SPANISH NATIONAL
CANCER RESEARCH CENTRE, CNIO

ANNUAL REPORT 2015

cnio Centro Nacional de Investigaciones Oncológicas

EXCELENCIA SEVERO OCHOA 2012 - 2019

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“Thanks to the ‘CNIO Friends’ initiative, close to 600 donors have given us their philanthropic support, and this number is increasing every day.”

MARIA A. BLASCO
Director

FOREWORD

Maria A. Blasco Director

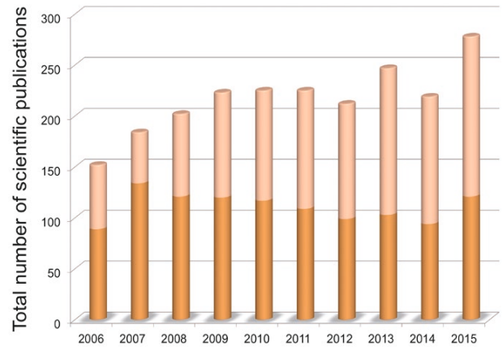
First of all, I would like to thank all of those who have once again collaborated on the elaboration of this Annual Report, with especial thanks to Sonia Cerdá who is responsible for our CNIO publications, as well as to our photographer Amparo Garrido and the underbau graphic design team.

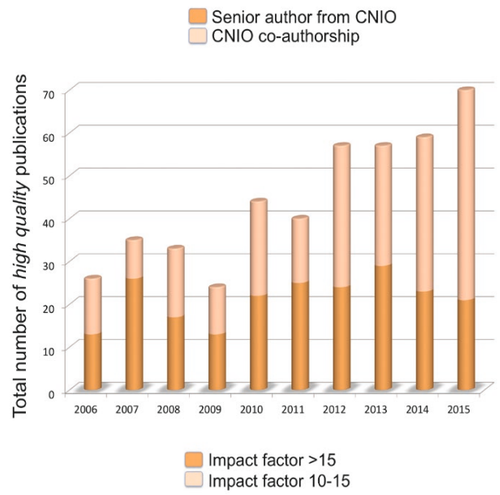
During 2015, the CNIO authored a total of 278 papers, 49 of which were published in journals with impact factors ranging from 10 to 15, and 21 papers in journals with impact factors greater than 15. Comparison with previous years (2006-2015) indicates that since 2010, the CNIO has further continued to increase its output of papers in top journals.

In line with our capabilities to produce top science, I am proud to mention that in 2015, the Spanish Ministry of Economy and Competitiveness renewed CNIO's accreditation as one of the "Severo Ochoa" Centres of Excellence, distinguishing our Centre as the flagship of Spanish cancer research. The "Severo Ochoa" award brings with it important associated funding for the CNIO as well as human resources.

During 2015, 2 new Junior Groups joined the Molecular Oncology Programme at the CNIO: the Microenvironment and Metastasis Group, led by Héctor Peinado, a former Assistant Professor at Weill Cornell Medical College (New York, USA); and the Brain Metastasis Group, headed by Manuel Valiente, a former postdoctoral fellow in the laboratory of Joan Massagué at the Memorial Sloan Kettering Cancer Center (New York, USA). In addition, during 2015, we completed the recruitment of a third Junior Group that will be incorporated into the Molecular Oncology Programme in January 2016. In particular, Alejo Efeyan, a former postdoctoral fellow in David Sabatini's Group at the Massachusetts Institute of Technology (MIT, Boston, USA), will lead the Metabolism and Cell Signalling Group at the CNIO. In 2015, Alejo received a European Research Council (ERC) starting grant.

In 2015, we created 2 new Core Units: a Protein Crystallography Unit, which is shared with the CNIO Experimental Therapeutics





Programme; and an Electron Microscopy Unit that works in collaboration with the laboratory of Óscar Llorca at the Centre for Biological Research (*Centro de Investigaciones Biológicas, CIB-CSIC*) in Madrid. These new Units have already started their work in collaboration with several CNIO Groups.

I am very pleased to announce that in December 2015, Óscar Fernández Capetillo, Head of the Genomic Instability Group at the CNIO, was appointed Vice-Director of Translational Research and Director of Innovation. We are very excited about having Óscar leading these key strategic areas for the CNIO. Given his former achievements in translating his basic research discoveries into drug development, in collaboration with the CNIO Experimental Therapeutics Programme, we are confident that Óscar will help to greatly enhance translational research at the CNIO.

I take this opportunity to wholeheartedly thank our former Vice-Director of Translational Research, Manuel Hidalgo. Thanks to his efforts, clinical research at the CNIO is now a reality. In particular, during the last few years, we have increased the number of Clinical Research Units at the CNIO, as well as our agreements with Hospitals. We have now a total of six leading oncologists working at the CNIO in some of the cancer types with the highest social impact (breast, prostate, GI tumours, lung, oncohaematological tumours and paediatric cancer). They all run early phase clinical trials and have clinical practice in the Hospitals associated with the CNIO. In addition, the CNIO also conducts important clinical activity through the Familiar Cancer Consultancy located at the *Hospital Universitario de Fuenlabrada*, as well as through the Molecular Diagnostics and Molecular Cytogenetics and Genome Editing Units at the CNIO.

Conducting world-class scientific research is paramount, but transferring the results to the life sciences value chain is of equal importance to the CNIO. In 2015, our partners have validated preclinical data in order to progress towards the clinical development of 2 of our previously licensed programmes. We expect that our collaborator Merck Serono will soon complete the preclinical development of ATR inhibitors, thereby accelerating the translation of CNIO's research into new potential treatment options for cancer patients. Our monoclonal antibody commercial pipeline has also been strengthened. Altogether, the income generated from Intellectual Property Rights (IPR) in 2015 amounts to about 800,000 €, thanks to the efforts of more than 40 CNIO Inventors who have contributed to this IPR. This amount represents half of the total revenue generated by all the University Systems in Spain, thus positioning the CNIO as a leading Public Institution in Technology Transfer and Innovation in our country. This income is expected to significantly increase over the next few years and will contribute to the economic sustainability of the CNIO, as well as to support its research activities.

We continue to build on our links with industrial partners. Significantly, in 2015, the CNIO renewed its collaboration with Roche for 2 more years. Furthermore, it entered into a new collaboration with Pfizer, which will enable 4 Research Groups at the CNIO to coordinate their efforts to better understand the biological processes underlying major tumours, such as those in breast, lung or pancreatic cancer. As a result of this 3 year collaboration, we expect to generate knowledge and tools to design new diagnostic and therapeutic interventions that will also be applied in personalised medicine. In 2015, 20 contracts were negotiated with companies and other stakeholders, thereby securing future revenues for collaborative research that amount to 4.5 million euros; this represents more than 10% of the total annual budget of the CNIO.

In recognition of these achievements, CNIO's 'Innovation strategy' was awarded with the Innovation Recognition Award handed out by the Spanish Association of Innovative Companies (*Foro de Empresas Innovadoras*, *FEI*); this award is an acknowledgement of the impact CNIO makes thanks to the innovative technologies that we foster and develop for the oncology sector.

The CNIO External Scientific Advisory Board (SAB), currently chaired by Mariann Bienz, is of outmost importance for guiding the strategic plans of the CNIO as well as for the review of our Research Groups. In June 2015, 2 Research Programmes were evaluated by the SAB, namely the Molecular Oncology Programme (MOP) and the Structural Biology and Biocomputing Programme (SBBP), as well as the 6 Units associated with the SBBP that provide technical support to the entire Centre. Overall, the SAB was impressed by the scientific excellence of the Programmes, which continue to position the CNIO at the forefront of international biomedical science as well as the most prominent institute in basic cancer research in Spain. Following the SAB's recommendations, we will start recruiting several new groups for the SBBP in 2016 with the aim of reinforcing structural biology at the CNIO.

During 2015, Julio Celis, from the Danish Cancer Society Research Center in Copenhagen, left the CNIO Scientific Advisory Board (SAB). Two new members have since been approved by our Board of Trustees to form part of the SAB, namely Stephen Frye, Director of the Center 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). We wholeheartedly thank Julio for his dedication to the CNIO during his 13 years with us. We would also like to welcome and extend our thanks to Drs. Frye and Yonath for their future commitment to the CNIO.

I would like to take this opportunity to thank all those who have 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 2015, the following scientists were the beneficiaries of the Jesus Serra Foundation's Visiting Researchers Programme: Chaitanya R. Divgi, Professor of Radiology; Vice Chair, Research Department of Radiology at Columbia University in New York (USA); Marcin Nowotny, Head of the Laboratory of Protein Structure, the International Institute of Molecular and Cell Biology in Warsaw (Poland); Eva Nogales, investigator with the Howard Hughes Medical Institute and Professor at the University of California in Berkeley (USA); and, Patrick Sung, Professor of Molecular Biophysics and Biochemistry and of Therapeutic Radiology, Yale University School of Medicine in New Haven (USA).

I also wish to thank the Foundation *Banc Sabadell* 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 2015, we had the privilege to listen to: Maria José García Borge, Head of the facility ISOLDE-CERN in Geneva (Switzerland); Ignacio Cirac, Director of the Theory Division, Max-Planck Institute for Quantum Optics in Garching (Germany); Thijn Brummelkamp, Group Leader in the Division of Biochemistry at the Netherlands Cancer Institute in Amsterdam (The Netherlands); and Elly Tanaka, Director of the DFG Research Center for Regenerative Therapies Dresden - Cluster of Excellence of the TU Dresden CRTD (Germany).

Thanks to the sponsorship of the *French Embassy* in Spain we were also able to invite the following speaker to our Distinguished Seminar series: Hugues de Thé, Head of Molecular Pathology and Virology Department, the University Institute for Haematology in Paris (France).

Furthermore, I would like to give my special thanks to the CNIO Women and Science (WISE) Office for organising an outstanding series of seminars on gender issues. We had the pleasure of listening to Natalia González-Valdéz, Director of l'Oreal Spain; María del Mar Martínez, Director at McKinsey's Madrid Office; Margarita Salas Falgueras, Professor of Research at the Centre of Molecular Biology "*Severo Ochoa*" (*CBMSO, CSIC-UAM*); Carmen Vela Olmo, Secretariat of State of Research, Development and Innovation of the Ministry of Economy and Competitiveness (*MINECO*); and Flora de Pablo, Professor of Research, Centre for Biological Investigation, Spanish National Research Council (*CSIC*).

In 2015, the presence of our Centre in the media continued its upward trend: over 2,600 mentions were tracked in the national and international press, representing a growth of 15% compared to the already remarkable figures of 2014. Likewise, our presence on radio and TV has also experienced a significant increase, almost tripling the hits of 2014. These include shots during the prime-time news bulletins of the main Spanish TV channels.

Two of our studies particularly caught society's attention this year: in May, the research on TRF1 and cancer immortality – published in 'EMBO Molecular Medicine' by the CNIO Telomeres and Telomerase Group – hit the front pages of the newspapers and was covered by numerous radio and TV news bulletins as well as international media outlets. In October, the international research published in 'Nature' – with the participation of Héctor Peinado, Head of the Microenvironment and Metastasis Group – on the first 'molecular labels' that can predict the organs where metastases will form, had a great resonance in Spain and abroad.

One of the most exciting adventures we faced in 2015 was the implementation of the 'CNIO Friends' initiative, a philanthropic platform launched in late 2014 to raise public awareness about the importance of supporting biomedical research in order to fight cancer. I am proud to say that this is revealing itself to be one of our most rewarding experiences in outreach activities conducted so far. After a year, the initiative resulted in us benefiting from several personal and unique interactions with our donors and friends. To the date, close to 600 donors have given us their philanthropic support, and this number is increasing every day.

In April, we received significant and remarkable support: the athlete Marcos Argumosa ran 10 consecutive marathons to familiarise society with 'CNIO Friends'. His action caught the attention of the media and boosted the involvement of new donors. And in December and at Christmas time, a video homage to our Friends, produced by the visual artist Amparo Garrido, travelled the length and breadth of Spain in the national high-speed long-distance trains thanks to an agreement with RENFE, the Spanish rail transport operator.

In 2016, during its first year of existence, 'CNIO Friends' will make it possible for us to launch the Postdoctoral Contract 'CNIO Friends' Programme, through which we will recruit 2 scientists to reinforce our lines of research over the next 2 years. This setup serves as a great example of how combining the efforts of the research community with the input of society can lead to results that can truly make a difference for the future of medicine.

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 (since March)
Brain Metastasis 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

Guillermo Montoya
Macromolecular Crystallography 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

David G. Pisano
Bioinformatics Unit

Víctor de la Torre (until November)
National Bioinformatics Institute Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Crystallography Unit

MANUEL HIDALGO 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 Programme Director

Manuel Hidalgo
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

Fátima Al-Shahrour
Translational Bioinformatics 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

DIRECTION OF INNOVATION

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director

Orlando Domínguez
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

Joaquín Pastor Programme Director

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 goal, as Vice-Director, is to strengthen CNIO´s Basic Research domain by encouraging scientific excellence and fostering collaboration, so we can continue to make strides in cancer research”

There have been several encouraging developments this year pertaining to the organisational aspects of CNIO’s Basic Research domain: 2 Junior Group Leaders, Héctor Peinado and Manuel Valiente, joined the CNIO to conduct new lines of research in the area of metastasis; the establishment of 2 new Core Units – the Electron Microscopy and Protein Crystallography Units – that have already started operations in collaboration with a number of CNIO Groups; and, the very positive evaluations of both the Molecular Oncology Programme and the Structural Biology and Biocomputing Programme by the corresponding external Scientific Advisory Board.

CNIO’s Basic Research areas will be further strengthened in the coming year with the incorporation of another Junior Group leader, Alejo Efeyan, who will conduct research focused on metabolism and cancer; new recruitments in strategic areas of structural biology; more collaborations with internal and external groups; and the consolidation of the projects developed with the Experimental Therapeutics Programme.

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It is my pleasure to introduce the highlights of the Molecular Oncology Programme in 2015. First of all, my enthusiastic and warm welcome to the two new Junior Groups, namely, the Microenvironment & Metastasis Group, led by Héctor Peinado, and the Brain Metastasis Group, led by Manuel Valiente. The two Groups will work on different aspects of metastasis, thereby filling an important gap in the research carried out at CNIO.

Héctor Peinado joins the CNIO after an extraordinarily successful postdoctoral period in the laboratory of David Lyden, at Weill Cornell Medical College, New York, working on tumour-derived exosomes and on the pro-metastatic conditioning of distant tissues by primary tumours. During 2015, Héctor successfully secured funding from different public and funding agencies, both nationally and internationally, including a generous donation from *La Sexta* and the AXA Foundation. His Group is now a reality, composed of 5 members and several students in practice.

Manuel Valiente is a pioneer in the molecular analysis of the mechanisms underlying the metastatic colonisation of the brain, work that he undertook in the laboratory of Joan Massagué, at the Memorial Sloan Kettering Cancer Center, New York. At CNIO, Valiente is going to dissect the process of brain metastasis in further detail. For this, he already has in place a Group of 5 members and several students in practice.

My best wishes to the two new Groups!

In the following pages, you will read about the exciting scientific advances made by each of the groups of the Molecular Oncology Programme. They are all at the forefront of their respective areas of research. There are two scientists in particular that I want to put in the spotlight due to their impressive outputs this year, namely, Marcos Malumbres and Oscar Fernández-Capetillo. This year, the Cell Division and Cancer Group, led by Malumbres, has published primary research in journals such as *Nat. Cell Biol.*, *Dev. Cell, Blood*, and *Mol. Cell Biol.* On a similar level, the Genomic Instability Group, led by Fernández-Capetillo, has published work in journals such as *Genes Dev.*, *Nat. Commun.*, *EMBO J.* and *Mol. Cell Biol.*

My congratulations go out to Marisol Soengas, Head of the Melanoma Group, for her remarkable achievements in securing funding for melanoma research in 2015. Two consortia led by Soengas have been granted generous funds for the next few years; one is funded by the *Asociación Española Contra el Cáncer* (€1.2M) and the other one is funded by the American Melanoma Research Alliance ($900,000). These grants put Soengas and the CNIO at the leading front of research against melanoma.

"The Molecular Oncology Programme continues its tradition of scientific excellence and innovation, with the ultimate goal of moving forward basic and translational research in the cancer field."

Oscar Fernández-Capetillo has been promoted to Vice-Director of Translational Research in order to capitalise on his experience in drug discovery. Also, Fernández-Capetillo has obtained a Chair as Professor in the prestigious Karolinska Institute in Stockholm. These are all major responsibilities that reflect Oscar's extraordinary leadership, efficiency and dedication. I wish him the greatest possible successes in these new challenges.

The outstanding careers of our investigators have been further recognised by prestigious awards. In this regard, Mariano Barbacid, Head of the Experimental Oncology Group, has received the Cancer Research Award given by the *Asociación Española de Investigación sobre el Cáncer*. Also, Oscar Fernández-Capetillo has received the *Carmen y Severo Ochoa Award*, given by the Foundation of the same name, and the award *Líder de Grupo Emergente en Investigación Biomédica*, given out by the AXA Foundation.

TUMOUR SUPPRESSION GROUP

Manuel Serrano
Group Leader

Staff Scientists
Susana Llanos, Daniel Muñoz, Cristina Pantoja

Post-Doctoral Fellows
Timothy Cash, Pablo J. Fernández-Marcos (until October), Cian J. Lynch, Gianluca Varetti

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

Technician
Maribel Muñoz (TS)*

Titulado Superior (Advanced Degree)

Student in practice
Isabel Calvo (since June)

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, in combination with additional mutations in other genes, can give rise to cancer. Understanding how these 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, including cellular pluripotency, cell senescence, and metabolism. Our Group aims to achieve an integrated understanding of cancer protection.

Our goals are:

- → To understand the mechanisms of tumour suppression and to identify new tumour suppressor regulators.
- → To study the interplay between tumour suppression and ageing.
- → To analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage.
- → To characterise cellular senescence as a tumour suppression mechanism.
- → To investigate cellular pluripotency and the involvement of tumour suppressors in the regulation of reprogramming to induced pluripotent stem (iPS) cells.
- → To explore the role(s) of cell plasticity in cancer, tissue regeneration, and ageing.
- → To search for new frontiers in cell plasticity.

"Pharmacological Notch inhibitors have anti-tumour effects in preclinical models of several types of cancers, including lung adenocarcinomas. However, caution must be taken in regards to their clinical application because Notch inhibition may increase the incidence of squamous-type tumours, like in the urinary bladder."

RESEARCH HIGHLIGHTS

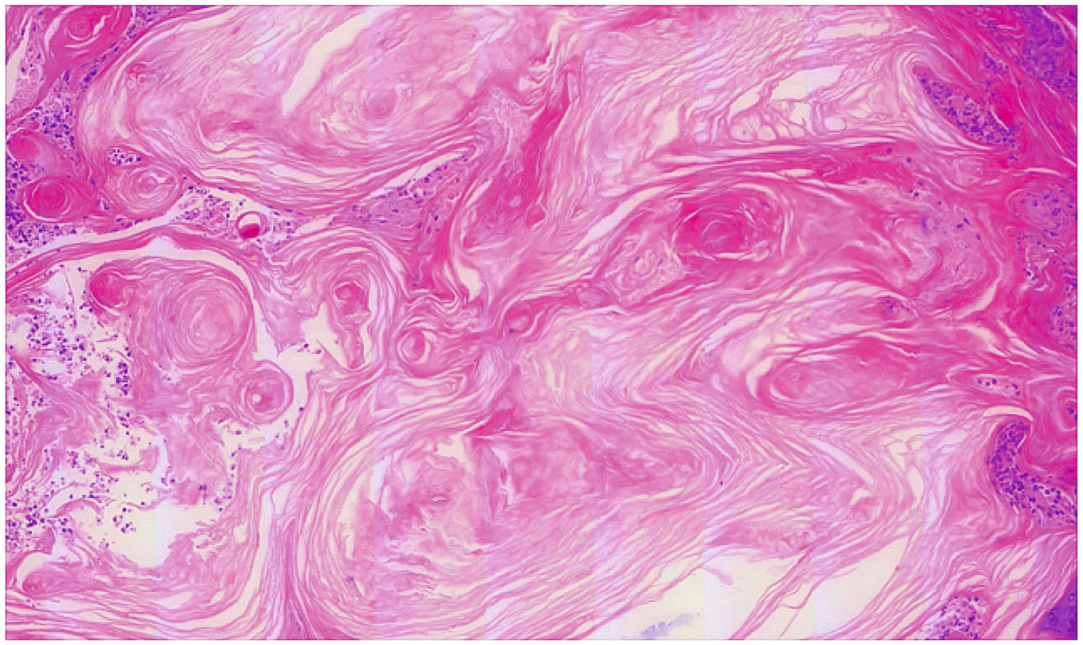


Figure 1 NOTCH inactivation induces squamous bladder carcinomas. Representative example of a squamous cell carcinoma, stained with haematoxylin and eosin, showing multiple layers of keratin.

NOTCH pathway inactivation promotes bladder cancer progression and epithelial-mesenchymal transition

The NOTCH pathway is frequently altered in multiple cancers. Interestingly, NOTCH mutations fall into two distinct patterns depending on the tumor type. On one hand, gain-of-function mutations are present in acute T-cell lymphoblastic leukaemias, chronic lymphocytic leukaemias, and lung adenocarcinomas, thereby showing that the NOTCH pathway is oncogenic in these malignancies. On the other hand, loss-of-function mutations are detected in myeloid leukaemias and in squamous cell carcinomas (SCCs) of different origins, implying that the NOTCH pathway plays a tumour-suppressive role in these cancers. Taking into account that the NOTCH pathway is tumour suppressive in several types of SCCs, we hypothesised that this could also be the case in the urothelium. We demonstrated that the genetic inactivation of the NOTCH pathway in the urinary bladder of mice, by two different genetic means, accelerates bladder cancer and promotes the formation of highly aggressive SCCs with areas of mesenchymal features (FIGURE 1). Mechanistically, we showed that loss of the NOTCH pathway promotes an epithelial-mesenchymal transition (EMT) in bladder cancer cells that is partly mediated by loss of its effector HES1. Our results indicate that NOTCH serves as a tumour suppressor in the bladder and that loss of this pathway promotes mesenchymal and invasive features.

Pharmacological inhibition of PI3K reduces adiposity and metabolic syndrome

The PI3K enzyme promotes cellular anabolism, a process that is necessary for normal cell growth and multiplication, but, when uncontrolled, it can also lead to cancer. Separate lines of research on longevity, dietary restriction, obesity, and metabolic syndrome have converged on the concept that moderate downregulation of PI3K signalling activity has the potential to improve health and protect against obesity and its associated diseases. We have found that oral administration of CNIO-PI3Ki – a PI3K inhibitor developed at the CNIO that is currently being studied for applications in cancer treatment – decreases adiposity in obese mice without affecting their lean mass. Long-term treatment of obese mice with low doses of CNIO-PI3Ki reduces body weight until reaching a balance that remains stable for months as long as the treatment is continued. CNIO-PI3Ki treatment also ameliorates liver steatosis and decreases glucose serum levels. Finally, in collaboration with Rafael de Cabo's group (National Institute on Aging, Baltimore, MD, USA), we have also demonstrated that daily oral treatment of obese Rhesus monkeys for three months with low doses of CNIO-PI3Ki decreased their adiposity and lowered their serum glucose levels, in the absence of detectable toxicities. In conclusion, pharmacological inhibition of PI3K is an effective and safe anti-obesity intervention that could reverse the negative effects of metabolic syndrome in humans.

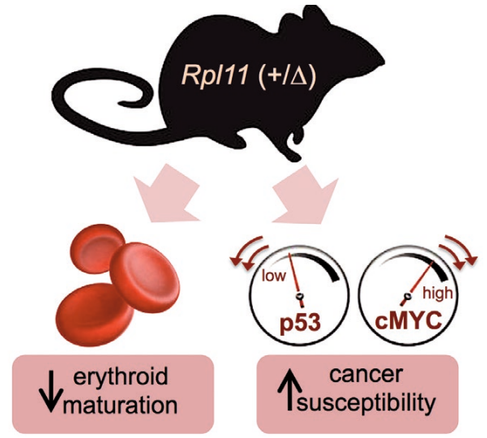


Figure 2 Heterozygous loss of *Rpl11* in adult mice recapitulates Diamond-Blackfan anaemia, including increased cancer susceptibility.

Partial loss of *Rpl11* in mice recapitulates Diamond-Blackfan anaemia and promotes lymphomagenesis

Mutations in ribosomal genes cause Diamond-Blackfan anaemia (DBA), a condition characterised by anaemia and cancer susceptibility. A subset of DBA patients carries loss-of-function haploid mutations in the *RPL11* ribosomal gene. The ribosomal protein RPL11 is particularly relevant because of its dual function: on one hand, it is an integral component of the ribosome; on the other hand, under conditions that perturb ribosome biogenesis, ribosome-free RPL11 activates p53 and inhibits cMYC. We have generated mice with an inducible *Rpl11* null allele and have shown that heterozygous loss of *Rpl11* in adult mice results in anaemia associated to decreased erythroid progenitors and defective erythroid maturation. Importantly, we have also observed that these mice have an increased susceptibility to radiation-induced lymphomagenesis. At a molecular level, cells and tissues from *Rpl11* heterozygous mice show compromised p53 activation upon ribosomal stress or DNA damage, and higher basal cMYC levels. This is the first DBA mouse model where cancer susceptibility is reported, thereby recapitulating the main pathologies of human DBA patients (FIGURE 2). ■

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AWARDS AND RECOGNITION

- Elected Member, Royal National Academy of Medicine of Spain.
- Elected Member, European Academy of Cancer Sciences.

EXPERIMENTAL ONCOLOGY GROUP

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Technicians
M. Carmen González (TS)*, Beatriz Jiménez (until December), Marta San Román, Raquel Villar

Titulado Superior (Advanced Degree)

External Collaborators
Jose Javier Berenguer (*Hospital Virgen De La Arrixaca*, Murcia), Juan Velasco (Eli Lilly Company, Madrid)

OVERVIEW

Our laboratory is interested in understanding the molecular events implicated in the development of K-Ras mutant lung and pancreatic cancers, 2 tumour types with some of the worst prognoses. We are also interested in identifying, and subsequently validating, targets of therapeutic value with the ultimate goal of establishing combination therapies that will have a profound effect on the progression of these tumours and could be ultimately translated to the clinic. We are addressing these ambitious goals by using a new generation of genetically engineered mouse tumour models that uses 2 independent recombinase systems to separate, both spatially and temporally, tumour development from target validation. Moreover, we are now conducting target validation by using conditional knocked-in mice that, upon Cre-mediated recombination, express a kinase dead isoform rather than ablate protein expression to better mimic pharmacological intervention. The outcome of these studies should pave the way for the development of more efficacious therapies in a clinical setting.

