Gómez is an expert in Genetic and Molecular Epidemiology. Her research mainly focuses on PanGenEU, a large European study involving six countries that delves into the relationship between multiple risk factors and pancreatic cancer, one of the carcinomas with the highest mortality rate today. These studies are aimed at defining the population with the greatest risk of suffering from pancreatic cancer, and thereby facilitate the early diagnosis of the disease.

Vera Pancaldi is an expert in Computational Biology. Specifically, she focuses on the study of how the DNA structure affects tumour aggressiveness and how it helps us to understand the context and impact of genetic modifications in patients. These studies could be used as a basis for improving tumour diagnosis and for developing personalised therapies in the clinic.

“CNIO Friends is crucial for raising public awareness about the importance of research and for fostering the responsibility we all have for actively participating in our future.”

“This financial contribution has enabled me to ensure the continuity of this project without having to worry about looking for new external funding; it gives me the necessary independence to develop this research.”

JUEGATERAPIA FOUNDATION-‘CNIO FRIENDS’

In July, the *Juegaterapia* Foundation, which helps children suffering from cancer, signed a collaboration agreement with the ‘CNIO Friends’ initiative to fund a 100,000 euro grant that will be dedicated to research projects related to childhood cancer. Alejandro Sanz came to the CNIO as *Juegaterapia*’s Goodwill Ambassador to commemorate this agreement. The singer visited the facilities and obtained first-hand information on the research being carried out. “The fight against cancer is a war and I am a soldier”, he said during his visit to the CNIO.

CNIO OPENS ITS DOORS TO ‘CNIO FRIENDS’

One of the most exiting events held in 2016 was the ‘Jornada Amigos del CNIO’, which took place in mid-May and welcomed our donors/CNIO Friends in order to bring CNIO science closer to them. Our donors spent an afternoon with us and once again demonstrated their great enthusiasm for being part of the important mission of conquering of cancer.

Last but not least, we would also like to extend our heartfelt thanks to all the anonymous benefactors who have donated their legacies to support cancer research at the CNIO (around €361,000 this year); in doing so they have contributed to society for generations to come.

BENEFACTOR FRIENDS/SPONSOR FRIENDS

→ Benefactor Friends

- **Alfonso Agüera Nieto**
  Santa Ana-Cartagena, Murcia
- **Álvaro Gil Conejo**
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- **Andrés Sánchez Arranz**
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  Mérida, Badajoz