RESEARCH HIGHLIGHTS

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

Patients with advanced K-*RAS* mutant lung adenocarcinomas have poor clinical outcome and are currently treated with standard chemotherapy due to the lack of targeted therapies. We have reasoned that the identification of novel mediators of K-Ras signalling during the early stages fop tumour development may unveil essential targets that would not be affected by the clonal heterogeneity observed in advanced and metastatic tumours. Indeed, transcriptional profiling of K-Ras^{G12V}-driven hyperplasias revealed inter-tumour heterogeneity with a subset exhibiting an aggressive transcriptional profile analogous to that of advanced human adenocarcinomas. This signature identified the tyrosine kinase receptor Ddr1 as having the top score. Genetic and pharmacological inhibition of Ddr1 blocked tumour development. Moreover, concomitant inhibition of Ddr1 and Notch signalling, a downstream mediator of Ddr1, thwarted progression of murine K-Ras^{G12V};p53-null adenocarcinomas. Importantly, this combined treatment induced regression of K-RAS;p53 mutant patient-derived lung orthoxenografts (PDX) with therapeutic efficacy superior to standard chemotherapy and with significantly less toxic effects. Our data indicate that the combined inhibition of DDR1/NOTCH could be an effective therapy for K-*RAS* mutant lung adenocarcinoma patients.

DDR1 and NOTCH are involved in a regulatory loop that maintains MAPK activity, an essential pathway for K-Ras-driven lung adenocarcinoma. In this setting, NOTCH inhibition has been shown to impair ERK activity by de-repression of DUSP1. In addition, DDR1 itself is a direct ERK transcriptional target. We propose that DDR1, NOTCH and RAS are connected in a robust signalling network, which could explain the additive effect observed upon DDR1/NOTCH co-inhibition. Yet, we cannot exclude the existence of DDR1 and NOTCH independent roles, ensuing additional therapeutic effect upon combined inhibition. For instance, DLL4 is the major NOTCH ligand in remodelling vasculature and its blockade induces decreased vessel function, thereby affecting tumour growth. Therefore, targeting DLL4 may avoid the gastrointestinal toxicity associated to pan-NOTCH inhibition while providing synergistic therapeutic effects. The fraction of DDR1+ tumours exceeds the prevalence of K-*RAS* mutations, suggesting that DDR1 might be associated to tumours driven by alternative oncogenes. Indeed, analysis of TCGA expression data in lung adenocarcinoma revealed a tendency for co-occurrence of EGFR mutations and DDR1 expression. This is in agreement with our TMA where most lung adenocarcinomas with p53, LKB1 or EGFR mutations display DDR1+ immunostaining. Intriguingly, all tested EGFR mutant cell lines express high levels of both DDR1 and HES1 and are sensitive to DDR1/NOTCH co-inhibition, suggesting that EGFR mutant patients could also benefit from this co-treatment. In summary, our results suggest that proper stratification of lung adenocarcinoma patients may lead to the identification of cohorts that can benefit from concomitant DDR1/NOTCH inhibition.

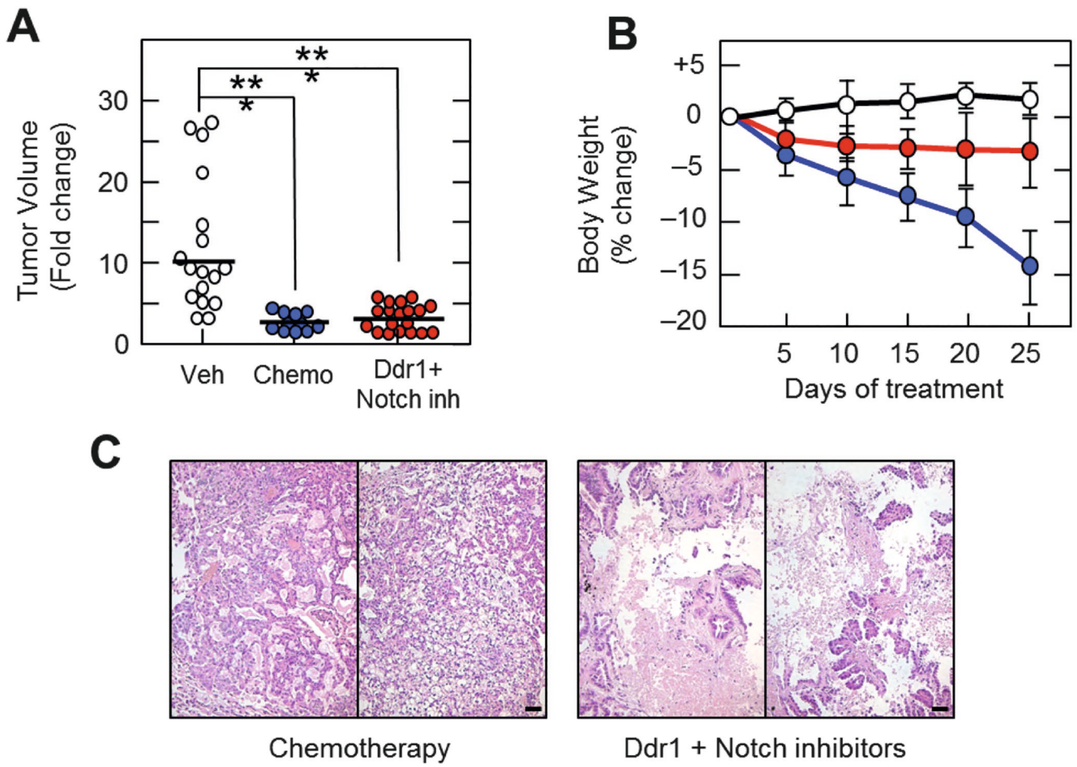

Figure 1 Combined inhibition of Ddr1 and Notch signalling has comparable therapeutic activity to standard chemotherapy regimens (cisplatin/paclitaxel) but is significantly less toxic. (**A**) Tumour volume change and (**B**) body weight loss in K-Ras$^{+/LSLG12Vgeo}$;TP53$^{lox/lox}$ mice exposed to Ad-Cre particles and treated with vehicle (open dots), standard chemotherapy (blue dots) and Ddr1+Noch inhibitors (red dots). (**C**) Ddr1-Notch-treated tumours displayed coagulative necrosis, a phenotype not detected in the chemotherapy-treated mice.

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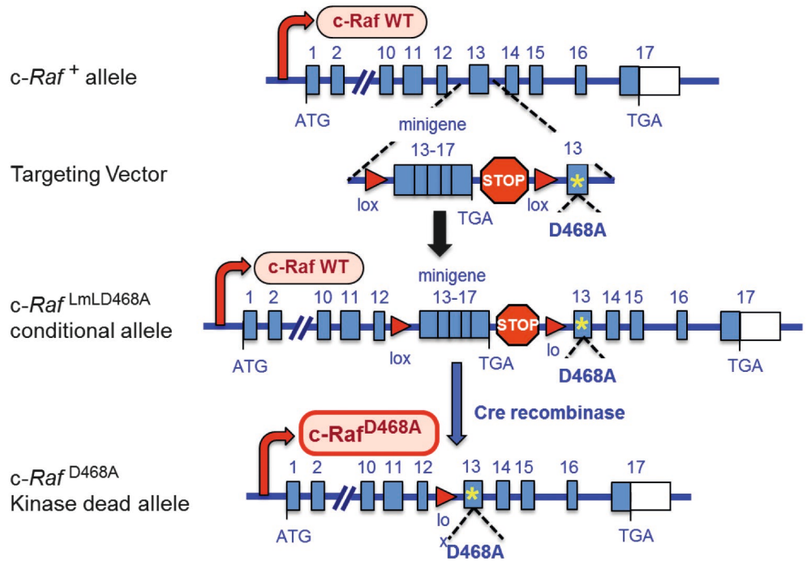

Figure 2 Schematic representation of the general strategy used to generate conditional knocked-in kinase dead strains. The diagram shown corresponds to the strategy followed to generate a strain expressing a c.RafD468A kinase dead isoform.

Generation of conditional knocked-in strains that express kinase dead isoforms of therapeutic targets for K-Ras driven lung and/or pancreatic cancers

The large majority of target validation studies in GEM models carried out thus far involve complete ablation of the target. Unfortunately, the pharmacological strategies that will be used in a clinical setting are unlikely to result in the elimination of protein expression. Most druggable targets studied thus far, including c-Raf, Cdk4, PI3K p110-alpha, EGFR and mTOR, are kinases. Thus, we have embarked upon a project to generate conditional knocked-in strains, which upon Cre-mediated recombination express kinase dead isoforms. As a mutational strategy, we have chosen to replace the aspartic residue that acts as the proton acceptor site in all kinases by an alanine. In all cases, we have used the 'minigene' targeting strategy for the c-Raf kinase (outlined in FIGURE 2). These mice will be crossed to our K-Ras driven lung and pancreatic GEM models to determine the role of the kinase activity of these druggable targets in tumour initiation (Cre-dependent models) as well as in tumour progression (Flp(o)- and Cre-dependent models). ■

AWARDS AND RECOGNITION

- 1st Cancer Research Award, ASEICA (*Asociación Española de Investigación en Cáncer*), Seville, Spain.
- Keynote Lecture, EACR-AACR-SIC Special Conference, Florence (Italy), June 2015.
- Keynote Lecture, Oncoforum, Leuven (Belgium), June 2015.
- Keynote Lecture, II International Frontiers in Oncology, Pamplona (Spain), October 2015.
- Keynote Lecture, 15th ASEICA International Congress, Seville (Spain), October 2015.
- Keynote Lecture, 10th GEICAM International Symposium, Cordoba (Spain), March 2015.
- Session Chair, 40th FEBS Congress, Berlin (Germany), July 2015.
- Session Chair, AACR-NCI-EORTC International Conference, Boston (USA), November 2015.
- Member of the Scientific Committee and Session Chair, ESMO Signalling Pathways Symposium, Barcelona, Spain.
- Member, Lilly Abemaciclib Global Scientific Advisory Board.
- Member, Novartis Lung Cancer Strategy Scientific Advisory Board.

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Titulado Superior (Advanced Degree)

Visiting Student
Miriam Popkes (September-November) (University Duisburg-Essen, Germany)

OVERVIEW

We study the mechanisms by which tumour cells are immortal and normal cells are mortal. The immortality of cancer cells is one of their most universal characteristics. 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. The 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 provided the first demonstration that induction of telomere dysfunction, independently of telomere length, by targeting the shelterin component TRF1, may represent a novel therapeutic approach for cancer treatment."

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

Telomere uncapping as a potential new therapeutic target for lung cancer

Since unlimited cell division in cancer requires activation of mechanisms that ensure maintenance of telomere length, telomeres are considered anti-cancer targets. The targeting of telomeres in human cancer has been approached via targeting telomerase activity. However, therapeutic strategies based on telomerase inhibition to treat cancer will be effective only when telomeres shorten below a minimum length. We investigated whether the induction of telomere dysfunction, independently of telomere length, by targeting the TRF1 shelterin component could be used as a more universal way to block the growth of dividing cells. We found that the genetic ablation of *Trf1* impairs the growth of p53-null K-RasG12V-induced lung carcinomas and increases mouse survival (FIGURE 1). This is accompanied by induction of telomeric DNA damage, apoptosis, decreased proliferation, and G2 arrest. This tumour-suppressive effect of Trf1 deficiency occurs already at the first mouse generation and is independent of telomere length. We also showed that chemical inhibition of TRF1 could be achieved *in vivo* by using small molecules, which effectively impair the growth of already established lung adenocarcinomas without affecting mouse and tissue viability. Our results constitute proof of concept that acute telomere uncapping by means of TRF1 abrogation is an effective therapeutic strategy to block the growth of aggressive lung

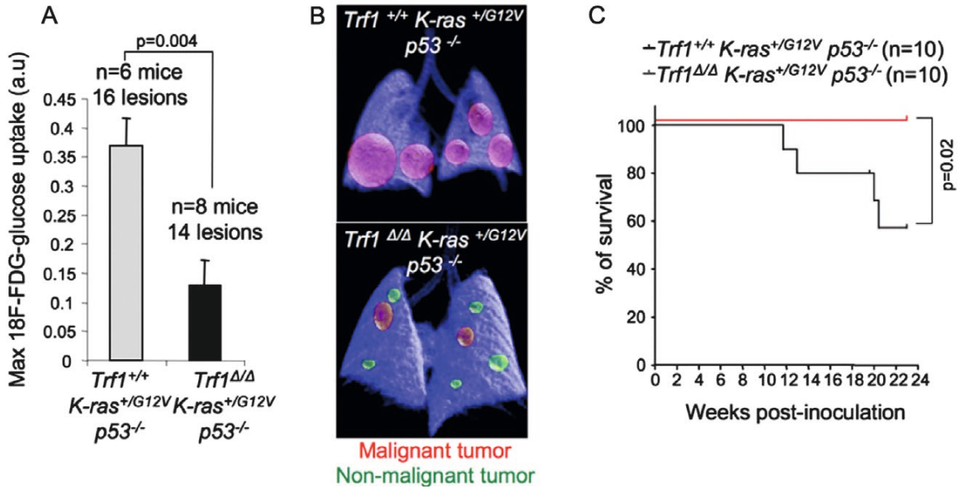

Figure 1 *Trf1* deficiency impairs *K-Ras*-mediated lung cancer development. (**A**) Maximum 18F-FDG-glucose uptake by *Trf1*$^{+/+}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ and *Trf1*$^{\Delta/\Delta}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ tumours 22 weeks after infection by PET. (**B**) Representative PET-CT image of *Trf1*$^{+/+}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ and *Trf1*$^{\Delta/\Delta}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ lungs. (**C**) Survival curve of *Trf1*$^{+/+}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ and *Trf1*$^{\Delta/\Delta}$ *K-Ras*$^{+/G12V}$ *p53*$^{-/-}$ mice.

carcinomas independently of telomere length and p53 status, and that it is possible to achieve this by small molecules able to target TRF1 *in vivo*. Since this strategy relies on a universal mechanism, namely induction of telomere uncapping, we speculate that it could be applied to many other cancer types.

Pulmonary fibrosis driven by telomere dysfunction

Idiopathic pulmonary fibrosis (IPF) is a degenerative disease of the lungs with an average survival, post-diagnosis, of 2-3 years. Mutations in components of telomerase or in proteins of the shelterin complex are found in both familial and sporadic IPF cases.

The lack of mouse models that faithfully recapitulate the human disease, however, has hampered new advances. We generated 2 independent mouse models that develop IPF owing to either critically short telomeres (telomerase-deficient mice) or severe telomere dysfunction in the absence of telomere shortening (mice with *Trf1* deletion in type II alveolar cells). We showed that both mouse models develop pulmonary fibrosis through induction of telomere damage (FIGURE 2). The severe telomere dysfunction associated with TRF1 deficiency, which induces a high burden of telomere-induced DNA damage, is sufficient for the development of pulmonary fibrosis (FIGURE 2). In the case of short telomeres owing to telomerase deficiency, additional damage was required to develop full-blown pulmonary fibrosis (FIGURE 2). We have provided proof of principle of the causal role of DNA damage stemming from dysfunctional telomeres in IPF development and identified telomeres as promising targets for new treatments.

A mutation in the shelterin component POT1 is responsible for cardiac angiosarcomas

Cardiac angiosarcoma (CAS) is a rare malignant tumour whose genetic basis is unknown. In collaboration with the CNIO Human Genetics Group and the Familial Cancer Clinical Unit, we have shown via whole-exome sequencing of a TP53-negative Li–Fraumeni-like (LFL) family including CAS cases, that a missense variant in the gene coding for the shelterin component POT1 is responsible for CAS. The same gene alteration is found in 2 other LFL families with CAS, supporting the causal effect of the identified mutation. We extended the analysis to TP53-negative LFL families with no CAS and found the same mutation in a breast AS family. Our functional and *in vitro* studies demonstrated that carriers of the mutation show reduced telomere-bound POT1 levels, abnormally long telomeres and increased telomere fragility, highlighting a new role of POT1 as a high susceptibility gene in familial cancer and opening therapeutic opportunities for prognosis and treatment in families with CAS. ■

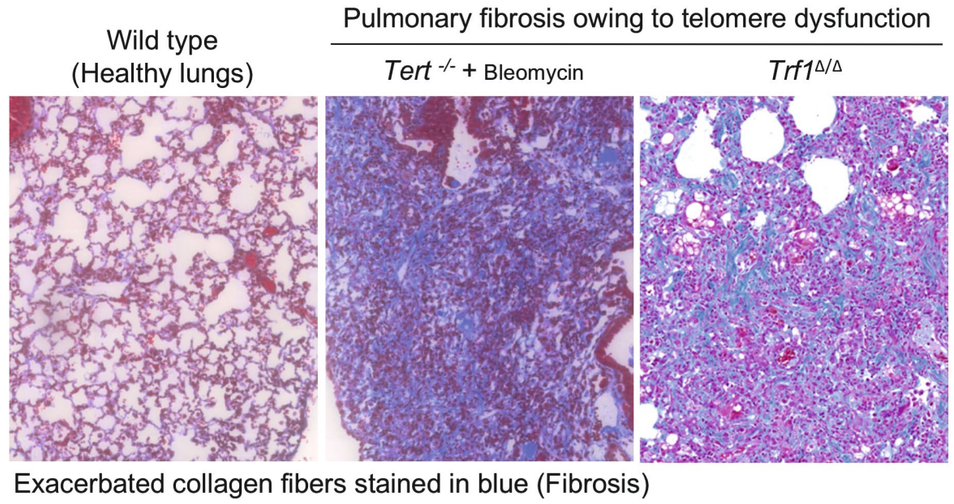

Figure 2 Exacerbated collagen deposition and fibrosis as visualised by blue Masson-trichrome staining in the lungs of mice with severe telomere dysfunction as a consequence of telomerase (Terc) or TRF1 deficiency.

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- Blasco MA, Bernades de Jesus B, Bär C, Serrano R.M, Bosch i Tubert F, Ayuso E, Bobadilla M (2015). Telomerase reverse transcriptase-based therapies. *PCT/EP14382312.*

AWARDS AND RECOGNITION

- Member of the Scientific Advisory Board, the *Institut Universitaire du Cancer de Toulouse* (*IUCT*), Toulouse, France.
- Member of the Scientific Advisory Board, the *Instituto de Neurociencias (IN) CSIC-UMH,* Alicante, Spain.
- Advisory Editorial Board Member, *Trends in Cancer*, Cell Press.
- Advisory Board Member, "Avenir" Foundation for Gender Equality, Spain-France.

CELL DIVISION AND CANCER GROUP

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Technicians
David Partida, Elisabet Zapatero (since September) (TS)*

Titulado Superior (Advanced Degree)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated. During the last few years, we have generated and characterised different mouse models in order to understand the relevance of several cell cycle regulators in the control of cell division and tissue physiology; these include cell cycle kinases and phosphatases, and proteins involved in ubiquitin-dependent degradation. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycles; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) characterising the function of microRNAs in cell biology and tumour development, and iv), understanding 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 towards improving therapeutic strategies against cancer cell proliferation.

"In 2015, we investigated the relevance of several mitotic regulators during cancer progression and therapy. We have also described the metabolic changes imposed by microtubule poisons that are used to treat cancer and their therapeutic relevance."

RESEARCH HIGHLIGHTS

In vivo relevance of cell cycle inhibitors and replicative stress

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 the prevention of 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. Moreover, ablation of these inhibitors prevents mouse development. This effect is most likely due to the hyperactivation of cyclin-dependent kinases, since the replicative stress can be prevented by slightly inhibiting the enzymatic activity of these proteins (Quereda et al., 2015).

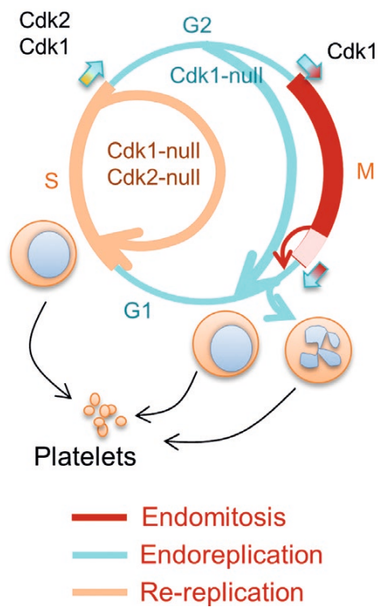

Figure 1 Different variants of the mammalian cell cycle are found in mammals. Megakaryocytes normally undergo endomitosis by skipping late mitotic events. In the absence of Cdk1, they undergo repeated S-G phases (endoreplication), whereas the DNA is replicated more than once (re-replication) in the absence of both Cdk1 and Cdk2.

Oncogenic effect of Aurora kinases in cancer

Aurora kinases are enzymes involved in the regulation of mitosis. These proteins are frequently overexpressed in human tumours and are currently considered as putative cancer targets. Yet, the effect of their overexpression *in vivo* is not well understood. We generated a new mouse model in which endogenous Aurora B can be overexpressed in a conditional manner (González-Loyola et al., 2015). Mice that overexpressed this kinase developed a wide variety of tumours. The molecular and cellular characterisation of these tumours suggested that Aurora B overexpression not only induces chromosomal instability, as previously expected, but also results in a dysfunctional p53 response, thus contributing to tumour development through multiple mechanisms.

Regulation of the megakaryocyte cell cycle

The cell cycle is widely considered as a universal mechanism for cell proliferation. However, some specialised cells display variants of the consensus cell cycle and understanding these differences may be crucial in the design of therapies against specific malignancies. Using mouse models with specific alterations in cell cycle regulators, we studied the relevance of endoreplication and endomitosis; two variants of the canonical cell cycle, in megakaryocytes. These cells undergo multiple rounds of genome amplification without generating daughter cells, thus increasing their ploidy. We have identified several mitotic kinases that, despite being essential for mitotic cell cycles (such as the ones used by cancer cells), are dispensable for the polyploidisation of megakaryocytes, thereby providing some new options for leukaemia treatment. Other kinases, such as Plk1, are still essential for megakaryocytes and their inhibition leads to thrombocytopaenia (Trakala et al., 2015).

Control of cellular metabolism in mitosis

Microtubule poisons, such as taxanes, block mitosis and eventually lead to cell death in a process frequently known as mitotic catastrophe. However, some cells are able to bypass this mitotic arrest and survive, thus contributing to chemo-resistance to those therapies. We have recently observed that mitotic arrest induces an early autophagic flux response, which results in

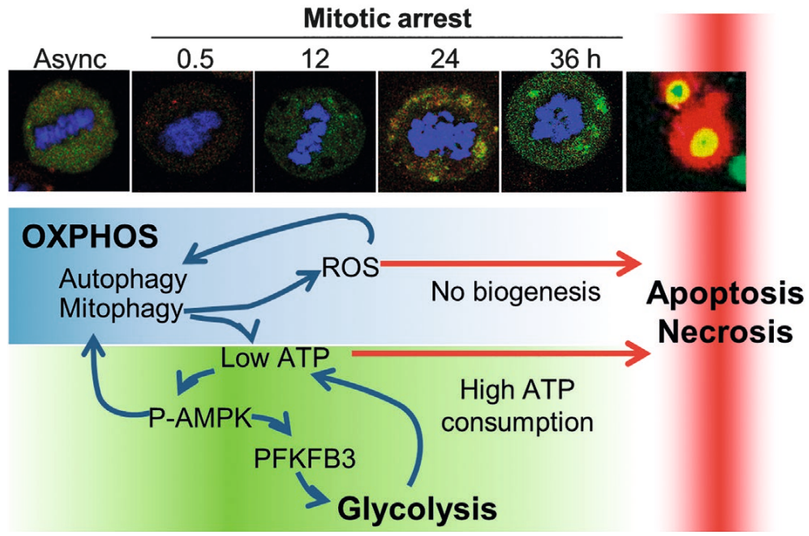

Figure 2 A metabolic switch during mitotic arrest. The extenuation of mitochondria during mitotic arrest results in the activation of an AMPK-PFKFB3-dependent pathway that induces glycolysis for the generation of energy and survival.

autophagy-dependent mitochondrial degradation and a dramatic energetic deficit (Doménech et al., 2015). The subsequent increase in the AMP/ATP ratio results in the activation of the metabolic sensor AMPK followed by phosphorylation and activation of PFKFB3, an enzyme required for glycolysis. Thus, mitophagy can be considered as a critical effector of the therapeutic effect of mitotic therapies, while both AMPK and PFKFB3 are critical for survival. The manipulation of these molecular routes may have therapeutic benefits in the presence of microtubule poisons (Esteban-Martinez et al., 2015). ■

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AWARDS AND RECOGNITION

- Board Member, Scientific Foundation of the Spanish Association Against Cancer (*AECC*).

GENOMIC INSTABILITY GROUP

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Titulado Superior (Advanced Degree)

OVERVIEW

DNA damage is the source of pro-cancerous mutations. In addition, recent evidence has suggested that the reverse connection might also exist; namely that oncogenes can promote the generation of DNA damage. However, the nature of the damage that is caused by oncogenes is still poorly understood. Our laboratory has centred its research on trying to understand how cells respond to 'replicative stress' (RS), a type of DNA damage that unavoidably occurs every time that a cell replicates its DNA, and which is mainly prevented by ATR and Chk1 kinases. Unfortunately, the essential nature of these kinases poses important limitations on their study, particularly at the organism level. In order to overcome these limitations, a significant part of our work over the last few years has been focused on the development of cellular and animal tools for the study of ATR and Chk1. These tools include mice with enhanced or limited ATR-Chk1 function, cell systems in which the pathway can be activated at will, and chemical inhibitors of the ATR kinase. Our studies have revealed the impact of replication stress on cancer and ageing, and have provided drugs that can be used to test our ideas on how to approach cancer therapy. 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 2015, we worked on the development of a new CRISPR-based pipeline for screening genes that are implicated in chemotherapy resistance, and described a new protein complex (SMC5/6) that is essential to suppress cancer and ageing in mammals."