→ Sponsor Friends

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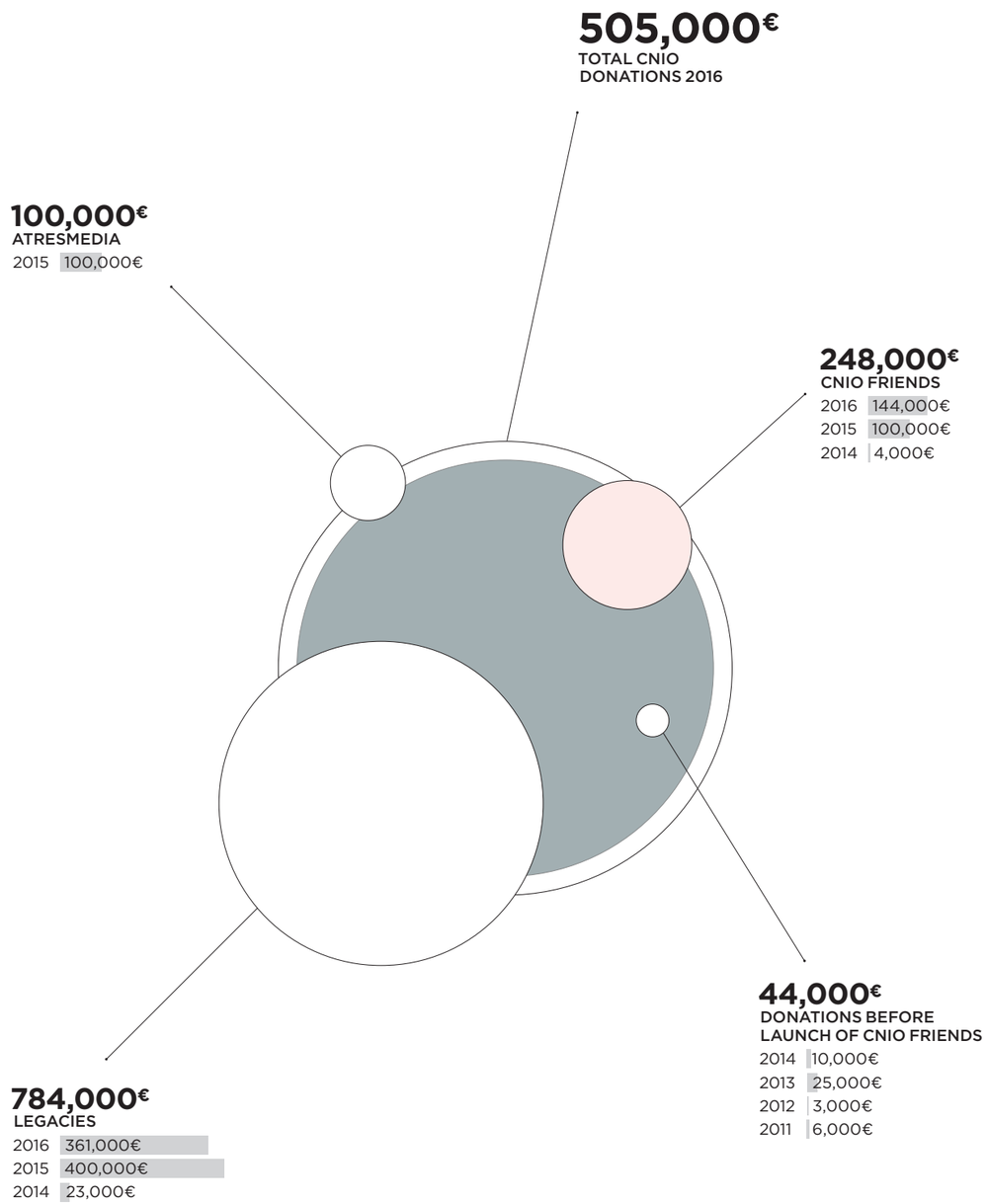
- **Freesia Group**
  Salou, Tarragona
- ***Fundación Juegaterapia***
  Madrid, Madrid
- **María Josefa Azcona Peribañez**
  Madrid, Madrid

DONATIONS TO THE CNIO



CREATIVE TEAM

In order to pour the Annual Report into a more creative concept, the CNIO works closely with selected professionals in the artistic and creative sectors who ensure delivery of an end product that is attractive in more ways than one. We extend our thanks to the creative team, the visual artist Amparo Garrido, and the graphic design studio underbau whose invaluable work created the images and design that illustrate this Annual Report.

AMPARO GARRIDO PHOTOGRAPHY

A Madrid-based visual artist working with photography and video, Amparo Garrido has been represented in individual and group shows both in Spain and abroad since 1998. Her work has been honoured in several prestigious competitions. She obtained the first place in the 2001 edition of the ABC Photography Prize, and second place in the 2007 *Purificación García* Prize. Other honourable mentions include the *Pilar Citoler* and *Ciudad de Palma* prizes. Her work can be found in major collections, including the *Museo Nacional Centro de Arte Reina Sofia* in Madrid, the photographic holdings of the Madrid regional government, the Coca-Cola Foundation, the *Es Baluard* Museum of Modern and Contemporary Art in Palma de Mallorca, and the 'Types and Trends on the Threshold of the 21st Century' Alcobendas Collection, among many others. Amparo's most recent solo exhibitions in Spain were shown at the *Sala Robayera de Miengo*, Cantabria 2017, *Galería Trinta*, Santiago de Compostela 2015, and the *Museo del Romanticismo*, Madrid 2012.

UNDERBAU DESIGN

Underbau is a design studio that emerged in 2008 from professional designers with 15 years of experience in the field of corporate design, publishing and advertising. From the very beginning, the studio has sought to maintain its primary focus on art and culture, working together with Spanish and international bodies (*Orquesta y Coro Nacionales de España*, *Instituto Cervantes*, *La Fábrica* and *Museo Thyssen-Bornemisza* among others). Underbau's total-design approach puts the emphasis on coherency. To achieve that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product.

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