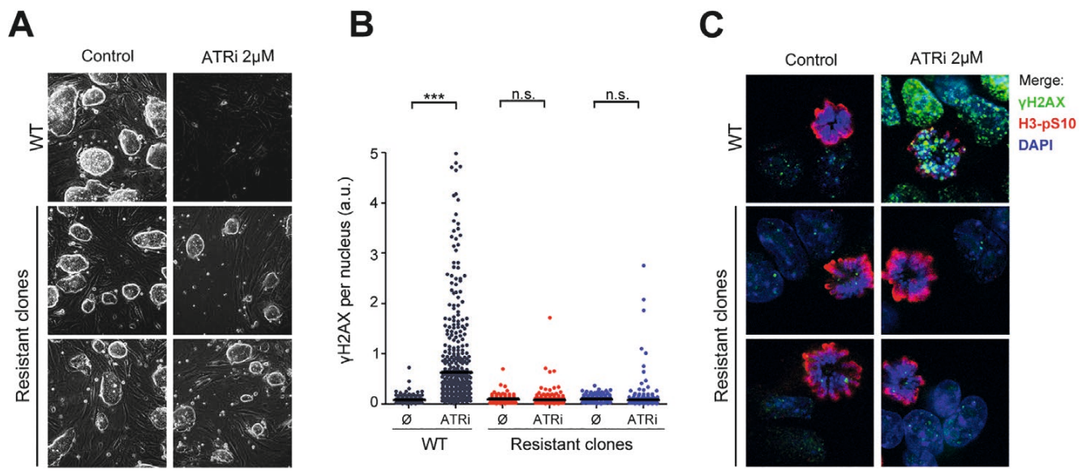

Figure 1 Isolation of mES cells resistant to ATR inhibition. (**A**) Growth of 2 different clones of mES cells at doses of ATR inhibitor (ATRi) that are toxic for wild type (WT) cells. (**B**) Quantification of the DNA damage generated by ATRi (measured by High-Throughput Microscopy as the nuclear intensity of gH2AX, per nucleus) in WT and ATRi-resistant clones. (**C**) Confocal images illustrating the lower levels of DNA damage (gH2AX staining) upon ATRi treatment in resistant clones compared to WT cells.

Searching for mechanisms of resistance to anticancer therapies

Cells are protected from the accumulation of replication stress through a phosphorylation-based signalling cascade that is coordinated by the ATR and Chk1 kinases. It is well known that cancer cells have high levels of replicative stress, and that massive accumulation of this type of DNA damage can rapidly lead to cell death. For this reason, targeting the RS-checkpoint kinase ATR has been extensively studied as an anti-cancer strategy in our laboratory. As a result, in collaboration with the Experimental Therapeutics Programme, we developed ATR inhibitors, some of which were licensed to the pharmaceutical company Merck Serono for clinical development. At present, we are trying to understand the mechanisms of resistance to this drug by making use of a novel CRISPR-based screening pipeline. Survival of therapy-resistant tumour cells is one of the main reasons for tumour relapse and, therefore, a capital problem in clinical oncology. During the last few years, the implementation of the prokaryotic innate immune system CRISPR-Cas9 into eukaryotic cells and animal organisms has become a very potent, worldwide-used tool for genome editing. Making use of this knowledge, we generated inducible-Cas9 mouse ES cells, in which we subsequently transduced a library of sgRNA-s targeting 20,000 genes of the mouse genome. We subsequently used the library for further screens and, for instance, identified mutations that render cells insensitive to ATR inhibitors (FIGURE 1). We are currently validating our findings, which we hope can help us to predict the mechanisms of resistance that might emerge in the clinic, as well as offer us a rational way to predict which patients will particularly benefit from a therapy with ATR inhibitors.

NSMCE2 suppresses cancer and ageing in mice

The structural-maintenance-of-chromosome (SMC) complexes play key roles in chromosome architecture and dynamics. Three heterodimeric SMC complexes have been identified in eukaryotes; these are named SMC1/3 (cohesin), SMC2/4 (condensin) and SMC5/6. The latter one has been shown to prevent DNA damage, but how or where it does this is still a matter of debate. During this past year, we focused our research on NSMCE2, a SUMO ligase that is part of the so-called SMC5/6 complex. By developing 3 independent murine alleles of NSMCE2 (constitutive KO, conditional KO and a sumo dead knock-in), we have found that

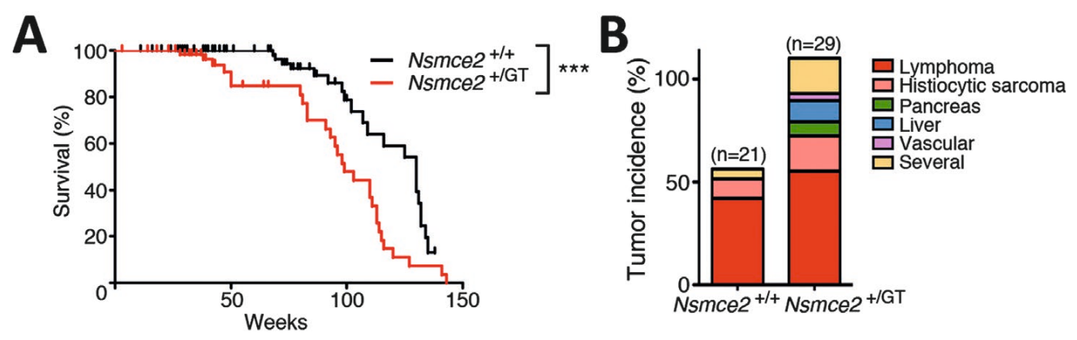

Figure 2 NSMCE2 is a haploinsufficient tumour suppressor. (**A**) Kaplan-Meyer survival curves of *Nsmce2*$^{+/+}$ and *Nsmce2*$^{+/GT}$ mice. (**B**) Tumour incidence found on Nsmce2$^{+/+}$ and *Nsmce2*$^{+/GT}$ mice at time of death.

this protein plays an essential role in limiting recombination and facilitating chromosome segregation in mammalian cells. Interestingly, this role seems to be independent of the SUMO ligase activity of NSMCE2 since mice lacking this activity had no obvious phenotype. Importantly, *Nsmce2* heterozygous mice showed a decreased lifespan associated with a higher incidence of tumours (FIGURE 2). Moreover, deletion of the gene in adult animals led to accelerated ageing and the presence of a number of pathologies also found in patients with a hereditary disease known as Bloom's Syndrome. Our study is the first to show that the genome protection activity of the SMC5/6 complex is essential for the suppression of cancer and premature ageing in mammalian organisms. ■

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AWARDS AND RECOGNITION

- 2015 *Fundación Carmen y Severo Ochoa* Research Award in Molecular Biology, Spain.
- 2015 *Constantes y Vitales* Research Award, *La Sexta* and the AXA Foundation, Spain.

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.

"Chromosome instability is a hallmark of many tumours and one key mechanism to prevent it is sister chromatid cohesion mediated by cohesin. During cell division, cohesion is particularly strong at centromeres. We have discovered an unanticipated role of CENP-C in promoting cohesion at this chromosomal region."

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-free system to explore additional aspects of cohesin regulation.

RESEARCH HIGHLIGHTS

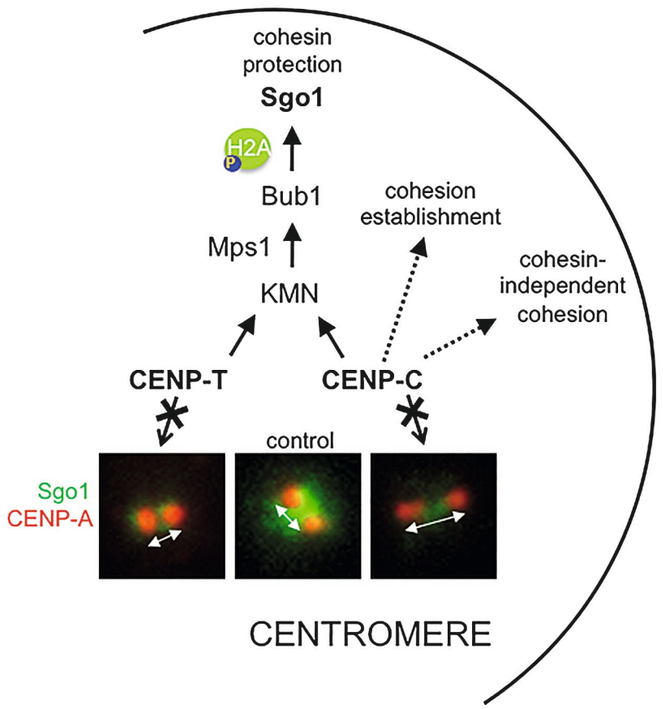

Figure 1 A model for the cross-talk between kinetochore assembly and cohesion machineries. Both CENP-C and CENP-T contribute to the recruitment of the cohesin protector Sgo1 to centromeres to a similar extent. However, prominent cohesion defects – measured as the distance between sister kinetochores – can only be observed in the absence of CENP-C. Additional possibilities for the contribution of CENP-C to centromeric cohesion are outlined.

A novel role of Centromere Protein C (CENP-C) in centromeric cohesion

Centromeric chromatin containing the Centromere Protein A (CENP-A) directs kinetochore assembly through a hierarchical binding of CENPs, starting with CENP-C and CENP-T. Centromeres are the chromosomal regions where cohesion, mediated by cohesin, is most prominently maintained in mitosis. Cohesion is essential for faithful chromosome segregation and therefore for preventing chromosome instability, a common feature of many solid tumours. While most cohesin dissociates from chromosome arms in prophase, Shugoshin 1 (Sgo1) prevents this process at centromeres. Centromeric localisation of Sgo1 requires histone H2A phosphorylation by the kinase Bub1. Using the *Xenopus* egg cell-free system, we have now found that both CENP-C and CENP-T can independently drive centromeric accumulation of Sgo1 through recruitment of Bub1 to the kinetochore KNL1, MIS12, NDC80 (KMN) network. Moreover, we have also shown that targeting of Bub1 is the only requirement for Sgo1 accumulation, since forced targeting of Bub1 kinase domain rescues Sgo1 recruitment in the absence of any kinetochore component other than CENP-A. The kinase Mps1 regulates this pathway. Even though Sgo1 targeting is similarly impaired in chromosomes assembled in extracts lacking Mps1, CENP-T or CENP-C, centromeric cohesion defects are most prominent in the absence of CENP-C. These findings reveal that CENP-C plays a second role in cohesion in addition to Sgo1 recruitment. We are currently investigating the nature of this role, which could be related to cohesin deposition at centromeres, cohesion establishment, or recruitment of some other cohesin-independent regulator of cohesion.

The contribution of cohesin to gene expression and chromatin architecture

To determine how cohesin contributes to the establishment of tissue-specific transcriptional programmes, we have compared the genome-wide distribution of cohesin, gene expression and chromatin architecture in the cerebral cortex and pancreas of adult mice. For this purpose, in close collaboration with the CNIO Bioinformatics Core Unit, we have used Next Generation Sequencing (NGS) technologies: Chromatin Immunoprecipitation (ChIP) sequencing, RNA sequencing and a Chromosome Conformation Capture technique known as 4C. We have found that more than one third of cohesin binding sites differ between the two tissues. The tissue-specific sites show reduced overlap with CCCTC-binding factor (CTCF) and are enriched at the transcription start sites (TSS) of tissue-specific genes. Analyses of chromatin contacts at the Protocadherin (*Pcdh*) and Regenerating islet-derived (*Reg*) gene clusters, mostly expressed in the brain and pancreas respectively, revealed remarkable differences in locus architecture that correlate with the differential distribution of cohesin. Since there are two versions of cohesin in somatic tissues, cohesin-SA1 and cohesin-SA2, we also interrogated their specific contributions. At the *Pcdh* locus, chromatin organisation is not significantly altered in brains of SA1 null embryos, suggesting that cohesin-SA2 can also perform the architectural function when SA1 is not present. In contrast, reduced dosage of SA1 altered the architecture of the *Reg* locus and decreased the expression of *Reg* genes in the pancreas of SA1 heterozygous mice (FIGURE 2). Given the role of Reg proteins in inflammation, such reduction may explain the increased incidence of pancreatic cancer observed in these animals. This is the first reported example of how heterozygous mutations in cohesin may contribute to tumourigenesis. ■

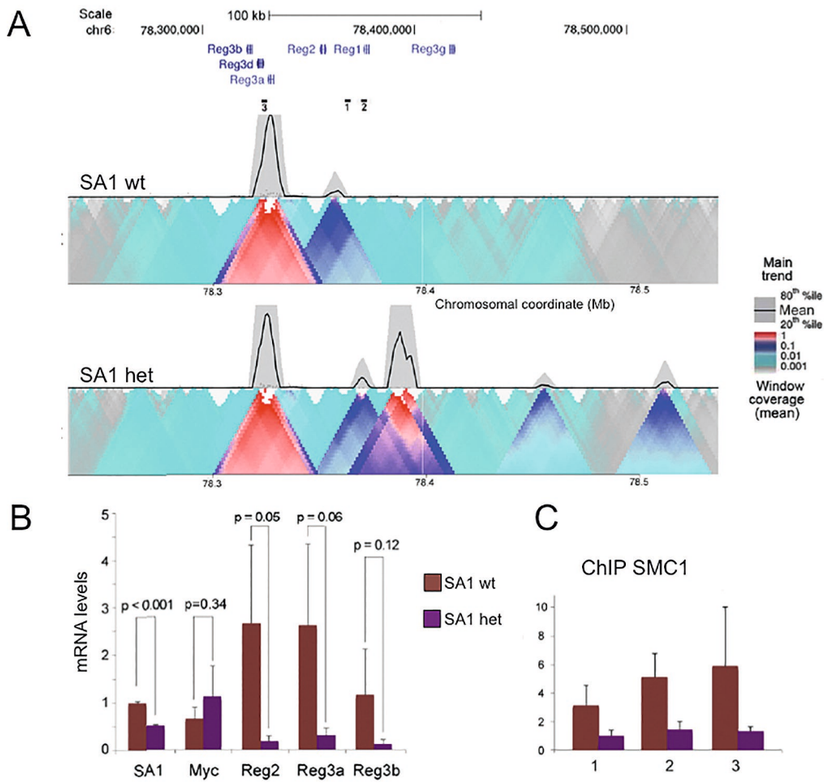

Figure 2 Distinct chromatin architecture at the *Reg* locus in the pancreas of wildtype and SA1 heterozygous mice, assessed by 4C (**A**) correlates with decreased genes expression of three *Reg* genes (**B**) and decreased cohesin occupancy (**C**). These changes could alter pancreas homeostasis and increase the risk of pancreatic cancer in SA1 heterozygous mice.

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DNA REPLICATION GROUP

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Post-Doctoral Fellow
Sara Rodríguez

Graduate Students
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OVERVIEW

Our laboratory studies the process of DNA replication. All cells that proliferate synthesise a replica of their genome before undergoing mitotic division. DNA replication makes the genome vulnerable to mutations and chromosomal reorganisations, and constitutes one of the unavoidable risk factors in developing cancer. On the other hand, as cancer cells are highly proliferative, the proteins that synthesise DNA are useful targets for chemotherapeutic drugs. Our current research interests are: (1) the control of DNA replication through the regulation of replication origins (the genomic positions where 'replisomes' are assembled to start the synthesis of new DNA); (2) the study of specific proteins that mediate replication of damaged DNA, such as the primase-polymerase PrimPol; (3) the consequences of deregulated replication in cancer and ageing. In our different projects we use a combination of technical approaches including biochemistry, molecular biology and mouse genetics.

"We have found that certain blood cell types are highly sensitive to replication stress. A partial loss of the replicative DNA helicase affects haematopoietic stem cells and erythroblasts, causing anaemia and increasing the frequency of haematological cancers."

RESEARCH HIGHLIGHTS

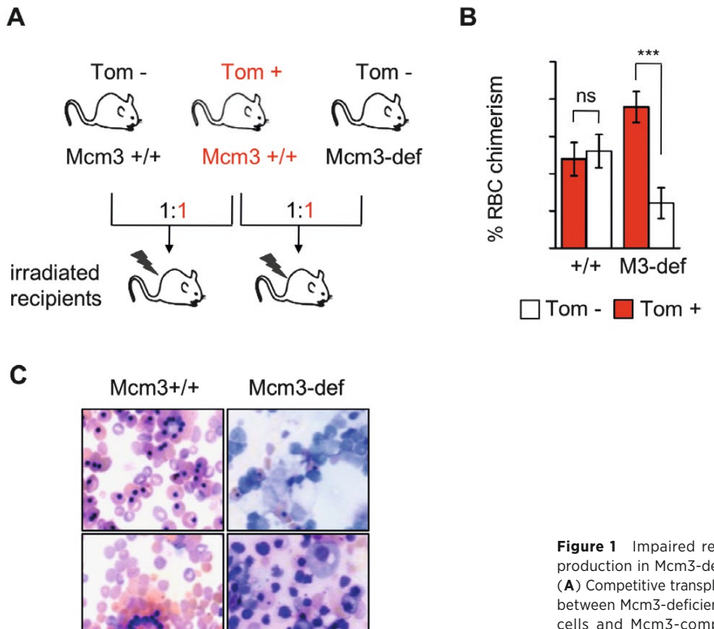

Figure 1 Impaired red blood cell production in Mcm3-deficient mice. (**A**) Competitive transplantation (1:1) between Mcm3-deficient foetal liver cells and Mcm3-competent bone marrow cells expressing fluorescent Tomato protein. (**B**) Red blood cell chimerism in recipient mice after transplantation. (**C**) Accumulation of immature erythroblasts in Mcm3-deficient peripheral blood. Adapted from Alvarez et al. (2015).

Lethal anaemia caused by hypomorphic expression of MCM helicase

We have shown, using a mouse strain with hypomorphic expression of the *Mcm3* gene, that limiting the number of potential replisomes *in vivo* affects the functionality of haematopoietic stem cells and the differentiation of rapidly-dividing erythrocyte precursors (FIGURE 1, A and B). In addition, the lifespan of *Mcm3*-hemizygous mice is reduced due to early-onset lymphomas and mesenchymal tumours. When the concentration of MCM3 protein becomes <1/3 of its normal levels, embryos die *in utero* because the foetal liver fails to make enough red blood cells to sustain oxygen delivery to all tissues. During the last year, we demonstrated the link between defective DNA replication and the anaemic phenotype. As erythrocyte precursor cells maturate in the liver, they undergo several rounds of rapid cell division that require a dynamic programme of DNA replication. In the last proliferation cycles of normal maturing erythroblasts, the speed of the replication machinery progressively decreases and, in turn, more origins are activated to complete replication before reaching mitosis. Mcm3-deficient erythroblasts cannot follow this programme and become blocked at the late basophilic stage, preventing the formation of mature red blood cells and causing a lethal form of aplastic anaemia (FIGURE 1, C). One interesting aspect of this study is that viability can be rescued by introducing an extra copy of checkpoint kinase Chk1, which alleviates replicative stress in other contexts (López-Contreras et al., *J Exp Med*, 2012). In collaboration with the Genomic Instability Group, we found that >50% of MCM3-deficient embryos carrying higher levels of Chk1 completed gestation and survived as adults with a mild anaemia (Alvarez *et al.*, 2015). This result opens up the interesting challenge of enhancing the cellular response to replicative stress as a means to counteract aplastic anaemias that are frequently associated with chemotherapy treatments.

Cdc6 overexpression affects papillomagenesis and influences hair growth

In 2015, we also completed a study to monitor the effect of Cdc6 deregulation *in vivo*. Cdc6 encodes a protein responsible for the recruitment of MCM helicase to replication origins and is overexpressed in several cancer types, including subsets of brain tumours, mantle cell lymphomas and non-small cell lung carcinomas. To date, no model has been described to test the proto-oncogenic effects of Cdc6 deregulation in mammalian tissues. The K5-Cdc6 mice strain generated at the CNIO displayed higher levels of CDC6 protein in the skin and other tissues with stratified epithelia. Cdc6 "gain of function" was revealed by the enhanced loading of MCM complexes in keratinocytes. Deregulated Cdc6 by itself did not promote skin tumours, but in combination with chemical carcinogens it favoured the formation of benign papillomas. Furthermore, older K5-CDC6 mice displayed better fur preservation than their wild-type littermates (FIGURE 2). This unanticipated 'anti-ageing' effect was analysed in collaboration with the laboratory of C. Blanpain (*Université Libre de Bruxelles*, Belgium); we found that CDC6 extended the resting stage of the hair follicle growth cycle (Bua et al., 2015).

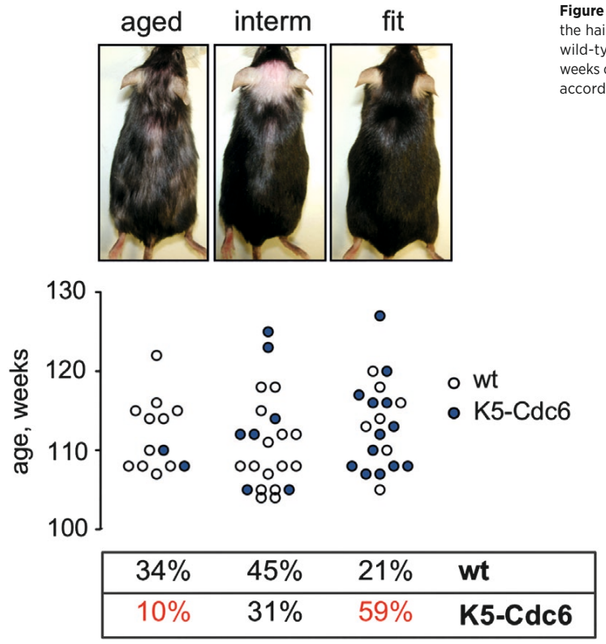

Figure 2 Cdc6 deregulation affects the hair growth cycle. Distribution of wild-type and K5-CDC6 mice (>105 weeks old) in 3 phenotypic categories according to their fur preservation. CDC6 overexpression extended the resting stage of hair follicles, increasing hair preservation. Adapted from Búa et al. (2015).

Applications of single-molecule analysis of DNA replication

One of our approaches for the study of DNA replication consists of the analysis of replisome progression and origin activity in individual DNA molecules. To this end, we extensively use a 'stretched DNA fibre' technique that has attracted the interest of other CNIO Research Groups. In 2015, single-molecule analysis of DNA replication was applied to several projects, including the study of megakaryocyte polyploidisation mechanisms led by Marcos Malumbres (Trakala et al., 2015) and the study of NSMCE3 in cancer and ageing, led by Oscar Fernandez-Capetillo (Jacome et al., 2015). We are currently working on the development of novel applications for this powerful technique. ■

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MELANOMA GROUP

María S. Soengas
Group Leader

Staff Scientists
Alicia Gónzalez (until March), David Olmeda

Post-Doctoral Fellow
Lisa Osterloh

Graduate Students
Daniela Cerezo, Metehan Cifdaloz, Panagiotis Karras, Raúl Martínez, Eva Pérez (until September), Cristina Tejedo (since November)

Technicians
Tonantzin Calvo, Estela Cañón (TS)*

Titulado Superior (Advanced Degree)

OVERVIEW

Cutaneous melanomas are a prime example of aggressive cancers, for which basic and translational research have significantly improved patient prognosis. Nevertheless, sustained clinical responses are still incomplete in this disease. The long-term goal of our Group is to identify new tumour drivers and progression biomarkers as a platform for a more rational drug design. Specifically, we are interested in stress response programmes (involving apoptosis, autophagy, senescence and endosome mobilisation), with a particular focus on lineage-specific oncogenes. RNA-binding proteins are also central themes in our research. Our experimental settings include human biopsies isolated from early, intermediate and late stages of melanoma development, combined with a unique set of animal models engineered for non-invasive imaging of metastatic processes. These studies are performed in the context of large multidisciplinary consortia and biotechnology companies in order to facilitate the translation of our discoveries to the bedside.

"We have identified oncogenic signals that are uniquely deregulated in melanoma. These lineage-specific drivers increase the risk for metastasis and inhibit the response to current targeted therapies, and as such, may have direct translational implications."

RESEARCH HIGHLIGHTS

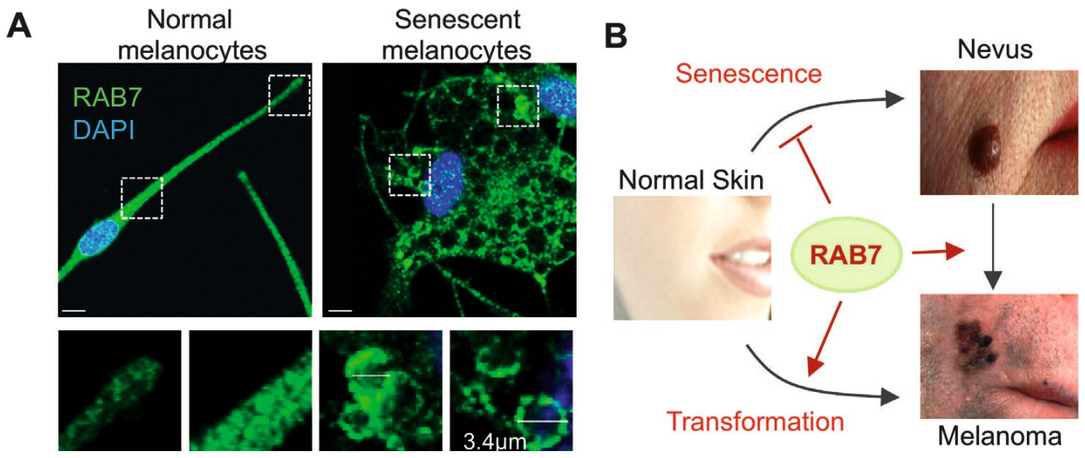

Figure 1 RAB7 as a new lineage-specific melanoma driver. (**A**) RAB7 macropinosomes (green) induced by a PI3K-activating oncogene in human senescent melanocytes (right panel) visualised by immunofluorescence. Normal melanocytes are included on the left. (**B**) Mode of action of RAB7 in melanoma development.

New lineage-specific roles of the endolysosomal machinery in melanoma progression

One of the long-term objectives of the Melanoma Group is the discovery of new melanoma drivers. In particular, we pursue lineage-specific oncogenes, which, due to their unique functions in melanoma, may represent alternative targets for therapeutic intervention. We have identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies. Within this gene cluster, melanomas were found to depend on the vesicular trafficking modulator RAB7 as a dose-dependent rheostat of progression and invasion (Alonso-Curbelo et al., *Cancer Cell* 2014 and *Oncotarget* 2015). In collaboration with the CNIO Experimental Therapeutics Programme, we have now traced this 'RAB7-based addiction' right back to the level of primary melanocytes. RAB7 was found to be essential to promote the degradation of an active influx of macropinosomes generated by early oncogenic signals (PI3K-induced), which otherwise would be toxic and lead to premature senescence (FIGURE 1). These data are relevant as they illustrate how melanoma cells wire the endolysosomal machinery to ensure their 'fitness' at all stages of tumour development. In collaboration with the group of P. Agostinis (the University of Leuven, Belgium), we are further exploring therapeutically-relevant regulatory mechanisms and functions of the endolysosomal machinery in different cell types (Maes et al., *FEBS J*, 2016).

Metastatic risk and resistance to BRAF inhibitors in melanoma defined by selective allelic loss of *ATG5*

We then questioned whether other lysosomal-dependent degradative processes, such as autophagy, could also be regulated and act in a melanoma lineage-specific manner. Curiously, a meta-analysis of mRNA expression and copy number variation, together with the assessment of prognostic values of all the genes constituting the core autophagy machinery, identified a unique feature in melanoma that involved selective heterozygous loses of *ATG5* (FIGURE 2A). This *ATG5* heterozygosity predicted poor overall patient survival, in melanoma, in a manner not shared by other autophagy factors and not recapitulated in other cancer types. Newly generated mouse models confirmed the functional relevance of the heterozygous deletion of *Atg5* in melanoma metastasis and importantly, in the resistance to targeted therapy (FIGURE 2B). These data have relevant translational implications for drug design, as an inadvertent partial blockade of autophagy may worsen (instead of counteracting) the malignant behaviour of metastatic melanomas (García-Fernández et al., *Autophagy*, in press).

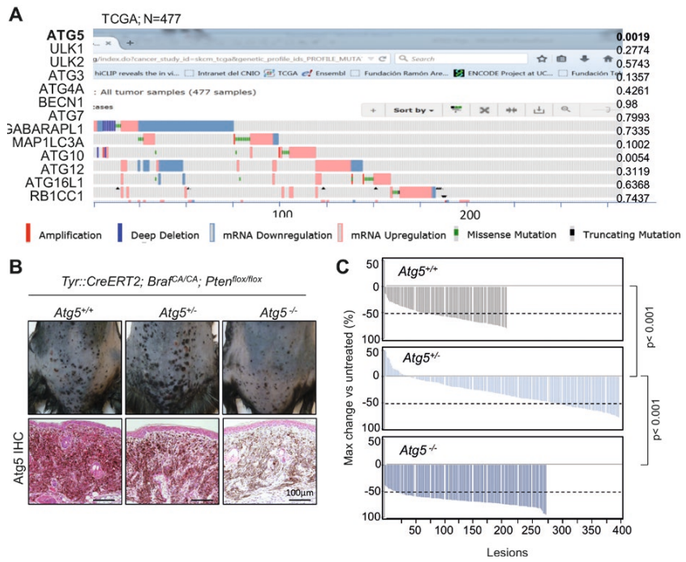

Figure 2 ATG5 in melanoma progression and drug resistance. (A) mRNA expression and genomic status of the indicated autophagy genes in TCGA melanomas, with p values corresponding to overall patient survival. (**B**) Impact of Atg5 copy number in the response to dabrafenib in *Tyr::CreERT2;Braf*$^{CA/CA}$*;Pten*$^{fl/fl}$-driven melanomas.

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

Melanomas are long-known for their highly deregulated mRNA gene expression profiles. Still, the specific contribution of RNA binding proteins and spliceasome modulators remains virtually unexplored in this disease. In collaboration with the groups of J. Valcárcel, F. Gebauer (the Centre for Genomic Regulation, CRG) and R. Méndez (the Institute for Research in Biomedicine, IRB), we have identified tumour-selective roles of modulators of mRNA stability, alternative splicing, transcription and translation, with targets involving master specifiers of melanocyte lineage. We are very excited about the prospect of RNA regulators as diagnostic markers and therapeutic targets in melanoma.

We have also made great progress in dsRNA nanoparticles as anticancer therapies. Comprehensive functional analyses *in vivo* now demonstrate a three-pronged activity of these compounds: (i) killing melanoma cells by further deregulating their endolysosomal machinery; (ii) abrogating the function of the neo-lymphangiogenic vasculature associated with malignant melanomas; and (iii) engaging a potent immunomodulatory activity (Olmeda et al., submitted). This information will support clinical trials that are under consideration by the CNIO spin-off company *Bioncotech Therapeutics*. ■

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- Maes H, Olmeda D, Soengas MS, Agostinis P (2015). Vesicular trafficking mechanisms in endothelial cells as modulators of the tumor vasculature and targets of antiangiogenic therapies. *FEBS J*. PMID: 26443003.

AWARDS AND RECOGNITION

- Established Investigator Award 2015, Melanoma Research Alliance.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García (since March)

Graduate Students
Ana I. Amor (since February), Lucía Robado (since October)

Technicians
Cristina Merino (since August), Marina Mazariegos (since November)

OVERVIEW

Metastasis is the most devastating phase of cancer. While most of the research effort to date has been focused on analysing the primary tumour, there is a lack of information on how the tumour microenvironment influences metastasis and pre-metastatic niche formation. The mechanisms underlying the evolution of the tumour microenvironment during metastasis may hold the key to converting cancer from a deadly disease to a manageable one. Prominent roles for stromal cells, such as fibroblasts, endothelial cells, lymphatic endothelial cells, bone marrow-derived cells, soluble factors and secreted vesicles have been established during pre-metastatic niche formation. Our novel studies suggest that tumour-secreted exosomes can fuse specifically to stromal cells acting locally in pre-metastatic niches and systemically reinforcing metastasis. We focus on understanding the interactions of the tumour with its microenvironment, thereby defining new targets to block metastasis.

"Our work has highlighted that tumour-secreted exosomes promote the 'education' of the tumour microenvironment, thus reinforcing metastasis."

Students in Practice
Diego Barba (May-July), Estrella Chavero (April-June), Teresa Gónzalez (since September), Sergio Haro (April-June), Macarena Tello (July-September), Bryan Zichang Wang (June-August)

RESEARCH HIGHLIGHTS

Role of tumour-derived exosomes in pre-metastatic niche formation

We have demonstrated that exosomes are biomarkers and functional contributors to pre-metastatic niche formation in metastatic organs. Exosomes can serve as vehicles for horizontal transfer of oncoproteins, thus promoting additional modifications in the tumour and metastatic microenvironments. We showed that melanoma-derived exosomes expressing c-MET influence bone marrow-derived cell mobilisation and recruitment to pre-metastatic and metastatic niches, thus promoting metastasis in a process that we have termed 'education' (Peinado et al., *Nature Med*, 2012). More recently, we have observed that pancreatic cancer-derived exosomes expressing macrophage migration inhibitory factor (MIF), preferentially acted upon Kupffer cells (Costa-Silva et al. *Nat Cell Biol*, 2015). Strikingly, MIF and c-MET levels in plasma exosomes demonstrate the potential of using exosomal protein levels as an early biomarker for liver pre-metastatic niche formation and for predicting patient outcomes, respectively.

Tumour-derived exosomes define metastatic organotropism

Our studies demonstrated that tumour exosomes are a major tumour-derived factor that acts systemically to promote bone marrow-derived cells (BMDCs) recruitment to the tumour and metastatic microenvironments (Peinado et al., *Nature Med.*, 2012). Our recent results demonstrate that tumour-derived exosomes are uptaken by organ-specific cells preparing the pre-metastatic niche. Exosome proteomics revealed distinct integrin expression depending on their specific organ of metastasis. Therefore, we postulate that exosome integrins could serve as a 'ZIP' code for exosomes to home in metastatic organs triggering local effects reinforcing organ-specific metastasis. Our clinical data indicate that the profiling of integrins in circulating exosomes could be used to predict organ-specific metastasis (Hoshino et al. *Nature*, 2015). ■

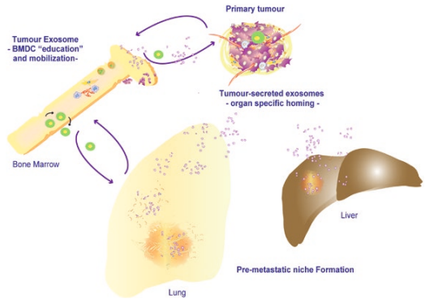

Figure Model of exosome-mediated metastatic dissemination. Tumour-derived exosomes educate BMDCs and are uptaken by organ-specific resident cells in future metastatic organs promoting the pre-metastatic niche formation.

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- Benito-Martin A, Peinado H (2015). FunRich proteomics software analysis, let the fun begin! *Proteomics* 15, 2555-2556.
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AWARDS AND RECOGNITION

- William Harvey International Translational Research Academy (WHRI-ACADEMY) COFUND Marie Curie Fellow.
- 1st Young Investigator Award, *ASEICA* (*Asociación Española de Investigación en Cáncer*), Spain.
- 2015 *Constantes y Vitales* Prize for Metastasis Research, *La Sexta* and the AXA Foundation, Spain.

BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente (since March)
Junior Group Leader

Staff Scientist
Marta I. Barradas (April-December)

"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."

Graduate Students
José Aguareles (July-November), Catia P. Domingues (since March), Maria Figueres (since September), Almudena Saiz (since October)

Students in Practice
Miguel Marín (June-October), Manon Mulders (since November), David Wasilewski (since July), Lucia Zhu (since November)

OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is always poor given that current therapy (i.e. surgery and radiation) has limited benefits for patients and the disease inevitably relapses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extracranially. Paradoxically, given the inefficiency of these therapies to work in the brain, they give cancer cells extra-time to colonise this highly demanding secondary site. In the laboratory we study why and how cells from different cancer types (breast and lung cancer among others) 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.

RESEARCH HIGHLIGHTS

The Brain Metastasis Group has established a new line of research at the CNIO focused on the progression of cancer to the Central Nervous System (CNS). During 2015, we have initiated various projects:

- → By exploring the role of commonly de-regulated genes in brain metastasis experimental models, we are now investigating unknown aspects of brain colonisation by cancer cells.
- → We have identified 2 distinct sources of resistance to irradiation, which we are currently testing using novel brain metastasis models that incorporate this therapy.
- → We are dissecting the heterogeneity present in the brain metastasis associated microenvironment by targeting transcription factors that we identified in subpopulations of glial cells associated with brain metastasis.
- → The lab has validated a new experimental platform to test brain metastasis sensitivity to drugs, which maintains cancer cells within their microenvironment and that is compatible with human specimens and medium-throughput optimisation. ■

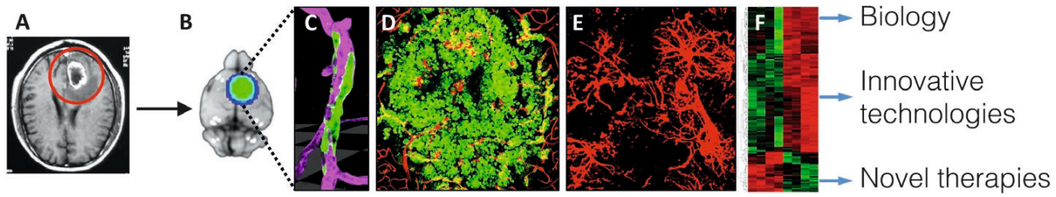

Figure (**A**) MRI of a human brain metastasis circled in red. (**B**) Mouse brain metastasis in experimental models identified with bioluminescence. (**C**) First days after brain metastatic cells (green) extravasated from capillaries (magenta). Metastatic cells grow along these vessels. (**D**) Established brain metastasis (green) in an advanced state surrounded by capillaries (red). (**E**) Reactive glia (red) is highly represented in the brain metastasis associated microenvironment. (**F**) Genomic analysis of brain metastatic cells under different experimental conditions.

AWARDS AND RECOGNITION

- Fero Grant for Translational research in Oncology (*IX Beca Fero 2015*), Fero Foundation for Oncology Research.

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 to liver, bone and brain tumours. The research covers various 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.

Mirna Pérez-Moreno's Group investigates the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer development. 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 (F-SB), studies how brain tumours, mainly glioblastomas and medulloblastomas, develop and how they respond to therapy. The Senior Group, led by Francisco X. Real, studies epithelial tumours focusing 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. 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, skin and bone. We are investigating the role of AP-1 in skin cancer and in inflammatory skin diseases, such as psoriasis, but we also aim to molecularly define the causes of lung fibrosis and cancer. We have also embarked on a new project in order to study how the whole organism responds to a growing tumour in the context of a complex metabolic disorder, termed cancer cachexia.

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

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

Staff Scientists
Latifa Bakiri, Nuria Gago, María Jiménez, Özge Uluçkan

Post-Doctoral Fellows
Albanderi Alfraidi, Rainer W. Hamacher (until March), Kazuhiko Matsuoka, Álvaro Ucero

Graduate Students
Lucía T. Díez, Stefanie Wurm (until May)

Technicians
Vanessa Bermeo, Ana Guío (TS)*, Stefania Tocci (until June)

**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 ultimate goal is to define molecular pathways leading 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.

"We aim to ensure that CNIO remains an international 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. Fifteen different nationalities from the 5 continents ensure an international science culture and all focus on unraveling the mysteries of 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, 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 screens.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins and their targets in bone development and disease using loss- and gain-of-function mouse models. c-Fos expression leads to osteosarcoma (OS) and chondrogenic hyperplasias. We found that c-Fos modulates Wnt signalling in OS cells, which constitutes a potential link between c-Fos/AP-1 and OS development. Furthermore, we generated adenoviral constructs expressing Cre recombinase under the control of cell-specific promoters, which allow restricting AP-1 expression or inactivation to specific cells of the bone lineage. Bone/cartilage dysplasias are observed when c-Fos is locally expressed in the knee joint using this approach.

Rheumatoid (RA), Psoriatic (PsA) and Osteo-Arthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the functions of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches.

Liver disease – inflammation, metabolism, fibrosis and cancer

Fra-1/2 proteins appear to be dispensable for liver fibrosis, while they are important modulators of hepatic lipid metabolism by controlling PPARγ transcription. Strikingly, specific AP-1 dimers can either induce or repress PPARγ expression. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, while both Fra proteins protect against steatosis, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to hepatomegaly and liver dysplasia in aged mice. Mechanistically, molecular analyses point to the involvement of the Wnt/γ-catenin pathway, often connected to human hepatocellular carcinoma (HCC).

Ectopic c-Fos expression and its dimers lead to spontaneous liver inflammation, fibrosis, hepatocyte/bile duct proliferation and tumours with human HCC gene signatures. Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas deletion in immune cells abrogates this protective effect. Moreover, a robust connection between c-Fos expression and the activity of the liver X receptor/retinoid X receptor (LXR/RXR) pathway, an important regulator of cholesterol homeostasis, was observed and most likely contributes to the oncogenic function of c-Fos in hepatocytes.

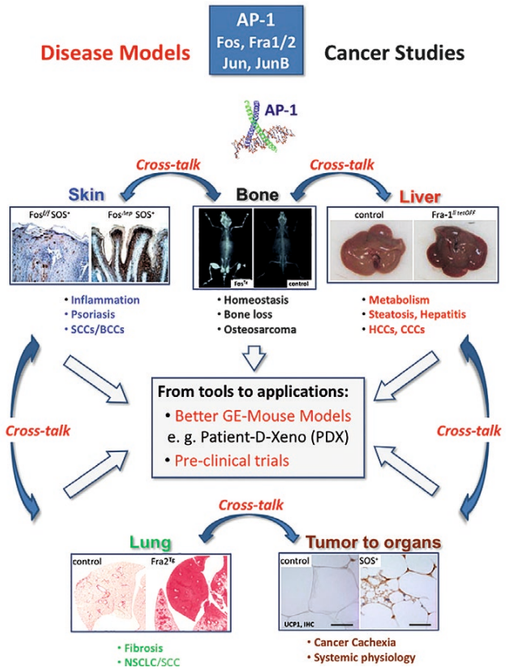

Figure Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse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

Cancer-associated cachexia (CAC)

We are studying the role of white adipose tissue (WAT) 'browning' as a contributing factor to the wasting process, thus providing a promising new target to prevent/delay cachexia in cancer patients. Other aspects of the syndrome are being investigated using GEMMs, such as the role of inflammation, the metabolic impairment and the systemic changes occurring in the neuro-endocrine system, in order to delineate the course of events during CAC and to potentially identify biomarkers for the onset of CAC.

A function for AP-1 in lung disease

We recently documented the connection between the Fos protein Fra-1 and major transcription factors controlling epithelial to mesenchymal transition, a process important in epithelial cancers. The contribution of Fra1/2 proteins to lung fibrosis and non-small cell lung cancer (NSCLC) is currently being studied using GEMMs, as well as lung cancer samples from patients, in order to unravel the relevant cellular and molecular mechanisms. Our preliminary data from the mouse models indicate that Fra-2 might be crucial for the development of fibrosis and protection from cancer in the lung. These findings may represent new diagnostic and therapeutic opportunities for these diseases and lay grounds for further pre-clinical testing. This study is conducted in collaboration with Mariano Barbacid's Experimental Oncology Group at CNIO and Daiichi Sankyo Company (Japan).

Skin cancer, inflammation and human disease

Since Squamous Cell Carcinomas (SCCs) have increased c-Fos expression, we modelled SCC development in mice with inducible c-Fos expression. We identified an essential role of c-Fos in modulating immune cell recruitment to the skin, which contributes to skin cancer development. We are currently evaluating the effect of p53 deletion in epidermal cells lacking c-fos. These mice develop SCCs upon ageing. The goal is to understand the initiation of SCCs in relation to ageing. We also demonstrated that loss of epidermal Fra-2 protein results in skin barrier defects. Mechanistically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters, which are co-occupied by the transcriptional repressor Ezh2 through post-translational mechanisms involving the ERK pathway.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone crosstalk. JunB represses the expression of pro-inflammatory cytokines that affect the differentiation of bone-forming osteoblasts. We extended our studies to psoriasis patients and have shown that they have bone loss. We are currently planning to evaluate the role of the microbiota in skin inflammation by antibiotic treatments, high-throughput microbiota sequencing and germ-free housing conditions.

New approaches including genetic and biochemical analyses by proteomics of mouse and human skin samples were performed; these unravelled novel pathways and molecules for targeted therapies, such as S100A8/A9 and complement C3. In addition, a potential role of specific miRNAs, e.g. miR21 involved in the pathogenesis of psoriasis, was established showing that miR21 inhibition can be a novel therapeutic intervention in psoriasis. Human skin samples are provided by our collaborator Esteban Daudén from the *Hospital Universitario de La Princesa* (Madrid, Spain). Another angle of research in psoriasis involves the understanding of the role of epidermal stem cells in the initiation and progression of psoriasis using state-of-the-art lineage-tracking models. ■

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EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Staff Scientist
Victor J. Sánchez-Arevalo

Post-Doctoral Fellows
Itxaso Bellón (since July), Enrique Carrillo, Luis C. Fernández, Eleonora Lapi, Miriam Marqués

Graduate Students
Isidoro Cobo, Francesc Madriles, Catarina Pereira

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

**Titulado Superior* (Advanced Degree)

OVERVIEW

Our Group focuses on pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC) by adopting a disease-oriented approach. Our strategy accords equal importance to the 3 models that we use: cultured cells, genetically modified mice, and patient samples. Our primary observations can be made at either of these levels and they are then expanded through additional work. We translate this knowledge to the "population-wide" level by harnessing information and samples from large patient cohorts.

Our research on PDAC focuses on the role of cell differentiation as a potent tumour suppressor mechanism that acts early on during carcinogenesis. We use the excellent genetic mouse models that are available because these processes cannot be readily studied using human samples. PDAC can originate both in pancreatic progenitors as well as in acinar cells. The elucidation of the contribution of these cell types to PDAC is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

In the context of UC, we focus our efforts on identifying new genes, using them for improved tumour taxonomy, as well as for characterising the mechanisms through which they participate in cancer. This knowledge is then applied for improved prediction of outcome and therapy.

"In 2015, we exploited the PDAC mouse models to highlight the role of GATA6 as a tumour suppressor and the requirement of BPTF for c-Myc- and KRas-driven carcinogenesis. We continued our genomic studies of UC and generated new information to improve the molecular classification of tumours and cell lines."

RESEARCH HIGHLIGHTS

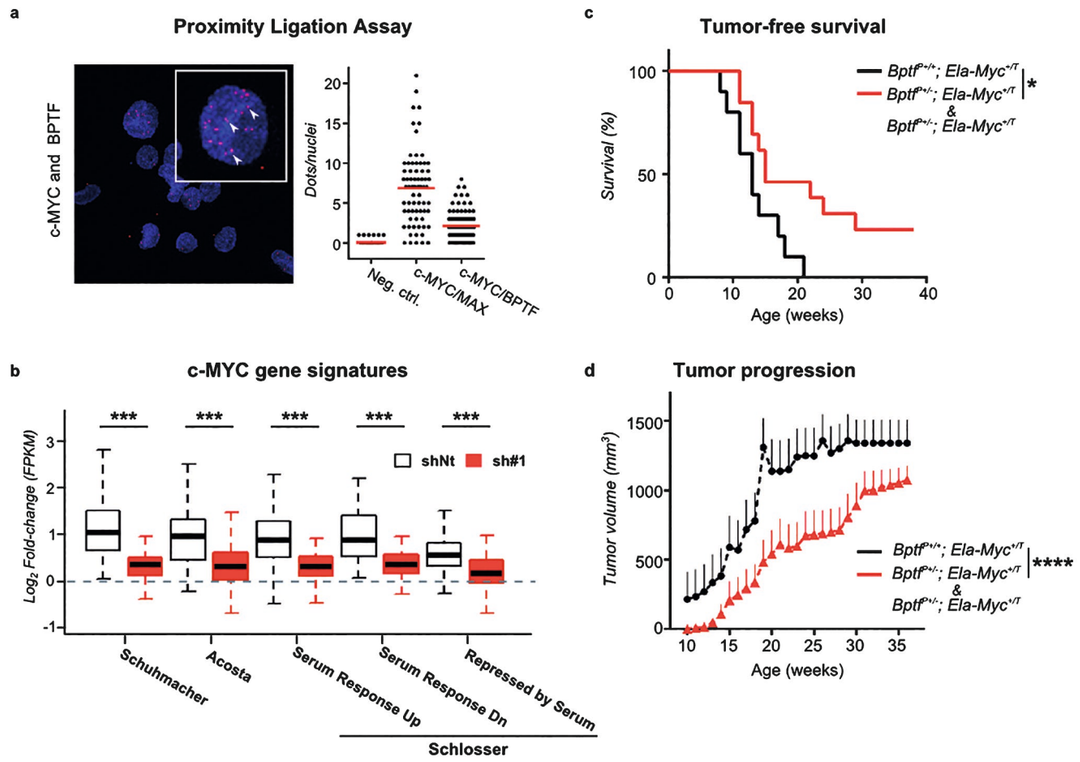

Figure The c-MYC-BPTF axis in human cancer. c-MYC and BPTF interact, as shown by the proximity ligation assay; the c-MYC/MAX interaction was used as a control (**A**). BPTF is required for the activation of c-MYC transcriptional signatures, as determined by the genetic knockdown of BPTF in human fibroblasts (**B**). Inactivation of 1 or 2 *Bptf* alleles in the pancreas hinders the initiation (**C**) and progression (**D**) of tumours in the Ela1-Myc mouse model of pancreatic cancer. These observations support the therapeutic relevance of this axis in c-MYC-driven tumours.

Pancreas cancer molecular pathophysiology

Cell differentiation as a tumour suppressor mechanism in the pancreas. PDAC is characterised by highly prevalent alterations in *KRAS, p16, TP53*, and *SMAD4*, as well as by low-frequency alterations in a plethora of other genes converging in a few critical genetic pathways. Our main interest is to identify new players involved in this tumour. We have continued our work on NR5A2 and have shown that the pancreas of heterozygous mice is histologically normal but that it displays a basal proinflammatory state similar to that of the early stages of acute pancreatitis. The activation of inflammatory genes is both direct and indirect, the latter through AP-1-dependent mechanisms. NR5A2 undergoes a 'genomic switch' from acinar genes to inflammatory genes both in haploinsufficiency and during pancreatitis. AP-1 proteins play a major role in these processes. We are now exploring whether similar effects take place in the pancreas of subjects carrying *NR5A2* variants associated with PDAC risk.

The BPTF-c-MYC axis as a therapeutic target. We identified BPTF as a regulator of PDAC and UC cell proliferation *in vitro*, and as a gene mutated in bladder cancer. We have now shown that BPTF is part of a complex containing c-MYC and that it is required for the full transcriptional and biological activity of c-MYC. In addition, we have shown that genetic inactivation of *Bptf* in mice hinders the initiation and progression of tumours driven by c-MYC, suggesting that this protein may be a good therapeutic target in cancers harbouring alterations in this gene/pathway. Importantly, these effects are also observed in haploinsufficiency. We are currently using mouse models of Burkitt lymphoma to further explore this notion.

This work benefits from a close collaboration with other groups working at the CNIO (Mariano Barbacid from the Experimental Oncology Group, Erwin Wagner from the Genes Development and Disease Group, and Núria Malats from the Genetic and Molecular Epidemiology Group).

Urothelial carcinoma (UC) genetics and biology

Our goal is to refine current knowledge on the genomic landscape of UC and to apply this in the clinical setting.

Over the last few years, a new molecular taxonomy of UC with broad implications has been emerging. A BAsal/SQuamous-like (BASQ) subtype has been defined by consensus at a meeting organised at the CNIO earlier this year. There was also agreement on the existence of a Luminal-Urothelial subtype that needs to be better defined at the molecular level. Together with the SOGUG cooperative group, we aim to assess the usefulness of this classification in the clinical setting. Approximately 30% of UC display a basal phenotype and the relationship with response to neoadjuvant and adjuvant therapy is being analysed.

Through exome sequencing we have identified new genes and pathways involved in UC and we are focusing on the STAG2 cohesin. We are analysing the mechanisms through which this gene contributes to UC, as well as the clinical significance of *STAG2* inactivation as it relates to patient outcome and response to therapy, and we are generating conditional *Stag2*-null mice. A relationship between *STAG2* inactivation and the Luminal phenotype has been established using several strategies.

UC cell lines have provided useful tools to study bladder cancer biology and to perform preclinical studies; we have generated the largest UC line genomic resource, which has evidenced the limitations of these strategies. Therefore, during the last year we have started to apply 'organoids' technology to study urothelial cells. We have established reproducible methods to produce normal mouse urothelial organoids and are characterising their requirements for growth and differentiation. Normal organoids have been cultured uninterruptedly for over 1 year. We have also initiated the culture of mouse tumour organoids and plan to move to using human samples in the near future.

This work is being conducted in close collaboration with the Group of Núria Malats at the CNIO, as well as with SOGUG and a European Consortium of collaborators. ■

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Book chapter

- Real FX, López-Otín C (2015). *Genes, herencia y cáncer*. In: Farreras-Rozman. *Medicina Interna*, Harcourt Brace de España, ed., Barcelona, in press.

AWARDS AND RECOGNITION

- 2015 European Pancreatic Club Meeting (President).

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

Mirna Pérez-Moreno
Junior Group Leader

Post-Doctoral Fellows
Donatello Castellana (until October),
Silvia M. Janeiro (since October)

Graduate Student
Daniel Peña (since February)

Technician
Francesca Antonucci (TS)*

**Titulado Superior* (Advanced Degree)

OVERVIEW

Tumour cells evolve into a progressively complex interplay of heterogeneous tumour cells with their tissue microenvironment, 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 in defining regulatory pathways controlling their proliferation and/or differentiation. However, the identification of extrinsic factors modulating stem cell behaviour is not yet fully established. Using the skin as a model system, based on mouse genetics and human samples, our research aims to understand how the interactions between epithelial progenitor cells, and also the interactions with their surrounding microenvironment, sustain skin homeostasis and regeneration, and how, when perturbed, this may lead to cancer. This information may provide insights for the future development of regenerative and anti-cancer therapies.

"During 2015, we continued our efforts to uncover novel events controlling the behaviour of skin stem cells, with the aim of opening up new insights into the mechanisms that control their regenerative characteristics and how, when disrupted, these may result in cancer."

RESEARCH HIGHLIGHTS

Regulation of epidermal progenitor cells' self-renewal and differentiation

We continue to explore how tissues acquire an adequate control of cell division and differentiation. In particular, we study the contributions of mitotic and cytoskeletal proteins in the regulation of skin progenitor's self-renewal through oriented cell divisions, using mouse epidermal development as a model system.

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

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

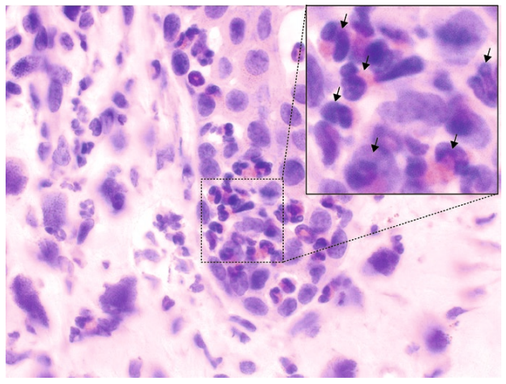

Figure (**A**) Skin carcinoma showing presence of a high density of inflammatory cells within the tumour. (**B**) Inset showing a magnification of immune infiltrates within the tumour. * Arrows point to some immune cells.

Contributions of inflammatory responses in cancer stem cell maintenance and tumour progression

The formation of tumours and their progression to malignancy undoubtedly involves the contributions of the tumour microenvironment. Identifying the signalling mechanisms and cell types that contribute to tumour initiation and progression to malignancy is instrumental for the identification of potential targets for clinical applications to eradicate tumours.

The microenvironment of many tumours is rich in cytokines, chemokines, and inflammatory enzymes. During 2015, 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 have directed our efforts towards 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. ■

PUBLICATIONS

- Sánchez-Martínez R, Cruz-Gil S, Gómez de Cedrón M, Álvarez-Fernández M, Vargas T, Molina S, García B, Herranz J, Moreno-Rubio J, Reglero G, Perez-Moreno M, Feliu J, Malumbres M, Ramírez de Molina A (2015). A link between lipid metabolism and epithelial-mesenchymal transition provides a target for colon cancer therapy. *Oncotarget* 6, 3879-3836.
- Villarejo A, Molina-Ortiz P, Montenegro Y, Moreno-Bueno G, Morales S, Santos V, Gridley T, Pérez-Moreno MA, Peinado H, Portillo F, Calés C, Cano A (2015). Loss of Snail2 favors skin tumor progression by promoting the recruitment of myeloid progenitors. *Carcinogenesis* 36, 585-597.
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AWARDS AND RECOGNITION

- Editorial Board Member, *Experimental Dermatology* Journal.
- Elected as one of the 101 most innovative minds in Spain by *Quo* magazine.

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Nabil Djouder
Junior Group Leader

Post-Doctoral Fellow
Hugo Bernard

OVERVIEW

The incidence of metabolic diseases and cancer has increased to epidemic proportions possibly due to a high-calorie diet and nutrient overload along with a more sedentary lifestyle. In this regard, we are interested in deciphering the growth factor and nutrient signalling pathways to find new effectors as well as in generating mouse models recapitulating human diseases. This will allow us to better understand how growth factors and nutrients impact on the patho-physiolological states of metabolic disorders and cancer. Using cell biological and biochemical techniques, combined with in vivo mouse models and human data, our lab devotes significant effort to develop innovative mechanism-based therapeutics, thereby providing the development of novel avenues to treat metabolic dysfunctions and cancer.

"Our research focus is to apply complex cancer models to guide clinical research perspectives and applications."

Graduate Students
Marta Brandt, Almudena Chaves, Amanda Garrido (since February), Ana Teijeiro, Krishna Seshu Tummala (until October)

Student in practice
Celia de la Calle (since February)

RESEARCH HIGHLIGHTS

Our lab studies molecular mechanisms of diseases associated to the dysregulation of the growth factor and nutrient signalling cascades. We have a particular interest in 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. Our task is to find new components of growth factor and nutrient circuitries and elucidate their role and functions in vivo by generating genetically engineered mouse models (GEMMs). Overall, Mouse model-based preclinical platforms (GEMMs and patient-derived xenograft (PDX) models) guide our research directives for future clinical applications.

Identifying new components of growth factor and nutrient circuits

Uncoventional prefoldin RPB5 interactor (URI). We reported URI as a direct downstream effector of the mammalian/mechanistic target of rapamycin (mTOR)/ribosomal protein S6 kinase -1 (S6K1), the growth factor- and nutrient-sensing node. We demonstrated that URI is an oncogene inducing nicotinamide adenine dinucleotide (NAD^+) depletion to induce hepatocellular carcinoma. Further, we developed URI-based fluorescence resonance energy transfer (FRET) probes to screen for new components of the growth factor and nutrient signalling cascade using live-cell imaging.

Microspherule protein 1 (MCRS1). We recently showed that MCRS1, in an amino acid-dependent manner, connects Rheb to mTORC1 activation. MCRS1 depletion inactivates mTORC1.

Genetically engineered mouse models

In order to better understand the role and functions of URI and MCRS1 in the liver, pancreas and intestinal disorders, the following GEMMs were generated in our laboratory:

- → 2 conditional knock-out mouse models (URI and MCRS1 loss-of-function).
- → 3 knock-in mouse models (over-expression of URI (wt), URI (S371A) and MCRS1).

Biological concepts from our laboratory

We have proposed the following concepts:

- → Oncogene-induced NAD^+ depletion results in DNA damage; thus suggesting that metabolic reprogramming initiates tumourigenesis prior to genomic instability.
- → Nicotinamide riboside, a vitamin B3 derivative and an NAD+ booster may be used for cancer prevention and treatment. ■

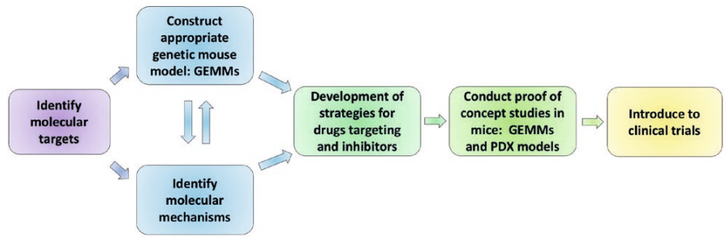

Figure Strategic development of growth factors and nutrients research at the CNIO: finding new effectors of the growth factor- and nutrient-sensing pathway, validating genomic predictors and oncogenic drivers in early stages of disease development, and generating new mouse models mimicking different steps of human sicknesses will offer new therapeutic strategies to prevent and cure metabolic dysfunctions and cancer.

PUBLICATIONS

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SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientists
Barbara Oldrini, Alberto J. Schuhmacher

OVERVIEW

Gliomas are a large group of brain cancers that include astrocytomas, oligodendrogliomas and oligoastrocytomas. Glioblastoma Multiforme (GBM), the highest grade of malignant astrocytomas, is the most common and lethal primary central nervous system tumour in adults. Standard therapy for GBM includes resection of the tumour mass, followed by concurrent radiotherapy and chemotherapy. Although the last decade has brought enormous advances in the treatment of other solid cancers, the median survival for GBM has stayed nearly the same over the last 50 years. Gaining insights into the pathways that determine this poor treatment response will be instrumental for the development of novel therapeutic modalities.

"The most effective treatment for GBM patients at present is a combination of radiotherapy and alkylating agents. Increasing the sensitivity of the tumour cells to these therapies will possibly extend the survival of the patients."

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

Graduate Students
Carolina Almeida, Alvaro Curiel

Technician
Claudia S. Troncone

Students in Practice
Paula Nogales, Anna Salamero

RESEARCH HIGHLIGHTS

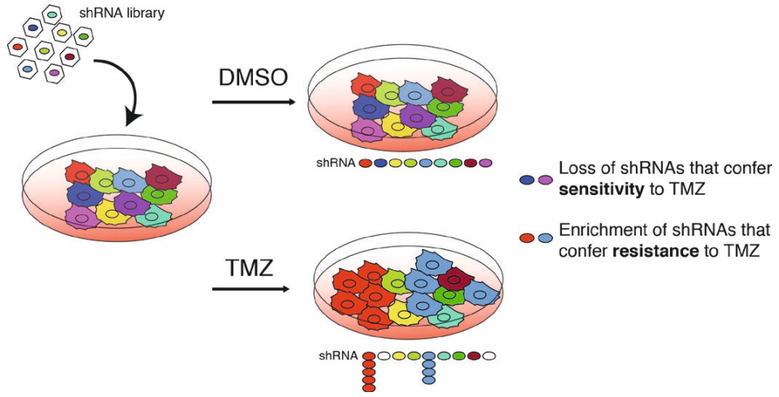

Figure shRNA screening strategy. The cells are infected with the shRNA library and then treated with DMSO or TMZ. Silencing of a specific gene can lead to 3 possible cellular responses: (**A**) the cells remain unaltered and identical to control cells; (**B**) the cells can become more sensitive to the treatment; and (**C**) the cells can acquire resistance to the treatment.

"aDDRessing" the issue of TMZ resistance in GBM

The standard therapies for GBM patients – ionising radiation (IR) and Temozolomide (TMZ) – generate double-strand DNA breaks (DSBs), which are the most deleterious form of DNA damage. DSBs 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 loss of components of a specific DNA repair pathway might be compensated by the increased activity of other components or pathways. Upregulated DNA repair pathways could lead to resistance to radiotherapy and DNA-damaging chemotherapy; therefore, inhibitors of these pathways could potentially increase the sensitivity of the cells to these therapies.

We have performed a series of shRNA-based genetic screenings to identify DDR genes that modulate the response to TMZ in glioma cells. In collaboration with Fátima Al-Shahrour from the CNIO Translational Bioinformatics Unit, we have been able to uncover novel biomarkers of TMZ response in GBM and, more importantly, novel targets to be inhibited in combination with TMZ treatment.

GlioVis, data visualisation tools for glioma datasets

We are currently living in the 'genomic era'. Scientific literature is flooded with an impressive amount of cancer genetic data: somatic mutations, copy number, gene expression, miRNA expression, DNA methylation, clinical information, etc. Most of the data are available in raw and processed forms; however, the analysis and interpretation of such information requires specialised software and training.

To render the data more accessible for the glioma research community we have developed GlioVis (http://gliovis.bioinfo.cnio.es): a user-friendly web application for data visualisation and analysis to explore brain tumour datasets. ■

PUBLICATION

- Oldrini B, Schuhmacher AJ, Squatrito M (2015). Turn it down a NOTCH in forebrain tumors. *Cancer Cell* 28,681-682.

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 as well as 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 cell adhesion and metabolism.
- Predict the consequences of cancer related alterations; we are focusing on alterations with a compensatory nature (co-evolutionary related mutations) as well as those affecting alternative splicing patterns.
- Contribute to the analysis of cancer epigenomic and genomic information within the framework of international genome projects.

"After a very positive initial period, the Programme is now in a phase of reorganisation, consisting of the renewal of the equipment, the replenishment of its Core Units, and most importantly, the recruitment of new group leaders in Structural and Computational Biology."

The Programme is currently composed of 3 Research Groups and 6 Core Units that provide support to the CNIO's research activities.

Recently, 2 key groups left the CNIO in order to follow their scientific development elsewhere. Francesco Gervasio, an expert in biophysical simulations, went to the University College of London, and Guillermo Montoya, a senior crystallographer, moved to the Novo Nordisk Foundation Center for Protein Research in Copenhagen, while maintaining during 2015 a reduced research activity.

In order to maintain a reasonable level of activity and to provide the necessary support to internal projects, we established 2 new Core Units this year: a Protein Crystallography Unit, which is shared with the CNIO Experimental Therapeutics Programme; and an Electron Microscopy Unit that works in collaboration with the laboratory of O. Llorca (*Centro de Investigaciones Biológicas, CIB-CSIC*, Madrid), where cell biology samples are prepared for further observation and imaging using our microscopes. These new Units have started their independent work by re-organising the equipment and operational aspects; they have already commenced work with a number of CNIO groups. Moreover, we also share the Translational Bioinformatics Unit, led by Fátima Al-Sharour, with the Clinical Research Programme.

Of particular relevance to the CNIO's activities in Computational Biology, was the finalisation of the negotiation for membership in the European Bioinformatics Infrastructure ELIXIR. ELIXIR is a large international consortium that is now associated to a European grant (EXCELERATE), in which the Spanish participation is led by the CNIO. CNIO's National Bioinformatics Institute Unit (*INB-ISCIII*) has played a key role in developing the technical aspects of the Spanish participation in ELIXIR and EXCELERATE.

During 2015, the 3-year extension of the 5+3 contracts of the 2 junior Group Leaders was evaluated by an *ad-hoc* expert committee and approved by the CNIO. Later in the year, the Programme was reviewed in depth by the CNIO's External Scientific Advisory Board (SAB). The SAB evaluated the activity of the Programme very positively and endorsed the reorganisation of its Units, for which it made very specific recommendations in regards to the need to focus on the proper equipping of these Units. Very importantly, the SAB strongly recommended the appointment of at least one senior structural biologist. The CNIO has taken these recommendations very seriously, allocating additional funds for the renewal of the equipment of the Units, and has started the search for a senior and a junior structural biologist.

STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Alfonso Valencia
Group Leader

Staff Scientists
Federico Abascal (until May), Andrea Nicole Dölker, Milana Morgenstern (until November), Tirso Pons, Daniel Rico, Michael Tress

Post-Doctoral Fellows
Simone Marsili, Vera Pancaldi, Miguel Vázquez

Graduate Students
Simone Ecker (until October), Maria Rigau, Juan Rodríguez, Jon Sánchez

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

'Titulado Superior (Advanced Degree)

Visiting Scientists
Dimitrios Morikis (University of California, Riverside, USA), Evangelina Nogales (July-September) (University of California, Berkeley, USA)

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, where 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 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.
- → Analyse the function, structure and specific interactions of cancer-related proteins.
- → Develop methods, tools and ideas to understand and model processes relating to genome structure, organisation and evolution, with a special focus on tumour progression.

"This year we carried out a new research effort regarding the analysis of genome/epigenome data, including chromatin 3D structure data, by combining methods for functional genome segmentation with network biology strategies. The results reveal interesting properties of chromatin organisation, including fundamental components for its organisation and dynamics."

RESEARCH HIGHLIGHTS

The work of our Group can be described in terms of our contributions to 3 large-scale community efforts.

Our Group contributes to the organisation of the data flow and analysis of the Spanish Chronic Lymphocytic Leukaemia (CLL) project, which is part of the International Cancer Genome Consortium (ICGC) that ended in 2015; and to the BLUEPRINT epigenome EU flagship project, which is now entering its final phase of data production. We have participated in the main published studies of the CLL consortium, as well as in the white papers on analysis technology of the ICGC consortium. In 2016, our main efforts will be dedicated to the analysis of the massive amounts of data produced by the largest cancer genome sequencing consortia, IGCG and TCGA, in what is known as PanCancer, as well as to the completion of the integrated analysis of the BLUEPRINT data.

Organisation of chromatin and the interaction between its components

Inspired by the work of the CLL-ICGC and BLUEPRINT projects, we have developed a new framework for the analysis of large scale epigenetic and chromatin capture data. Based on concepts developed in the area of network biology, we have used the available information to reconstruct the network of interactions between a large set of more than 70 chromatin features, including DNA and histone modifications, as well as a large number of chromatin binding proteins. The analysis of the network reveals interesting properties of the components related to their specific functional activity and evolutionary history, including detection of the importance of the 5hmC modification of DNA as a network organiser.

The additional incorporation of information on the three dimensional organisation of the chromatin in the nucleus (HiC and PChiC experiments) opens up new avenues for the study of the dynamics of the interactions between active chromatin features, such as the different isoforms of RNA polymerase II.

Alternative splicing at the protein level

This year we continued the work on alternative splicing in the context of the NIH-funded GENCODE project.

We have produced a new release of the APPRIS system for the systematic annotation of protein isoforms. APPRIS annotates binding sites, evolutionary rates, the existence of complete protein structures or models, as well as the presence of membrane regions, and determines the most likely complete isoform for each gene ("Principal" Isoform). APPRIS annotations are part of the information used for the annotation of the human genome.

The Principal Isoform is the only one that has protein-like features, including presence of binding sites, coverage of known protein structures, evolutionary conservation and normal rates of evolution, and it is the genuine representative of the function of the corresponding genes. The systematic analysis of the Principal Isoforms has several interesting consequences.

Firstly, it allows a better definition of the mutations that might be relevant in cancer studies, rejecting others that map outside the boundaries of the Principal Isoforms, and therefore are expected to not have functional consequences at the protein level.

Secondly, we have been able to compare the evolutionary consequences of splicing versus gene duplication by reconstructing the history of a selected set of genes that after duplication have retained 2 very similar paralogs, or have produced 2 splice isoforms differing solely by the presence of 2 homologous exons.

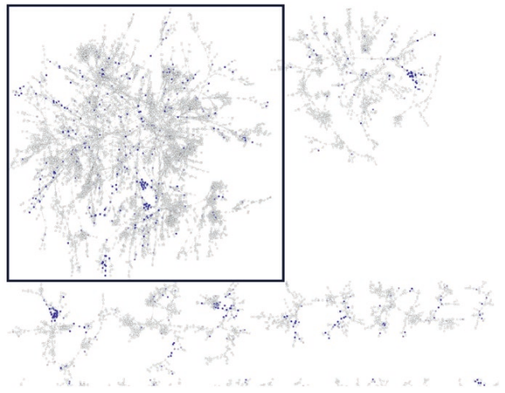

Figure 1 Promoter-Capture HiC (PCHiC) network. In the network each node represents a genome fragment containing at least 1 promoter. The central connected component is highlighted. The blue nodes correspond to chromatin fragments binding at a significant level of the EZH2 protein. The analysis of the networks shows that these fragments tend to cluster together more frequently than what could be expected by their overall abundance. In network language this type of clustering is known as 'assortativity'. *Experimental data from Peter Fraser (The Babraham Institute).*

Thirdly, by combining APPRIS with the systematic exploration of all the available large scale MS data sets – carried out in collaboration with the group of Jesus Vazquez (CNIC) – we have demonstrated that the immense majority of genes only express 1 isoform at the protein level; at least for those genes with medium-high levels of expression. This finding is in clear contradiction with studies that have analysed expression at the level of transcription but, perhaps not surprisingly, the finding seems to fit well with the large scale analysis of gene expression in multiple tissues, carried out by the ENCODE/GTEx (www.gtexportal.org) consortium.

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Figure 2 Interface to the toxicology data. Example of a keyword search (query 'mitochondrial') showing sentences that mention chemical compounds and some other informative terms (e.g. 'steatosis'). The system assigns confidence scores to each sentence based on the confidence in the identification of terms related with toxicity (shown on the left of the panel).

Biological text mining

Text mining is an increasingly important component of computational biology with numerous applications in biology and biomedicine.

As part of the BioCreative international effort for the evaluation of text mining systems, we organised the 2015 competition (Seville, Sept 2015), with a special focus on the extraction of mentions of chemicals (drugs and other compounds), diseases and gene names from patient records. For the competition, we created a large, systematically annotated corpus, as well as the corresponding annotation guidelines and tools to score the results of the teams participating in the competition.

Beyond the BioCreative benchmarking effort, in the context of the IMI eTOX project, we completed the development of a system specialised in the extraction of information related to the toxicology of biological compounds and drugs from scientific publications and toxicology reports. The relationships between administered drugs, symptoms, toxic end-points, target genes and cytochrome variants are systematically linked to the related databases and underlying text.

We have also completed the development of a text mining system to extract information related to melanoma from the literature. The system systematically explores melanoma related papers to detect mentions of genes, mutations and drugs, as well as a number of medical/pathology related terms, and exposes the information to the end-users together with facilities for navigating the information in the context of the bulk of information available from the large cancer genome projects. ■

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MACROMOLECULAR CRYSTALLOGRAPHY GROUP

Guillermo Montoya
Group Leader

Staff Scientist
Jesús Prieto

Post-Doctoral Fellow
Rafael A. Molina (until September)

Technician
Igor Yefimenko (until September)

OVERVIEW

Macromolecules and their interactions underlie all biological processes and play either, dynamic roles in catalysis or signalling, or static roles in scaffolding or information storage. Our Group focuses on the molecular understanding of the role played by macromolecules involved in oncogenic processes. There is an information gap between our current knowledge and our understanding of the molecular mechanisms that govern the function of different cellular machines. Structural determination reveals an unparalleled view of the design principles of living systems at levels that span from basic mechanistic questions regarding protein function, to the evolutionary relationships between cellular components. To achieve this, our work focuses on the structural and dynamic interactions of these biomolecules and their complexes.

"We have visualised, for the first time, the dynamics of DNA phosphodiester hydrolysis by an endonuclease."

RESEARCH HIGHLIGHTS

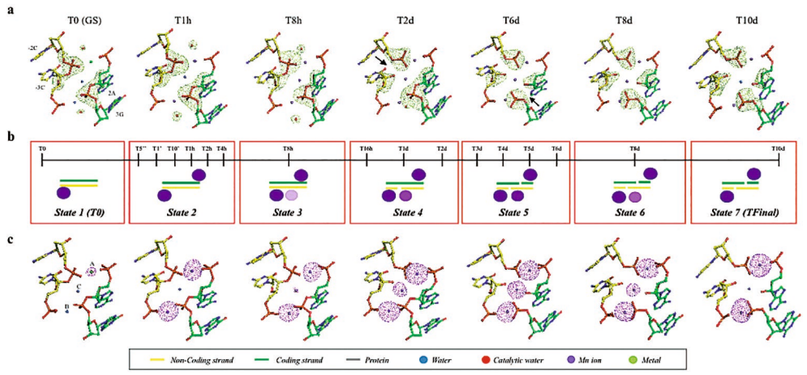

Figure 1 (**A**) Detailed views of the active centre, with F_o–$F_{c(0\,d-10\,d)}$ omit maps superimposed onto their corresponding refined structures. The omit maps' density is contoured at 5σ. The reaction time course displays the 7 different structural reaction intermediates captured in this work. (**B**) Sketch of the reaction depicting the entrance of cations and the cleavage of the bonds of the noncoding and coding strands during the course of the reaction. (**C**) Mn^{2+} anomalous maps of the 7 reaction intermediates, displaying the sequential entrance of the cations in sites A, B and C, and the exit of the crucial metal ion in the central site after DSB generation. All anomalous maps show density contoured at 6σ except in state 3, for which density is contoured at 4σ to show the entrance of Mn^{2+} in site C.

Structural design of protein-DNA interactions for gene targeting

We have observed, for the first time, the hydrolytic reaction performed by a specific endonuclease on its target DNA; we watched how an endonuclease generates a double-strand break in a DNA molecule following a two-metal ion mechanism. To investigate this process we developed a procedure to slow down the enzymatic reaction. This method allowed us to monitor the kinetics of enzyme catalysis using a time-resolved crystallography approach capturing the structures of successive reaction intermediates. Thus, we have provided a uniquely detailed view of the dynamic processes of this key biological reaction. Our work interlaced structural and molecular dynamic analyses to dissect the hydrolysis of a phosphodiester bond, thereby precisely defining the catalytic mechanism of an endonuclease. We have solved more than 150 crystal structures to obtain key snapshots of different catalytic stages, showing the orchestrated conformational changes in the amino acids, nucleotides and metals during catalysis. This work provides the first 'live' and visual proof of this key biological mechanism (FIGURE 1). This information may be used to engineer even more precise 'cutters'. These scaffolds can present new perspectives for a wide range of applications, such as the correction of mutations linked to monogenic inherited diseases. Our Group has solved the crystallographic structures of different variants, revealing the molecular basis of new target DNA recognition domains. In addition, we have shown that the repair of the damaged gene can occur at its locus in human cells, thereby opening up avenues for the identification of possible therapeutic applications.

The telomeric nucleosome

The telomere is a specialised region of the chromatids that contains repetitive DNA sequences. This 'buffer' DNA is truncated during chromosome replication and needs to be further expanded by the telomerase. Different proteins and protein-DNA complexes are implicated in the assembly of a supramolecular structure of the telomeric chromatin that caps the telomere end avoiding the activation of the DNA Damage Response and harmful chromosome fusion. One of the essential protein components involved in this assembly is the shelterin complex, consisting of 6 different proteins: TRF1, TRF2, TIN2, POT1, TPP1 and RAP1. The current literature contains scarce knowledge of these mechanisms. In this proposal we aim to decipher the molecular basis of shelterin telomere capping and telomere organisation (FIGURE 2). These data will help us to understand how the loss of telomere protection contributes to genome instability.

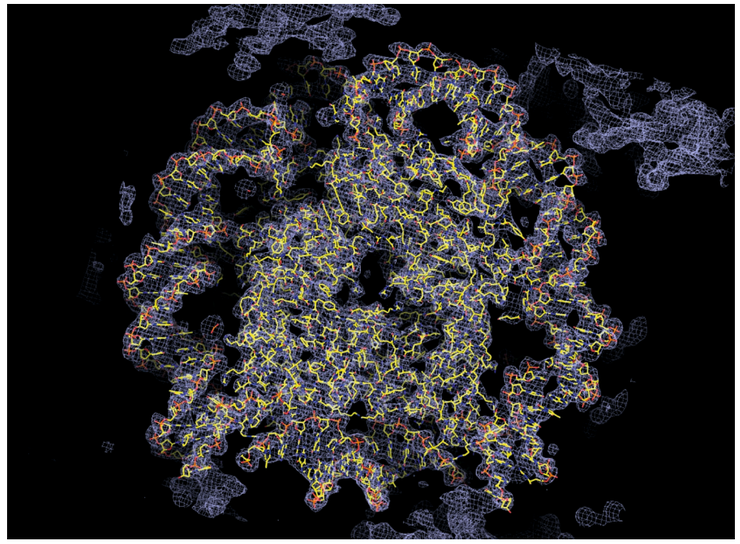

Figure 2 Electron density map at 1s of the crystal structure of the telomeric nucleosome particle at 3.15 Å resolution in complex with associated factors.

Mitotic complexes

Cellular growth and division are regulated by an integrated protein network that ensures the genomic integrity of all eukaryotic cells during mitosis. Microtubules play an important role in several cellular processes, particularly in the formation of the mitotic spindle. The regulation of microtubule dynamics during mitosis is key for spindle formation. Spindle defects, arising from failures in setting up the microtubules, lead to chromosomal instability and aneuploidy, a common cause of tumour development. One of the most effective strategies for cancer treatment so far has been to interfere with the highly dynamic mitotic spindle microtubules; tubulin remains the most successful spindle targeted molecule in cancer. To date, novel anti-mitotic agents have demonstrated limited efficacy in clinical trials and classical anti microtubule drugs are still considered as being the best approach for cancer therapy. We are attempting to dissect the molecular working mechanism of CCT/TRiC, the molecule responsible for the folding of tubulin and actin, which are the essential building blocks of the cytoskeleton. This molecular machine is essential for sister chromatid separation through the folding of key anaphase promoting factor subunits, such as Cdc20. Using a hybrid approach, we are aiming to dissect the molecular recognition of these key substrates by the chaperonin. ■

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AWARDS AND RECOGNITION

- Co-organiser, EMBO Workshop "Cell division: molecular machineries and cancer targeted therapies" with co-sponsorship by UNIA.

CELL SIGNALLING AND ADHESION JUNIOR GROUP

Daniel Lietha
Junior Group Leader

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Graduate Students
Marta Acebrón, Marta Camacho, José Vicente Velázquez

Technician
Pilar Redondo

"We elucidated mechanisms by which the SHIP2 inositol phosphatase is regulated to reduce PIP3 levels. This information can aid in the design of novel small molecules, targeting SHIP2, to reduce oncogenic signals."

OVERVIEW

Our Group studies regulatory mechanisms of key signalling switches that control 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 deregulate these events. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

We focus on mechanisms of growth and adhesion signalling that occur at the plasma membrane and involve specific phosphoinositides. Specifically, we aim to answer the following questions: (i) how does the phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP_2) activate Focal Adhesion Kinase (FAK); (ii) how does phosphoinositide 3-kinase (PI3K)-generated phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) lead to activation of serine/threonine protein kinase B/Akt (PKB/Akt); and iii), how are the SH2-domain-containing inositol 5-phosphatases (SHIP) regulated to reduce PIP_3 levels in the plasma membrane.

RESEARCH HIGHLIGHTS

We showed that Focal Adhesion Kinase (FAK) activation occurs via PIP_2-mediated FAK clustering and conformational changes at the cell membrane, which induce FAK autophosphorylation and Src recruitment. Src in turn phosphorylates the FAK kinase to induce full opening and activation of FAK. At present, we are studying the architecture of FAK clusters bound to lipid membranes by electron microscopy. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ a fragment-based approach guided by structural studies to extend initial fragments into inhibitory lead compounds.

Regarding Protein Kinase B (PKB), we performed biochemical and cellular studies to understand which regulatory mechanisms control intrinsic activity and which ones regulate selective phosphorylation of specific PKB substrates. We confirmed that PKB phosphorylation is essential for activity, however, surprisingly, we found that membrane targeting, which has been regarded as essential for PKB activity, mainly affects substrate specificity. Furthermore, we showed that in addition to the canonical activation mechanism via PI3K, initial C-terminal phosphorylation and association of PKB with PDK1 in the cytosol can also activate PKB, resulting in phosphorylation of different PKB substrates.

SHIP phosphatases remove the 5-phosphate from PIP_3 and thereby, like PTEN, negatively regulate PIP_3 levels. Despite their importance, little is known about mechanisms of SHIP regulation. We recently solved a crystal structure of the catalytic and C2 domains of SHIP2, showing an extensive interface between the 2 domains (FIGURE). Although the C2 domain interacts with the phosphatase domain far from the active site, biochemical studies showed that the C2 interaction greatly enhances the catalytic activity of SHIP2 and, interestingly, affects substrate recognition. We employed molecular dynamics (MD) simulations to guide a mutagenesis study that revealed how the C2 domain, via an allosteric mechanism, affects the dynamics of loops close to the substrate binding site, affecting SHIP2 catalysis. ■

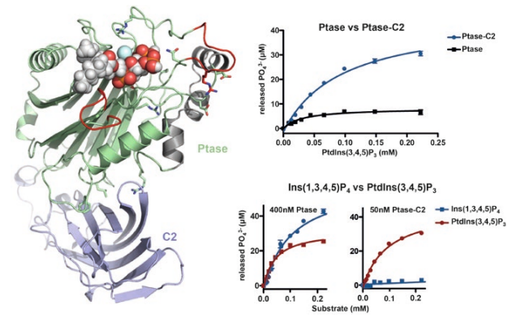

Figure A crystal structure of the phosphatase (Ptase) and C2 region of SHIP2, MD simulations and a mutagenesis study suggest an allosteric path from the C2 domain via a helical region (grey) to loops at the active site (red). Substrate atoms are shown as spheres and mutated residues as sticks. Right: The C2 domain increases activity and affects substrate preference.

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STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

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Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

Graduate Students
Francisco del Caño, Alba Ruiz

Technician
Araceli Grande (TS)*

Titulado Superior (Advanced Degree)

OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and for preventing 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 provide further insight into the design of compounds to modulate protein activity, as well as 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. We proposed novel mechanisms of DNA targeting during transposition."

RESEARCH HIGHLIGHTS

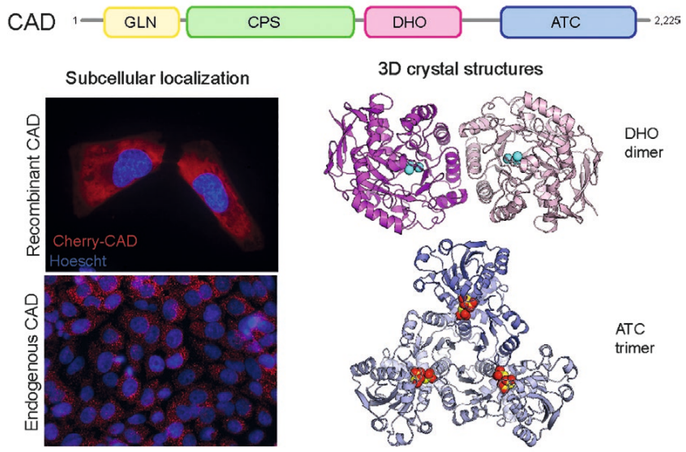

Figure CAD is a multifunctional protein that initiates and controls *de novo* biosynthesis of pyrimidines. We followed the cellular localisation of CAD by using specific antibodies or by expressing a fluorescence construct. We also determined the crystal structures of the DHO and ATC domains of human CAD.

Revealing the structure and functioning of CAD, a metabolic gatekeeper of cell proliferation

The *de novo* synthesis of pyrimidines is essential to fuel the high demand of nucleotides during cell proliferation. This pathway is initiated by CAD, a 243 kDa multifunctional polypeptide with 4 enzymatic activities: glutaminase, carbamoyl phosphate synthetase (CPS), aspartate transcarbamoylase (ATC) and dihydroorotase (DHO). CAD also limits the flux through the pathway and its activity is allosterically controlled and it is also modulated by phosphorylation. Despite its central metabolic role and its potential as an anti-tumour target, there is no detailed information about CAD other than that it self-assembles forming a 1.5 MDa complex. We are interested in characterising the architecture of CAD to understand its catalytic and regulatory mechanisms. Giving its large size and modular organisation, we tackle this challenge by combining single-particle EM and X-ray crystallography. Recently, we reported the crystal structure and biochemical characterisation of the DHO domain of human CAD. Now, we have determined the crystal structure of the ATC domain and we are producing larger CAD complexes for EM studies. In addition, we have labelled full-length human CAD with GFP or Cherry to investigate the localisation of CAD in the cell during the cell cycle.

Basic mechanisms of DNA recognition

MuB is an ATP-dependent nonspecific DNA-binding protein that selects the target DNA for transposition. A detailed mechanistic understanding of how MuB juggles the DNA is unknown. We demonstrated that MuB is an AAA+ ATPase that assembles into helical filaments around the DNA, and identified critical residues for the ATPase activity, DNA binding and filament assembly, as well as for the interaction with the transposase. However, the function of a 7 kDa N-terminal domain (NTD) has remained uncharacterised. In collaboration with the CNIO NMR Unit, we have determined the structure of the NTD, which reveals a striking similarity to DNA-binding proteins. We have also demonstrated that the NTD directly mediates the ability of MuB to establish filament-filament interactions. We propose a 'zippering' mechanism by which the NTD favours filament clustering and the bridging of distant DNA regions during transposition. ■

PUBLICATIONS

Dramićanin M, López-Méndez B, Boskovic J, Campos-Olivas R, Ramón-Maiques S (2015). The N-terminal domain of MuB protein has striking structural similarity to DNA-binding domains and mediates MuB filament-filament interactions. *J Struct Biol* 191, 100-111.

Book Chapter

Ruiz-Ramos A, Grande-García A, Ramón-Maiques S (2015). Dihydroorotase domain of human CAD. In: Encyclopedia of Inorganic and Bioinorganic Chemistry. John Wiley & Sons, Ltd. DOI: 10.1002/9781119951438.eibc2321

AWARDS AND RECOGNITION

1st Prize Poster Award, XXXVIII Congress of the *Sociedad Española de Bioquímica y Biología Molecular* (*SEBBM*): *Francisco del Caño, Araceli Grande-García, Santiago Ramón-Maiques. Subcellular localisation of the multifunctional protein CAD and production of knock-out cell lines by the CRISPR/Cas9 system.*

SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (since March) (TS)*

Titulado Superior (Advanced Degree)

"In 2015, we quantified abundant cell metabolites, thereby contributing to the understanding of the metabolic response to chemotherapeutic drugs causing mitotic arrest and the metabolic plasticity of pancreatic cancer stem cells, which are crucial aspects of tumour treatment and biology."

OVERVIEW

The Unit consolidates the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and other biophysical instrumentation made available by 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.

RESEARCH HIGHLIGHTS

Our 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, analytical ultracentrifugation, and a surface plasmon resonance (SPR) machine. Research Groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2015. Two important intra-Programme collaborations carried out this year are: the NMR solution structure determination of the N-terminal domain (NTD) of MuB, an essential protein for DNA transposition specificity (with the Structural Bases of Genome Integrity Group), as illustrated in the FIGURE; and lipid binding studies of different PKB/Akt constructs using SPR (with the Cell Adhesion and Signalling Group).

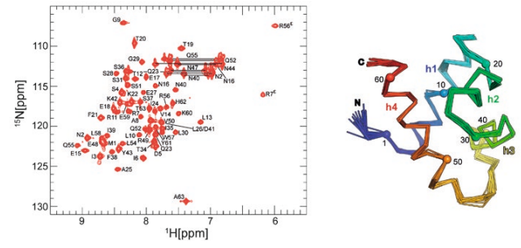

Figure (**Left**) ^{1}H-^{15}N HSQC NMR spectrum of uniformly ^{15}N labelled MuB NTD with residue assignments indicated according to the sequence numbering. (**Right**) Superimposed 20 best structures of MuB NTD represented in Ca trace, with spheres every 10 residues and coloured in rainbow gradient.

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

PUBLICATIONS

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BIOINFORMATICS UNIT

David G. Pisano
Unit Head

Technicians
Ángel Carro (TS)*, Coral Fustero (since December) (PEJ-L) **,
Gonzalo Gómez (TS)*, Osvaldo Graña (TS)*, Miriam Rubio (TS)*

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

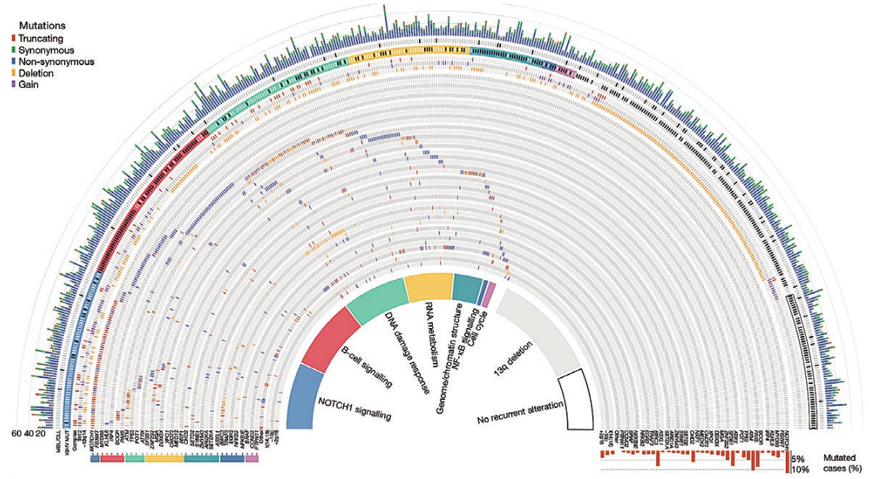
OVERVIEW

The Bioinformatics Unit is devoted to assisting CNIO's researchers with data analysis and interpretation using statistical and computational methods. It also maintains the scientific computing facilities at the CNIO and provides training in bioinformatics tools and methods.

It is important for people to realise that a research centre like the CNIO conducts a massive amount of experiments that generate data at the same rate as large companies do. Current biotechnology techniques enable us to capture molecular pictures of biological systems and to observe, in a unique experiment, the status and composition of millions of these elements. This generates huge amounts of 'big data' that we have to manage and analyse using computational technologies, which are essential for the understanding of the genetic and molecular bases of cancer.

RESEARCH HIGHLIGHTS

In 2015, as part of the International Cancer Genomic Consortium (ICGC) and in collaboration with E. Campo's group at the Hospital Clinic in Barcelona and C. López-Otin's laboratory at the University of Oviedo, we contributed to the work reporting a comprehensive genomic characterisation of Chronic Lymphocytic Leukaemia (CLL) and its precursor in more than 500 patients (Puente *et al.* 2015). The study extends the number of CLL driver alterations found in the coding portion of the genome and also identifies novel recurrent mutations in non-coding regions, including the 3' UTR of *NOTCH1*, which cause aberrant splicing events, as well as mutations in an enhancer that result in reduced expression of the B-cell-specific transcription factor PAX5. It also confirms the insights provided by previous works on more limited patient datasets: each tumour carries an individual, distinct and personal signature of genomic alterations (FIGURE).



In collaboration with the laboratory of C. Heeschen (Barts Cancer Institute, London), we helped to unveil specific metabolic features of pancreatic Cancer Stem Cells (CSCs) (Sancho *et al.* 2015), and also to describe the pancreatic ductal adenocarcinoma (PDAC) microenvironment in order to better understand its biology (Sainz *et al.* 2015). Our long-standing collaboration with CNIO's Chromosome Dynamics Group (A. Losada) yielded interesting insights into cohesin's contribution to the establishment of tissue-specific transcriptional programmes, by jointly interpreting genome-wide cohesin distribution, gene expression and chromatin architecture in the cerebral cortex and pancreas of adult mice (Cuadrado *et al.* 2015).

Other bioinformatics analyses were performed together with M. Serrano's laboratory (CNIO) (Morgado-Palacin *et al.* 2015, Palla *et al.* 2015), J. Benitez (CNIO) (Matamala *et al.* 2015, Vaclová *et al.* 2015), and A. Muñoz (IIB) (Aguilera *et al.* 2015).

We helped the Confocal Microscopy Core Unit (CNIO) to design and implement iMSRC, a new software tool that converts a conventional automated microscope into an intelligent screening platform (Carro *et al.* 2015). In collaboration with D. Glez-Peña (University of Vigo) we published miRGate (Andres Leon *et al.* 2015), a curated database of miRNA-mRNA targets with more than 125 million predictions on a consistent sequence space. Another genomic resource for the UBC-40 urothelial bladder cancer cell line (Earl *et al.* 2015) was released in collaboration with F.X. Real's laboratory (CNIO). Previously published works have allowed us to deliver additional data and protocols as genomic data resources (Tanic *et al.* 2015, Foronda *et al.* 2015). ■

PUBLICATIONS

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- Vaclová T, Gómez-López G, Setién F, Bueno JM, Macías JA, Barroso A, Urioste M, Esteller M, Benítez J, Osorio A (2015). DNA repair capacity is impaired in healthy BRCA1 heterozygous mutation carriers. *Breast Cancer Res Treat* 152, 271-282.
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NATIONAL BIOINFORMATICS INSTITUTE UNIT

Victor de la Torre Russis
(until November)
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 programme of the National Infrastructure of Biomolecular and Bioinformatics Resources Platform (*Plataforma en Red de Recursos Biomoleculares y Bioinformáticos, PRB2*). The INB itself is also the Spanish Node of the European Bioinformatics Infrastructure ELIXIR. The INB is organised as a network composed of 10 nodes distributed across 9 centres. The INB Unit at the CNIO is the Central Node of the network. As a Central Node, the main goals of the INB Unit are to:

- → Coordinate the activities of the Institute.
- → Design (with the support of all the nodes) the INB scientific/technical programme and to ensure its execution.
- → Design (with the support of all the nodes) the INB training programme.
- → Coordinate the participation of Spain in ELIXIR.
- → Mediate the collaboration between the INB and third parties including: National and International research consortia, other infrastructures, SMEs and the Industry.

"The Unit has contributed to the creation of a text-mining infrastructure that identifies, for a large number of compounds, the associated toxicological effects based on the evidences extracted from several literature corpus."

The INB Unit differs from the other Units in the Structural Biology and Biocomputing Programme in the sense that its offering is not restricted to the CNIO Groups, and that its budget is funded entirely by an external agency, the ISCIII.

RESEARCH HIGHLIGHTS

The INB service offering is distributed amongst three horizontal Work Packages (WP) as well as the Training WP. These services are integrated into six major research areas: Health, Genomics, Functional Genomics, Structural Biology, Biological Networks and Data mining. Besides from its coordination role, the Unit also participates in the delivery of the services.

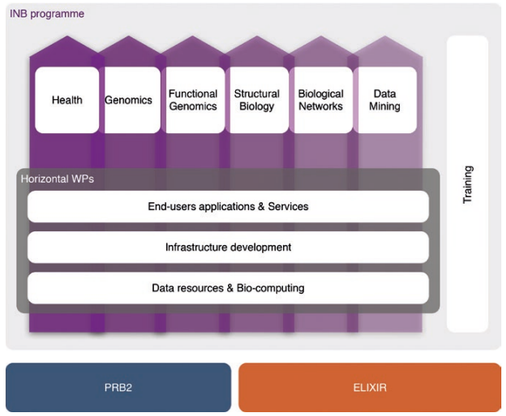

Figure Diagram of the organisation and activities of the Spanish National Bioinformatics Unit (INB).

Data resources and Bio-computing

The storage and processing of data have become fundamental tasks for 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://blueprint-dev.bioinfo.cnio.es/#/). BLUEPRINT is a high impact FP7 project aimed at producing a blueprint of haemopoetic epigenomes. In the current version, the data portal provides an epigenomic analysis obtained from 439 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).

Infrastructure development

Within the infrastructure development, special attention is paid to the **text-mining infrastructure** for the processing of biomedical texts. The LiMTox system (http://limtox.bioinfo.cnio.es) is the first text mining approach that extracts 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.

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.

The application allows clinicians and researchers to explore the possible causes of the disease by combining patient's genomic data with phenotypic annotations. The Unit has also developed APPRIS (http://appris.bioinfo.cnio.es/#/), a service that automatically annotates genes and transcripts. The GENCODE consortium uses APPRIS to annotate the principal isoforms of several species. ■

PUBLICATIONS

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- Lees JG et al. (incl. Fernández JM, Kralinger M, Valencia A) (2015). FUN-L: Gene prioritization for RNAi screens. *Bioinformatics* 31, 2052-2053.

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Technician
Alberto Buscató (since December) (PEJ-L)*

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

"PIKKs are attractive targets for anti-cancer therapies as they regulate their kinase activity in response to diverse stimuli. In collaboration with Óscar Llorca from *CIB-CSIC*, we have summarised the findings on the structure of PIKKs in a review article."

OVERVIEW

The Electron Microscopy (EM) Unit is a research laboratory and a central core facility that provides CNIO researchers, and the wider research community, with access to Transmission Electron Microscopy, as well as supplying expertise in EM image analysis. As a core facility, we offer standard specimen preparation techniques for proteins and protein complexes, data collection and data processing tailored to the specific needs of the users.

We also provide training for regular users on the use of equipment, as well as guidance regarding specimen preparation. For cell biology samples, we have established collaboration with the *Centro de Investigaciones Biológicas* (*CIB-CSIC*, Madrid), where the samples are prepared for further observation and imaging with our microscope.

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit is a research facility that provides support for biological science projects at scales ranging from the cellular level to the macromolecular complex level. The Electron Microscopy Unit implements sample preparation protocols and data collection methods, as well as performing 2D and 3D data processing.

In collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) we demonstrated that PI(4,5)P_2 induces Focal Adhesion Kinase (FAK) clustering at the cell membrane. We are currently employing 2D electron crystallography to elucidate the arrangement of the FAK protein and its conformation within the clusters. We have obtained preliminary 2D crystals of the 4.1-ezrin-radixin-moesin (FERM)+kinase domain of FAK on a lipid monolayer.

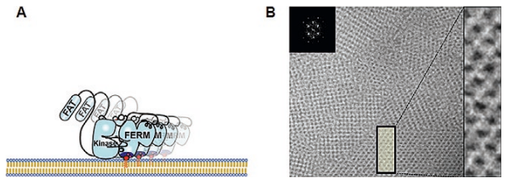

Figure 2D electron crystallography of Focal Adhesion Kinase (FAK) clusters. (**A**) Schematic representation of the FAK cluster. (**B**) Preliminary 2D crystals of FERM+kinase domains (middle), higher magnification (right) and Fourier Transform of an array (left). This work was carried out in collaboration with the CNIO Cell Signalling and Adhesion Group.

In collaboration with the CNIO Telomeres and Telomerase Group (Molecular Oncology Programme) we are pursuing the structural characterisation of TRF1, a central component of the shelterin complex, which is known to protect mammalian telomeres and regulate telomerase activity. We have obtained negative-staining images of the purified TRF1 oligomer. 2D averages and the preliminary 3D structure indicate that TRF1 forms dimers, as previously suggested. Furthermore, in collaboration with the CNIO Melanoma Group (Molecular Oncology Programme), we have developed a protocol that enables, via electron microscopy, the visualisation of different extracellular vesicles produced by melanoma cell lines.

Additionally, under the framework of a recently established agreement with the *CIB-CSIC* and in line with the needs of CNIO's researchers, we also provide ultrastructural analysis and immunodetection by EM at the cellular level. Thus, cell and tissue samples that are prepared by the Electron Microscopy Facility at the *CIB-CSIC* can be visualised and analysed at the CNIO. ■

PUBLICATIONS

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CRYSTALLOGRAPHY UNIT

Inés Muñoz
Unit Head

Technician
Alicia Virseda (since December) (PEJ-L)*

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

"Our goal is to provide CNIO investigators with 3D structural information of their target macromolecules to understand the mechanism(s) that regulate their biological functions, including their modulation by novel therapeutic ligands."

OVERVIEW

The aim of the Crystallography Unit is to provide the CNIO Research Groups with a three-dimensional characterisation of the structure of biological macromolecules at high-resolution (X-ray crystallography) and low-resolution (SAXS). The knowledge of the 3D structure of proteins and protein complexes is essential for understanding their function in cellular processes. The crystal structures give us a picture – at atomic resolution – of the protein. With this knowledge, we know where to introduce mutations that can alter (or improve) the specificity of the protein and its affinity to other molecules. In turn, this can lead to the design of drugs to block or control the activity of proteins involved in disease. Small-angle X-ray scattering (SAXS) is a complementary technique to X-ray crystallography. It permits delineation of the dynamic changes in shape and size undergone by the proteins in solution, giving a structural picture of the thermodynamic behaviour of these biological molecules, including changes induced upon ligand binding.

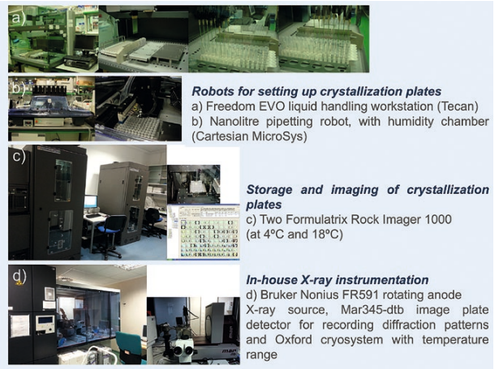
This Unit is shared between the Structural Biology and Biocomputing Programme and the Experimental Therapeutics Programme.

RESEARCH HIGHLIGHTS

The Crystallography Unit began its journey in February 2015, with the aim of providing state-of-the-art, high-throughput protein crystallization, X-ray crystallography and SAXS services to meet the demands of the Research Groups at the CNIO and special collaborative efforts outside our institute.

The full-service Unit provides access to sophisticated equipment and technologies, including the European synchrotron light sources. We also offer consultancies, guidance, and technical assistance at every stage of the 3D structure determination process. Non-crystallography groups benefit from expert aid in the use of existing 3D structures for the design and interpretation of experiments, including the possibility to crystallize their target proteins in the presence of inhibitors, for structure-based drug design.

Since its formation, the Unit works in close collaboration on the drug discovery projects led by the Experimental Therapeutics Programme. We also run a number of collaborations with different Groups at the CNIO involved in the following Programmes: Molecular Oncology (Telomeres and Telomerase and Brain Metastasis Groups), Clinical Research (Gastrointestinal Cancer Clinical Research Unit), and Cancer Cell Biology (Epithelial Carcinogenesis Group). Additionally, the Unit has initiated external collaborations with the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CIB-CSIC), and the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA). ■



Robots for setting up crystallization plates
a) Freedom EVO liquid handling workstation (Tecan)
b) Nanolitre pipetting robot, with humidity chamber (Cartesian MicroSys)

Storage and imaging of crystallization plates
c) Two Formulatrix Rock Imager 1000 (at 4ºC and 18ºC)

In-house X-ray instrumentation
d) Bruker Nonius FR591 rotating anode X-ray source, Mar345-dtb image plate detector for recording diffraction patterns and Oxford cryosystem with temperature range

Figure Crystallography Unit Equipment.

PUBLICATION

Wu W, Xu C, Ling X, Fan C, Buckley BP, Chernov MV, Ellis L, Li F, Muñoz IG, Wang x (2015). Targeting RING domains of Mdm2-MdmX E3 complex activates apoptotic arm of the p53 pathway in leukemia/lymphoma cells. *Cell Death Dis 6*, e2035.

Vice-Direction of Translational Research

MANUEL HIDALGO
Vice-Director of Translational Research

“CNIO is a leading institution in translational research areas such as molecular epidemiology, biomarker discovery and novel therapeutics.”

The activity and impact of CNIO’s scientists in the field of translational cancer research continues to be outstanding. While the contribution of the Centre towards making discoveries in basic science is undoubtedly critical, the resolution to making these discoveries more clinically applicable is fundamental. In 2015, several important advances in translational studies have taken place at the CNIO, ranging from studies of cancer risk factors, biomarker discovery, to the advanced preclinical testing of new treatments and clinical trials. A key aspect is the expanding portfolio of ongoing clinical trials conducted in collaboration with hospitals in the Community of Madrid, other Regional Communities and abroad.

HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Director

The Human Cancer Genetics Programme is currently composed of 3 Research Groups: Human Genetics, Endocrine Cancer, and Genetic and Molecular Epidemiology Groups; and 3 Units: Human Genotyping-*CEGEN*, Molecular Cytogenetics and Genome Editing, and the Familial Cancer Clinical Unit. In addition, the Programme includes a Familial Cancer Consultancy for the evaluation of cancer families and the selection of appropriate candidates for genetic studies in order to perform a correct diagnosis and to provide genetic counselling. The Consultancy is located at the *Hospital Universitario de Fuenlabrada* and works in close collaboration with the Hospital's Oncology Service. This year, we have doubled the number of consultancy days undertaken due to the increase in the number of families who attended for genetic counselling; 320 families versus 180 in 2014. This increase in families has led to a higher number of genetic and genomic diagnosis studies being possible with the incorporation of a massive sequencing platform in the Programme.

"Genetics, lifestyle, exogenous factors... one cause alone is unlikely to lead to cancer; it takes all of them to cause cancer. It is a difficult task to decipher the weight that each factor carries, living in our 'contaminated first world'. However, that's exactly what our work aims to uncover."

The core goals of the Programme are geared towards research, training and diagnosis. Our main interest in clinical diagnosis is based on the genetic characterisation of families with cancer. The Programme's research priorities are the genetic and cytogenetic study of tumours, the search for diagnostic and prognostic markers, as well as the discovery of novel cancer-related genes. These research activities are complemented by another area of work that studies the genetic and environmental factors that confer cancer susceptibility and modulate drug response (pharmacogenetics). 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 training and education. During this year, the groups in the Programme hosted 6 resident physicians from different hospitals in Spain for 3-month periods. Furthermore, we offer professionals from different international research centres the opportunity to join us either as visitors, or for a short training of 1-3 months (a total of 6 international visitors from Europe (3) and Latin America (3) were hosted in 2015).

In terms of education, since the beginning of 2015, 1 foreign Erasmus Master's student and 8 national and 5 international PhD students worked on their research projects; 4 of them have already successfully defended their thesis.

We participate in many international and national Consortia, which enables us to apply for international projects, hold international meetings and publish in the best journals. This year we were awarded a COST Action and a European project. Likewise, a good collaboration with other CNIO Groups and Units is one of our main characteristics, enabling us to benefit from the valuable internal feedback generated by people, techniques, technology and knowledge.

Milestones and major achievements of the Programme:

- The co-organisation of the Conference 'The human microbiome. Present status and future prospects' in collaboration with B-Debate.
- The co-organisation of the European Pancreatic Club.
- The organisation of the VII Congress of the Spanish Society of Pharmacogenetics and Pharmacogenomics.
- The identification of 3 new genes responsible for families with rare cancers.

HUMAN GENETICS GROUP

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**Titulado Superior* (Advanced Degree)

OVERVIEW

The Human Genetics Group has been working on research aiming to better understand the genetic bases of familial cancer for several years. Our main objective is to translate our discoveries into clinical practice. We continue working on our Familial Cancer Exome Project, which aims to identify new high-susceptibility genes, via whole exome sequencing, in order to explain some families with infrequent tumours. Similarly, we are interested in the identification of modifier genes that modulate factors such as age of onset, disease evolution and cancer risk. Finally, we are gaining further insight into the role of miRNAs as early diagnostic biomarkers of breast cancer.

"We have discovered two high-susceptibility genes that explain two different types of rare familial tumours, and we have advanced in the identification of genetic risk factors for breast and high-grade ovarian tumours."

Our strategic goals are:

→ To better define the genetic landscape of familial breast and ovarian cancer.
→ To discover new genetic markers associated with diagnosis and prognosis.
→ To improve our knowledge of families with rare or infrequent cancers by using massive sequencing.

RESEARCH HIGHLIGHTS

Breast cancer

We previously identified an SNP in the *OGG1* gene that conferred a higher susceptibility to ovarian cancer in carriers of the *BRCA1* gene (HR: 1.12, p= $4.8x10^{-3}$). This gene is related to oxidative stress. We have now demonstrated that carriers of this SNP and the *BRCA1/2* mutation have lower expression levels and shorter telomeres than non SNP carriers, suggesting a higher genomic instability in the carriers, which would explain their higher risk of cancer.

We have found that normal cells derived from heterozygous *BRCA1* mutation carriers are haplo-insufficient for DNA repair. Moreover, we have demonstrated that certain missense mutations in *BRCA1* seem to make the cells more sensitive to Poly (ADP-ribose) Polymerase (PARP) inhibitors than those mutations that give rise to the absence of the protein (frameshift mutations). We are currently investigating the mechanisms underlying these differences with the aim of identifying new markers of sensitivity or resistance to these agents.

Ovarian cancer

By using array-comparative genomic hybridisation, we have defined and validated a region of genomic loss at 6q24-26 that is associated with an improved outcome in patients with high-grade serous ovarian cancer (HGSOC). We have selected several candidate genes in the region and have performed functional analyses in cell models as well as survival association analyses. We have found that low expression of the NER-related gene *GTF2H5* is associated with a better prognosis in HGSOC patients and may also be predictive of the response to platinum-based chemotherapy.

Familial cancer exome project

There are a number of families that have rare or infrequent cancers with an unknown genetic base. We have started a massive sequencing project with the objective of identifying some of these high-susceptibility genes. In 2014, we discovered that the ATP4a gene was responsible for type I gastric carcinoid. In collaboration with S. Ortega's Transgenic Mice Core Unit, we generated a knock-in mouse model to decipher the pathological evolution of gastric carcinoid. We generated heterozygous and homozygous (Hz) mice for the ATP4a mutation and demonstrated that Hz mice mimic the human biochemical and pathological alterations; however, they only get the preneoplastic step (dysplasia). We have also demonstrated that by adding hydrogen chloride (HCl) at low concentrations (3%) to the water, it is possible to prevent and/or recover the 'normal clinical, morphological and analytic conditions' of the stomach (FIGURE).

A second gene, *POT1*, which is responsible for cardiac angiosarcoma in families with multiple types of cancer, has also been discovered by our Group in collaboration with M. Blasco's Telomeres and Telomerase Group. The gene is related to telomeres; several functional and structural studies demonstrate how this alteration prevents the binding of telomerase to POT1, resulting in telomere lengthening, genomic instability and an increased risk of cancer. We have started analysing the genetic landscape of these tumours by massive sequencing in order to identify putative drug targets.

We are also sequencing families with breast cancer following a recessive model in order to search for new susceptibility genes. Among the candidates, we have found a splice-site deleterious mutation in a gene that is involved in the maintenance of genomic stability and DNA repair pathways. This excellent candidate is currently undergoing functional studies in order to explore its implication in the disease.

In parallel, some families with ovarian cancer were also sequenced. One non-described variant belonging to the DNA repair pathway is under functional analysis to determine its pathogenicity. In both groups of patients, around 60 variants of unknown significance are being investigated in a case-control study that aims to define their possible implication in cancer development as moderate susceptibility genes.

The genetic aetiology of families with testicular cancer is unknown. In collaboration with several urological and oncological groups, we are selecting, sequencing and analysing families with this tumour type. Our preliminary results of 10 families have identified some altered genes that are present in many families, suggesting a role in the disease through a polygenic model. We are sequencing a second group of 10 families that will allow us to confirm these findings. ■

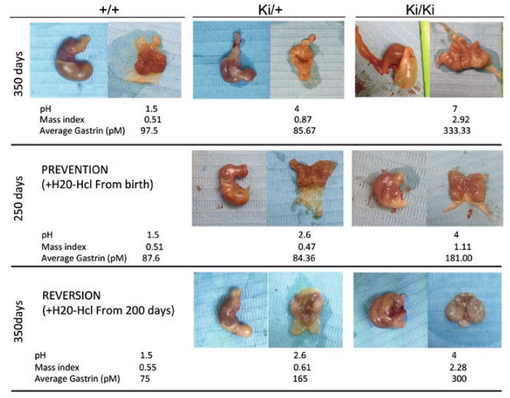

Figure Top: pH (basic), stomach mass index (hyperplasia) and gastrin level (hipergastrinemia) corresponding to the Homozygous (Hz) mouse for the mutation (right), compared with the Heterozygous (Htz) (middle) and normal mice (left). By adding 3% HCl to the water from birth (middle) or from day 250 up to day 350 (down), it is possible to prevent or rescue the constitutional abnormalities.

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HEREDITARY ENDOCRINE CANCER GROUP

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Technicians
Rocío Letón
Lara Sánchez (until September)

OVERVIEW

Our Group is interested in identifying high and low genetic risk factors involved in endocrine tumour susceptibility. To this end, we analyse tumour samples and look for differences between genomic features according to the different individual genetic backgrounds. Such comprehensive characterisation allows us, not only to define diagnostic and prognostic markers associated with primary mutations, but also to pinpoint specific altered pathways that can lead to the identification of future therapeutic targets.

We are also interested in defining genetic markers associated with differences in anticancer drug response and toxicity. We are applying a candidate gene approach, as well as whole genome association studies, to a large series of biological material with associated data, in order to identify therapeutic interventions and other clinically relevant outcome variables. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

"We proposed a methylome profile to stratify phaeocromocytoma patients according to their metastasis risk, identified miRs that act as 'master regulators' of thyroid transformation, and proved that EPHAs play a key role in chemotherapy-induced neuropathy."

RESEARCH HIGHLIGHTS

DNA methylation profiling in phaeochromocytoma and paraganglioma reveals diagnostic and prognostic markers

Phaeochromocytoma and paraganglioma (PPGL) are rare tumours that often present highly variable, post-operative outcomes. Current therapeutic options for metastatic PPGL are very limited. Therefore, novel prognostic markers for metastatic PPGL are urgently needed. We investigated, identified and validated novel prognostic and predictive markers for PPGLs presenting metastases by using whole-genome DNA methylation profiling data from 2 large, well-characterised discovery and primary validation series of tumours (FIGURE). Even after correcting for the succinate dehydrogenase subunit B (*SDHB*) genotype, 48 of these CpGs showed significant associations with time to progression, indicating their potential utility as novel molecular predictive markers. Our findings suggested that aberrant DNA methylation might affect nervous system development and transcriptional regulation networks in PPGL with metastases, thus providing potential clues for future therapeutic strategies. Specifically, these analyses indicated that *RDBP* hypermethylation might contribute to metastatic disease by altering transcriptional networks, including those involved in response to apoptotic stimuli, invasion, and maintenance of DNA integrity. Our results suggest that these CpGs may represent potential markers of malignancy and also serve as prognostic predictors independent of genetic background.

MicroRNA deep-sequencing reveals master regulators of follicular and papillary thyroid tumours

We found that follicular and papillary thyroid tumours present distinct microRNA profiles that are closely linked to specific mutations. It was especially interesting to uncover a few miRNAs consistently up-regulated across all tumour classes, suggesting they play a role as 'master regulators' of thyroid transformation. By integrating these miRNA data with gene expression data, we were able to identify target genes for these key miRNAs, which is a novel finding for thyroid pathologies. Furthermore, based on an analysis of clinical follow-up information, we propose a prediction model for disease relapse based on the expression of 2 miRNAs (miR-192 and let-7a) and clinicopathological features. On the whole, our Group has contributed with a comprehensive and clinically relevant characterisation of orphan thyroid subtypes, so far neglected by international consortia. These findings could be crucial to stratify patients and improve their clinical management.

Polymorphisms in ephrin type A receptor *(EPHA)* genes are risk factors for taxane-induced neuropathy

Improving the quality of life for cancer patients is of enormous clinical and social relevance. In this regard, the peripheral neuropathy induced by anti-cancer drugs, can result in symptoms and disabilities in up to 40% of cancer patients as a consequence of the chemotherapeutic drugs and also the new targeted agents. The neuropathy limits the dose and efficacy of these drugs, and diminishes the quality of life of the patients, sometimes permanently. In a previous GWAS analysis, we proposed that genetic variants in the *EPHA* genes were important factors influencing taxane-induced neuropathy. To follow-up on our initial results, we analysed data from patients treated with first-line paclitaxel and with exceptional neuropathy data, recorded cycle by cycle. Polymorphisms in *EPHA5*, *EPHA6* and *EPHA8* genes were confirmed as risk factors for the neuropathy (rs7349683: HR=2.3, P=0.007; rs301927: HR=1.9, P=0.006; rs209709: HR=1.9, P=0.012, respectively). From a biological perspective, Eph receptors represent a family of receptor kinases, involved in axon guidance and other neural-related functions. Furthermore, because EPHA proteins mediate neural injury repair, these polymorphisms could act as broad-spectrum neuropathy risk markers relevant for many neurotoxic drugs. ■

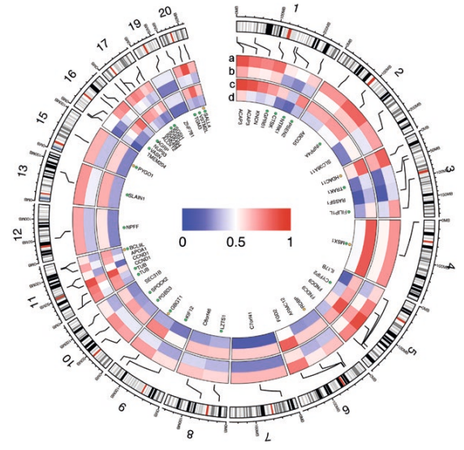

Figure DNA methylation patterns associated with metastatic PPGLs. (**A**) Outermost track provides ideogram for chromosomes. Heatmaps show methylation levels for 52 confirmed CpGs associated with metastatic PPGL: a) metastatic tumours in discovery series (DS); (**B**) tumours without metastases in DS; (**C**) metastatic tumours in validation series (VS); (**D**) tumours without metastases in VS. Green dots indicate genes with functions in nervous system development, while genes involved with transcriptional regulation at gene promoters are indicated by orange dots. Hyper- and hypomethylated CpGs are indicated in red or blue, respectively.

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GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

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**Titulado Superior* (Advanced Degree)

Students in practice
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Visiting Scientists
Kristel Van Steen (University of Liège, Belgium), Yves Allory (H. Créteil, Paris, France)

OVERVIEW

The scope of the 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 their 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 developed and applied statistical approaches to integrate *omics* data, predict cancer risk and outcome and to identify the role of both genetic and non-genetic factors in cancer development and progression."

RESEARCH HIGHLIGHTS

Research findings

During 2015, the Group has mainly focused its research on pancreatic and bladder cancers.

In **pancreatic cancer** (PC), we have substantially contributed to the field by characterising the reverse association between atopic disease and risk of PC, analysing data from the PanGenEU study. A meta-analysis, including our own study, concluded on the protective effect of asthma in PC development (FIGURE 1). We have participated in consortia studies on the role of vitamin D metabolic pathway genes and PC risk, as well as in 2 studies that have identified proteins in urine and plasma with a potential for PC early diagnosis. We continue to support the Spanish Registry of Familial Pancreatic Cancer with colleagues at the *Hospital Ramón y Cajal* in Madrid. This project includes a screening programme for high-risk relatives. A study was conducted assessing the detection of circulating tumour cells and kras mutant circulating free DNA in peripheral blood as biomarkers in patients diagnosed with exocrine pancreatic cancer. In regards to **bladder cancer** (BC), we have conducted several studies on the role of inflammation-related genetic factors in both the development and progression of this cancer, and have contributed to a large international study on the role of large structural genetic mosaicism in human autosomes and the risk of BC, among other neoplasms. Jointly, with colleagues from the National Cancer Institute in Bethesda (USA), our Group has participated in the identification of a novel susceptibility locus at 13q34 and refinement of the 20p12.2 region as a multi-signal locus associated with BC risk in Europeans, as well as in assessing the modification effect of occupational exposures on bladder cancer risk by common genetic polymorphisms. We actively participate in the Seventh Framework Programme funded TransBioBC study, aimed at identifying proteomic biomarkers for BC primary diagnosis and surveillance. In line with this study, a patent has been submitted on the BC biomarkers identified in the DeCanBIO project. The Group also participates in a collaborative effort focused on T1 high-grade/Grade 3 BC by assessing the effect of re-transurethral resection and Bacillus Calmette-Guérin treatment on clinical outcomes.

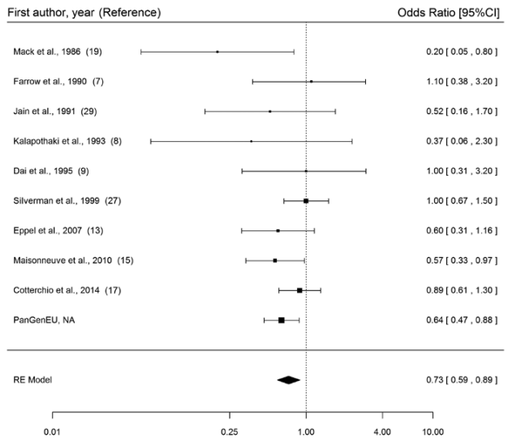

Figure 1 Meta-analysis of case-control studies of the association between asthma and pancreatic cancer risk. The pooled estimate and the 95 % confidence interval for a random-effects model is shown in the bottom of the figure.

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Methodological contributions

By adopting an integrative research approach, we participate in large, international multidisciplinary studies requiring the development of methodological innovations in all aspects of epidemiology. In this regard, we have proposed an integrative framework that allows the identification of relationships at the whole-genome level, providing some new biological insights and highlighting the importance of integrating *omics* data (FIGURE 2). Furthermore, we developed a permutation-based method to concomitantly assess significance and to correct by multiple testing with the MaxT algorithm, and applied it with penalised regression methods (LASSO and ENET) when exploring relationships between common genetic variants, DNA methylation and gene expression measured in bladder tumour samples. In addition, Bayesian-based approaches are being explored for predicting BC risk and outcomes using whole exome and genome sequencing data.

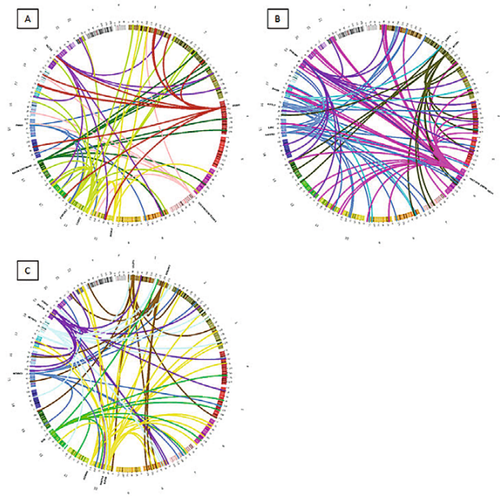

Figure 2 Circular representation of the 'hotspots' found for SNPs (**A**), CpGs (**B**) and gene expression probes (**C**) extracted from the relationships on the triplet SNP-CpG-Gene expression. Each chromosome is represented by a different colour and the colour of the lines corresponds to the SNPs, CpGs or gene expression probes that are located in the chromosome that it shares the colour with. The name of the genes is located in the gene with the 'hotspot'.

Translational activities

We coordinate and lead certain projects of the COST Action BM1204 *EU_Pancreas platform (www.eupancreas.com).* This Action includes 200 multidisciplinary members from 22 EU countries, EU governmental and nongovernmental institutions, as well as private companies. Several scientific, training, and dissemination activities have been conducted during 2015. By endorsing the European Multi-stakeholder Platform on Pancreatic Cancer, we have actively participated in several activities aimed at increasing PC awareness in the general population, the medical community and among health policy makers, as well as in setting a European-based clinical registry of PC (PancreOS). A White Paper on PC has been jointly written with the European Alliance of Personalised Medicine and has been distributed among European Parliamentarians. ■

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FAMILIAL CANCER CLINICAL UNIT

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Technicians
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Fátima Mercadillo

OVERVIEW

Genetic susceptibility plays an important role in several types of cancer. The identification of mutations in genes that predispose to cancer has clinical utility for patient management. Furthermore, a better knowledge of these genes will improve our understanding of the molecular pathways involved in cancer initiation.

The Familial Cancer Clinical Unit (FCCU) aims to identify genetic alterations that confer cancer susceptibility. Patients with hereditary cancer syndromes carry constitutionally rare alterations in high-penetrance genes. The most widely known examples are represented by mutations in *BRCA1* and *BRCA2* genes, which confer a high risk to develop breast and ovarian cancers, as well as mutations in mismatch repair genes (*MLH1, MSH2, MSH6* and *PMS2*) involved in Lynch syndrome, with an increased risk of colorectal cancer, endometrial cancer and other associated tumours.

"The search for prevention strategies represents the 'holy grail' of oncology. Given the high risk of cancer associated with hereditary cancer syndromes, there is a broad field of research into strategies for preventing cancer beyond the avoidance of carcinogens and making other lifestyle changes."

Characteristically, carriers of mutations in these high-penetrance genes show an early age of tumour onset, bilateral tumours and a tendency to develop several primary tumours.

CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The FCCU visits patients with suspected genetic susceptibility to cancer at our consultancy in the Medical Oncology Service of the *Hospital Universitario de Fuenlabrada* (HUF). The referral system for the HUF area was clearly established during 2015. Discussions of protocols and clinical guidelines are held in monthly sessions conducted by the hospital's Hereditary Cancer Clinical Committee, which was created in March 2015.

During this year, we have doubled the number of patients visited in our consultancy at HUF: 318, as opposed to 163 during 2014. Moreover, 328 genetic diagnostic studies were performed in the FCCU laboratory (314 in 2014). The analysis of the *SMARCE1* gene, whose mutations explain some cases of familial meningiomas, has now also been incorporated into our catalogue of services.

The FCCU, in collaboration with other CNIO and non-CNIO research groups, has actively contributed to the search for new genes implicated in cancer susceptibility. The recognition of mutations in *MDH2* responsible for familial paragangliomas, in *FAN1* causing familial colorectal cancer type x or, finally, mutations in *POT1* as the cause of hereditary cardiac angiosarcoma, are some of the findings in this field. These findings once again illustrate the great complexity of the genetic heterogeneity that characterises cancer predisposition syndromes.

We have continued the characterisation of early-onset colorectal cancer (EOCC). On the premise that the carcinogenetic mechanism and the progression of colorectal cancer may differ with the location, we analysed the molecular and clinical characteristics of EOCC according to the tumour location in order to identify more homogeneous subgroups of colorectal cancer. Right-sided EOCC is a subset in which most Lynch syndrome cases are found, with earlier stages at diagnosis and better prognosis.

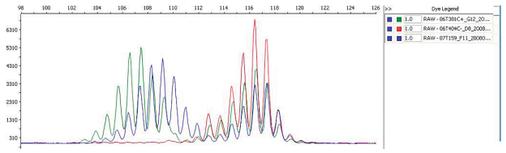

Figure Microsatellite instability. This is the consequence of a deficient mismatch repair system, therefore associated with Lynch tumours and a right-sided location.

At this location the CpG Island Methylator Phenotype (CIMP) is predominant and Chromosomal Instability (CI) is rare. Left-sided EOCC appears as a transitional or intermediate location, except for CI tumours that seem to predominate at this location. Finally, rectal EOCC shows microsatellite stability, low CIMP, and low CI, possibly in relation with MACS (Microsatellite and Chromosomal Stable) tumours (FIGURE).

Establishing relationships with cancer patient associations is one of FCCU's main goals. During 2015, the FCCU strengthened its relationships with ASACO (*Asociación de Afectados por Cáncer de Ovario*) and AEAS (*Asociación de Afectados por Sarcomas*). Members of ASACO are regularly evaluated in the FCCU consultancy for genetic counselling. We also participated in the ASACO annual meeting last May. In addition, we have started a specific collaboration with AEAS members focused on the evaluation of familial antecedents of cancer. A survey was distributed among the members during the first semester of 2015 and the global results were presented in the AEAS annual meeting – hosted by the CNIO – that took place last September. ■

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PATENT

- Perea García J., González Sarmiento R., Urioste Azcorra M., Rueda Fernández, D., Arriba Domènech, M., García Hernández J.L., Pérez García. J (2015). *Biomarcador para el diagnóstico, pronóstico y seguimiento de cáncer colorectal de aparición precoz. ES201531891.*

MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

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**Titulado Superior* (Advanced Degree)

OVERVIEW

Improvements in whole-genome sequencing have generated abundant data from cancer cells, revealing the complexity of the cancer genome, which undergoes small changes in the DNA sequence as well as structural rearrangements (translocations, inversions, deletions and amplifications). We study the role of acquired chromosomal abnormalities in cancer through: (1) the molecular characterisation of genetic and cytogenetic markers, and (2) the design of human stem cell models carrying chromosome rearrangements. The Molecular Cytogenetics and Genome Editing Unit is a state-of-the-art facility that performs customised conventional and molecular cytogenetic analyses of cells from human and animal sources. Our experience with the versatile CRISPR-Cas9 genome editing technology enables us to engineer human cells to recapitulate cancer genome alterations. The Unit also participates in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

"We identify aberrant genomic profiles in leukaemias and sarcomas, opening up new avenues for targeted therapies. By genome engineering, we create human stem cell models with unique genetic events, thereby providing invaluable tools for future basic and clinical research."

RESEARCH HIGHLIGHTS

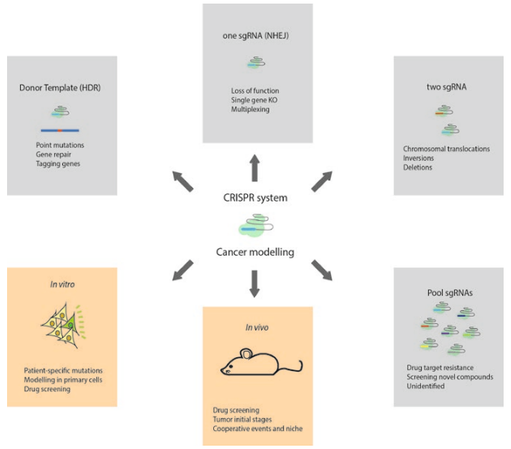

Figure Types of genome-engineering CRISPR-Cas9 system applications to study cancer. HDR: homology-directed repair; sgRNA: small guide RNA; NHEJ: non-homologous end joining.

Optimising CRISPR technology to model human cancer aberrations in primary cells

Methodologies to reproduce tumour-associated chromosome aberrations are necessary to perform the functional identification and detailed characterisation of cancer genes. We have developed an efficient strategy, based on the CRISPR genome editing system, to generate human cell models bearing tumour specific chromosomal alterations (*Nat Commun* 2014), demonstrating that the *in vitro* modelling of tumour-initiating cells is feasible with CRISPR. We have optimised our CRISPR protocol to increase the efficiency of engineered chromosome aberrations in poorly transfectable human primary cells, reducing the labour and costs associated with single cell cloning and genotyping after cell modelling.

From the patient chromosome translocations to the human haematopoietic progenitor (hHSC) cell models

We have genetically engineered 2 human hHSC models to carry novel fusion genes: i) a RUNX1 truncated protein as a result of the t(1;21)(p32;q22) translocation identified in a leukaemia patient – this model demonstrates that C-terminally truncated RUNX1 proteins can contribute to leukaemogenesis in a similar way to the *RUNX1-ETO* fusion gene –; ii) an hHSC model bearing the *NUP98/HOXA9* fusion gene that has allowed deciphering its leukaemogenic molecular mechanisms, opening up new therapeutic possibilities.

Technological and translational activities

Our Unit provides state-of-the-art molecular cytogenetic and genome editing services. We are developing various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as *in situ* analysis of RNA expression by FISH analysis, and implementing the use of CRISPR libraries to perform high-throughput functional analysis for the identification of cooperative driver genes essential for cancer development. In 2015, we carried out over 1,500 assays for experimental and clinically-oriented projects. ■

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AWARDS AND RECOGNITION

- Nominated Member of the Scientific Committee of the *Fundación Leucemia y Linfoma*: a charitable organisation that provides funds for research and educational projects within the field of onco-haematological disorders.

HUMAN GENOTYPING-*CEGEN* UNIT

Anna González Neira
Unit Head

Graduate Student
Sara Ruiz

Technicians
Charo Alonso, Núria Álvarez,
Belén Herráez, Tais Moreno (TS)*,
Guillermo Pita (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 us to identify genes underlying complex diseases, such as cancer, and drug response. In this Unit we implement different high-throughput and cost-effective methods to measure from one, to millions of SNV and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in the Unit. Complementarily, research focused on the identification of predictive biomarkers for personalised cancer therapy is also undertaken.

RESEARCH HIGHLIGHTS

Exome array analysis identifies new loci and low-frequency variants associated with anthracycline-induced cardiotoxicity (AIC) in children

Anthracycline chemotherapeutic agents are widely used to treat childhood cancers. Their clinical use is limited due to dose-dependent AIC, especially for late-onset manifestations such as the observed dilated cardiomyopathy. We aimed to identify genetic factors contributing to AIC in paediatric oncology patients. We genotyped 93 DNAs from anthracycline-treated children, who survived at least 5 years after the completion of therapy, for 247,870 variants on the Illumina HumanExome Beadchip. We found a polymorphism in a gene encoding a cardiac transcription factor that is involved in structural atrial remodelling, conferring a risk of AIC (OR= 8.61; 95%CI= 2.57-28.9; p= 4.86x10-4). We further explored the joint effects of common and low-frequency variants (MAF<5%) on AIC, taking into account genes with at least 3 variants that were genotyped using a gene-based test (SKAT). We identified a protein-coupled receptor (p= 1.70x10-4) as the most strongly associated gene. This study demonstrates that both single-variant and gene-based tests – taking into account the joint effects of common and low-frequency variants – can elucidate genetic markers predictive of AIC in children.

Identification of rare variants associated with capecitabina-induced hand-foot syndrome (HFS)

Capecitabine (Xeloda) is an oral prodrug of 5-fluouracil (5-FU) that is used in the standard treatment of breast and colorectal cancer. One of the most relevant dose-limiting adverse effects of capecitabine is HFS, characterised by redness, tenderness, and peeling of the palms and soles. With this study we aimed to identify genetic variants associated with HFS. Using the Illumina HumanExome Beadchip, we investigated 239,800 variants across the genome in 636 Spanish and UK patients suffering from extreme HFS toxicity grades (grade 0 vs. grades 3 & 4) and diagnosed with breast and colorectal cancer. We also found a new intronic variant in *ENOSF1* not previously associated with HFS (OR=0.61, p=6.681x10-05) . Recently, polymorphisms in *ENOSF1* have been reported to be associated with capecitabine-related severe toxicity, mainly HFS. Remarkably, ENOSF1 has been proposed to regulate *TYMS* (one of the main targets of 5-FU) expression through degradation of TYMS mRNA via an antisense mechanism.

Replication analysis in Ewing's sarcoma (ES) survival genes

Ewing sarcoma (ES) is relatively uncommon, despite being the second most frequent primary malignant bone tumour in children and adolescents after osteosarcoma. Despite considerable progress made during the past decades, many individuals still relapse or suffer from adverse drug reactions; this has motivated the search for predictive factors. We selected 24 genes reported to be involved in the biotransformation of the 6 agents used as the standard chemotherapy regimen for ES, and a total of 384 SNPs were selected across these candidate genes. We identified 3 SNPs in the Spanish population – rs7190447, rs4148737 and rs11188147, located in the *ABCC6*, *ABCB1* and *CYP2C8* genes, respectively –that are significantly associated with overall survival. These associations were confirmed in a large, independent, replication cohort of 495 patients from 5 European countries. ■

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CLINICAL RESEARCH PROGRAMME

MANUEL HIDALGO 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.

"We focus on developing novel and more effective treatments against cancer."

The CRP is composed of 5 Clinical Research Units (CRU) and 3 support Units. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo, 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, and 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, focuses on the implementation of molecular markers in clinical trials, and the Clinical Trials Management Unit coordinates our clinical trials activities. Finally, the Translational Bioinformatics Unit, led by Fátima Al-Shahrour, works on applying knowledge of cancer genetics to patient care.

In 2015, we continued the expansion of our clinical trials activities in collaboration with several hospitals in Spain. Operational units at the *Hospital Universitario de Fuenlabrada*, *Hospital de Madrid*, *Fundación Jiménez Diaz*, *Hospital Ramon y Cajal*, *Hospital Niño Jesus*, *Hospital "12 de Octubre"* and the *Hospital Virgen de la Victoria* in Malaga, have treated over 350 patients in early clinical trials this year. We have also established multicentre clinical trials in breast, prostate and pancreatic cancers that involve the participation of several Spanish hospitals. Finally, we launched the 'Avatar' Clinical Trial aimed at personalising the treatment of patients with pancreatic cancer.

GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

Manuel Hidalgo
Clinical Research Unit Head

Staff Scientists
Rodrigo De Almeida (since September), Pedro P. López, Sofía Perea (since September)

Post-Doctoral Fellows
Lucía Fernández, Camino Menéndez, María Vela (since September)

Graduate Students
Spas Dimitrov, Beatriz Salvador

Technicians
Soraya Ardila (since April) (TS)*, Natalia Baños, Victoria B.bonilla (since May), Yolanda Durán (since May), Marina Mendiburu-Eliçabe (until February) (TS)*, Manuel Muñoz, Gemma M. Sánchez (since December) (TS)*, Francesca Sarno (since September) (TS)*

*Titulado Superior (Advanced Degree)

Clinical Investigator
Victor Moreno (until February)

Visiting Scientists
Lucas Moreno (CNIO-HNJ Clinical Research Unit, *Hospital Infantil Univesitario Niño Jesús*, Madrid), Raul Calero (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. Our work 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 we have implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action. An important development in this area has been the recent report that *nab*-paclitaxel – an agent that we helped to develop – has demonstrated improved survival in patients with pancreatic cancer; this has led to the approval of the drug to treat this disease. Our Group has demonstrated that SPARC expression is not a predictor of *nab*-paclitaxel activity.

"In 2015, we initiated phase 2 clinical trials with demcizumab, a new drug that targets cancer stem cells in *KRAS* mutant tumours, and phase 1 studies of palbociclib, a CDK4/6 inhibitor that has substantial preclinical activity in mouse models."

Key to our work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. We have developed and have characterised the largest collection of these models in pancreatic cancer. We use the Avatar models 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, we are using the Avatar models for personalised cancer treatment integrated with next generation sequencing. We have initiated several projects, the generation of 2D and 3D models, using Zebrafish to generate ZaVATAR models, and the generation of Avatar models suitable for immunotherapy studies.

RESEARCH HIGHLIGHTS

In 2015, we continued the below summarised lines of work that were initiated in the previous year:

Avatar mouse model development and characterisation

Our Group has continued its efforts to develop and characterise Avatar models from patients with GI malignancies, as well as other tumour types, for drug screening, development of drug combinations, biomarker discovery, and personalised medicine. This collection of patient-derived xenografts (PDX) from pancreatic ductal adenocarcinomas (PDA) is the largest and best characterised collection available so far, and represents an important resource for academic and industry investigators; it is also part of the European PDX Consortia. We have collaborated in several published studies (listed below) that use our collection for drug development and biological studies. Examples of some of the most recent agents that we have tested include the PanHer inhibitor SYM013 – which has been shown to have activity in *KRAS* mutant *p53* WT tumours – and Palbociclib, an inhibitor of CDK4/6 that targets p16 defective cancers (FIGURE 1). These 2 agents are already in clinical trials. We have also initiated projects to develop 2D and 3D cultures from Avatar models, using Zebrafish as a platform for Avatar development (ZaVATAR), as well as modifications in the host mice to incorporate immunotherapy strategies.

Development of novel anticancer agents

We have significantly expanded the portfolio of early clinical trials in patients with GI and other malignancies. At present, the GI Cancer Unit is conducting several clinical studies with

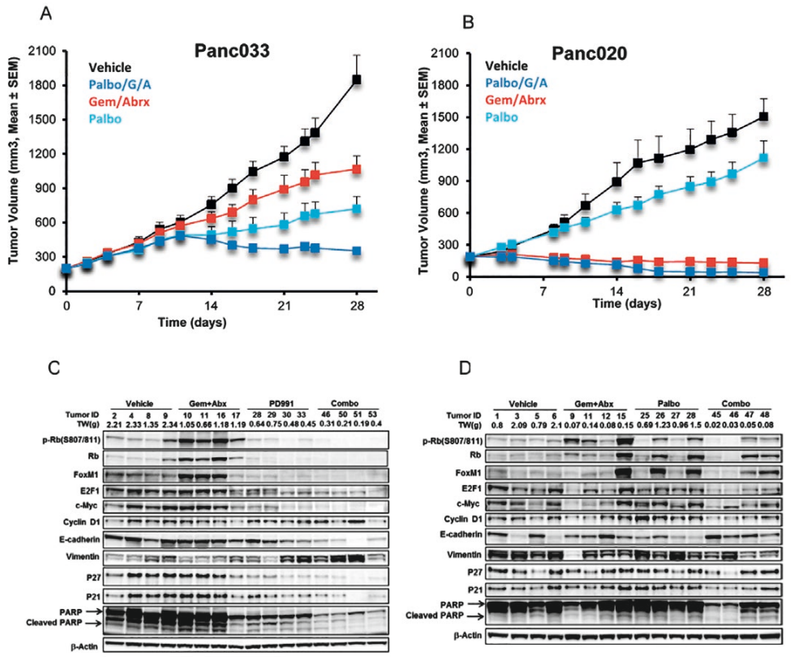

Figure 1 Preclinical assessment of palbociclib in PDAC Models. Palbociclib shows differential single agent efficacy across models. (**A**) Palbociclib single agent activity exceeds Gemcitabine and *Nab*-paclitaxel in a rapid autopsy derived PDX model (PANC-033) and the combination provides a significant increase in growth inhibition. (**B**) Panc020, a resection derived model in which Gemcitabine and *Nab*-paclitaxel treatment was very efficacious and Palbociclib did not provide additional benefit to tumour growth inhibition. (**C, D**) Western blot analysis of samples from the Panc033 (**C**) and Panc020 (**D**) models showing biomarker modulation.

novel anticancer agents, spanning a wide range of mechanisms of action, such as signalling inhibitors, Notch inhibitors, stroma-directed agents and conventional chemotherapy. More recently we have been involved in studies with immune targeting agents and oncolytic adenoviruses. These studies include first-in-class/first-in-human clinical trials and analyses of clinically important biomarkers, as well as co-clinical studies in mouse models. We are particularly interested in building upon the *nab*-paclitaxel gemcitabine regimen that we helped to develop. Based on positive results obtained in both preclinical and phase 1 clinical studies, we are now leading a multicentre phase 2 study of demcizumab in patients with advanced PDA. Furthermore, we have initiated the phase 1 development of the CDK 4/6 inhibitor palbociclib in combination with *nab*-paclitaxel in patients with advanced PDA. In addition, we continue to test multiple new agents, alone and in combination, in animal models of PDAC in preclinical studies. The overarching goal is to identify agents that warrant clinical studies. In order to increase the number of agents that can be tested, as well as to explore combinations and perform studies in a more rapid and efficient manner, we have developed 2D and 3D screening platforms, as well as a new strategy to use Zebrafish (ZaVATARS) to this end.

Personalised treatment of pancreatic cancer

Our goal in this area is to implement an integrated approach that combines next-generation sequencing with Avatar mouse model development. In a pilot study, we performed whole-exome sequencing analyses in 25 patients with advanced solid tumours in order to identify putatively actionable tumour-specific genomic alterations. Avatar models were used as an *in vivo* platform to test proposed treatment strategies. A total of 13 patients received a personalised treatment, of which 6 achieved durable remissions. Based on these results we launched the Avatar clinical trial, in which patients with advanced PDA are randomised to either a standard of care approach or to a personalised approach in which we perform a tumour biopsy followed by exome analysis and generate an Avatar model to experimentally test treatment options resulting from the genetic analysis. With funding from a European Research Council (ERC) Advanced Grant, we are now launching a multicentre randomised study in collaboration with hospitals in Madrid. This unique clinical trial will provide an important collection of biomaterials and a prospective clinical follow-up of patients with pancreatic cancer. Furthermore, in regards to colorectal cancer (CRC), we have conducted a clinical trial in the first-line setting to show that the monitoring of *RAS* mutation status in circulating cell-free DNA (cfDNA) predicts patient outcome (FIGURE 2). ■

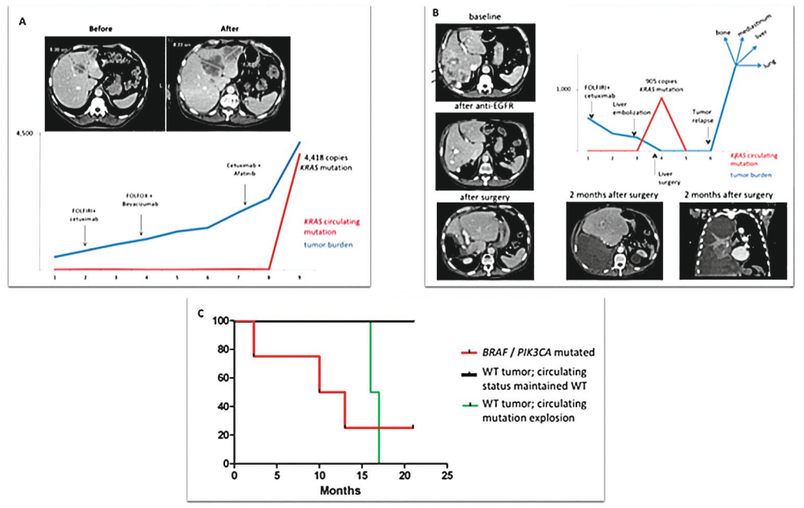

Figure 2 Analysis of RAS mutations in cfDNA of CRC Patients. (**A, B**) Representative plasma levels of mutant *RAS* in patients with CRC treated with EGFR inhibitors. (**C**) Survival of patients with CRC as a function of the emergence of mutations in cfDNA.

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AWARDS AND RECOGNITION

- "III Premio Albert Jovell" Award for Social and Scientific Research in the Oncology Field, the Spanish Group of Patients with Cancer (*GEPAC*).

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientists
María José Bueno, Juan Manuel Funes (until April), Silvana A. Mouron (since February), Paloma Navarro (until April)

Graduate Student
Ivana Zagorac

Technicians
Verónica Jiménez, José Francisco López (since June) (TS)*, Esperanza Martín (since December), Jesús Sánchez (until April) (TS)*

Titulado Superior (Advanced Degree)

Clinical Research Fellow
Laura M. Medina (since April)

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.

"This year, we completed a taxonomic project that enables the detection of virtually 100% of the patients with early hormone-receptor positive breast cancer who will experience distant relapse."

RESEARCH HIGHLIGHTS

FASN in breast cancer

Carbon tracing experiments have demonstrated that, in the absence of FASN, the carbon skeletons incorporated through glucose in the glycolysis process are not redirected to other anaplerotic pathways such as the pentose-phosphate shunt or the Krebs cycle. We are currently pinpointing the mechanism that can explain why glycolysis comes to a stop after the synthesis of pyruvate in FAS$^{-/-}$ cells.

Breast cancer taxonomy

We have identified a set of 3 genes that, when altered (mutated, amplified, or showing deregulated levels of RNA expression), are almost invariably linked to relapse of early hormone-receptor positive breast cancer. Alterations in these genes explain almost half of the relapses in this subset. They are also implicated in hormone resistance.

Resistance to targeted therapies

We have continued our research in antiangiogenic agents. Antiangiogenic agents can exert a hypoxic or normalising response. Last year, we pinpointed the mechanism that explains resistance after induction of a normalising response. This year, we discovered that when these drugs induce hypoxia in the microenvironment, they also induce a change in the immune infiltrate that promotes tumour tolerance. This mechanism of resistance is reversible.

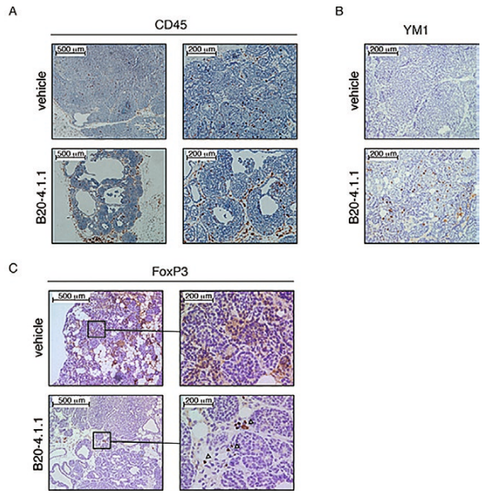

Figure Breast tumours chronically treated with an antiangiogenic monoclonal antibody are infiltrated by immunosuppressive leukocytes. (**A**) CD45 staining reveals how in the B20-4.1.1-treated (bevacizumab) animals the leukocytes (CD45+) remain arrested in the stroma, as opposed to infiltrating the tumour cell nests. (**B**) and (**C**) show infiltrates of M2 macrophages and FOXP3-positive Treg immunosuppressive lymphocytes, respectively. The brown staining in the vehicle-treated tumours is non-specific.

Clinical trials

We have launched the trials CNIO BR-007, 008 and 009 along 2015. All these trials are based on information generated in our preclinical discoveries. More information about the trials can be found in the research group website. ■

PUBLICATIONS

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- Hernández-Agudo E, Mondejar T, Soto-Montenegro ML, Megías D, Mouron S, Sanchez J, Hidalgo M, Lopez-Casas PP, Mulero F, Desco M, Quintela-Fandino M (2015). Monitoring vascular normalization induced by antiangiogenic treatment with 18F-fluoromisonidazole-PET. *Mol Oncol*. PMID: 26778791.

PATENT

- Gold D.P., Quintela-Fandino M. (2015). Combination therapies. US62/111030.

PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro

Clinical Research Fellow
Nuria Romero

Post-Doctoral Fellow
Carolina Navas (since March)

Graduate Students
Elena R. Cutting (until May), Paz Nombela, Floortje Van De Poll, Ylenia Cendón

Technician
Patricia Cozar

Visiting scientists
Teresa Garcés (January-September) (*Fundación de Investigación Hospital Madrid*), Fernando Lopez (*Hospital Ramón y Cajal*, Madrid), Maria I. Pacheco (*Fundación de Investigación Hospital Madrid*)

OVERVIEW

Prostate cancer (PrCa) is the most common cancer and the second leading cause of cancer mortality among men in Western countries. Despite the advances in PrCa diagnosis and early-disease treatment achieved over the last 25 years, up to 20% of PrCa patients will still develop metastatic disease at some point. The majority of these metastatic PrCa patients will succumb after the acquisition of a castration-resistant status (Castration Resistant Prostate Cancer; CRPC), even when treated with novel therapies that have shown to improve survival and quality of life (QoL) in this advanced-resistant setting. The early identification of PrCa patients who have a more aggressive biology and a greater predisposition to develop aggressive metastatic disease could lead to improved 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

DNA repair defects in early prostate cancer

The driving androgen receptor (AR) signalling in PrCa has been implicated in the acquisition of DNA damage, such as single- (SSBs) and double-strand breaks (DSBs). Interestingly, AR activity also regulates a network of DNA repair genes. Genes directly regulated by the AR are involved in homologous recombination (HR), non-homologous end-joining repair, DNA mismatch repair, Fanconi anaemia and base-excision repair pathways. To date, a small number of familial cancer syndromes have been associated with an increased risk of prostate cancer. The majority of these genes are associated with inherited mutations in HR DNA repair genes (e.g. *BRCA2, BRCA1, PALB2, and NSB1*) or DNA damage sensors/check points (e.g. *CHK2*) that directly activate HR. We have investigated the effect of inherited *BRCA* mutations on conventional treatments for localised and locally advanced PrCa as a model of sporadic aggressive PrCa. We have previously shown that *BRCA* carriers have worse outcomes than non-carriers, when conventionally treated with radiotherapy or prostatectomy, as they relapsed and progressed earlier to lethal metastatic disease. In 2015, we have shown that, despite of their aggressive behaviour, *BRCA* mutated tumours are androgen-dependant, which is of relevance for tailored treatment. We are currently working on the molecular characterisation of *BRCA* mutated PrCa, in collaboration with the Institute of Cancer Research (UK), KConFab and the Peter McCallum Cancer Centre (Australia), as well as several Spanish centres. In 2015, we have also identified several features, previously associated with poor PrCa outcome, to be significantly more common in *BRCA2* mutated PCa than in sporadic tumours, which may help to explain their adverse prognosis and be of relevance for targeted therapies.

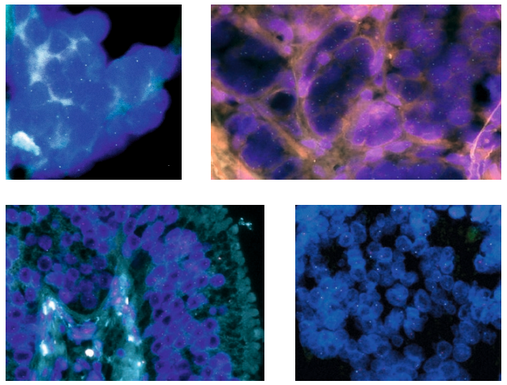

Figure Different patterns of *BRCA2* loss in prostate tumours.

Circulating biomarkers in CRPC

In the current metastatic CRPC scenario, there are several drugs with diverse mechanisms showing activity in a subset of patients, while others remain primarily resistant. The efficient 'a priori' discrimination between both populations is still required. The development of novel biomarkers, which are truly indicative of the tumour biology and/or the tumour-host interaction, should facilitate individual patient risk stratification and improve treatment benefit prediction. We have launched a network of 55 centres across Spain (the PROCURE platform) in order to conduct several prospective, multicentre, and parallel biomarker studies in patients receiving docetaxel, cabazitaxel, radium-223 or abiraterone acetate; these studies will involve over 800 CRPC patients during the next 5 years. With these studies, we aim to analytically qualify and clinically validate a series of blood-borne biomarkers including: ctDNA, ctRNA, exosomes and CTCs. Its characteristics make this study unique in the CRPC field. ■

PUBLICATIONS

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- Tolcher AW *et al.* (incl. Olmos D) (2015). Anti-tumour activity in RAS-driven tumours by blocking AKT and MEK. *Clin Cancer Res* 21, 739-748.
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- Climent MÁ, León-Mateos L, González Del Alba A, Pérez-Valderrama B, Méndez-Vidal MJ, Mellado B, Arranz JÁ, Sánchez-Hernández A, Cassinello J, Olmos D, Carles J (2015). Updated recommendations from the Spanish Oncology Genitourinary Group for the treatment of patients with metastatic castration-resistant prostate cancer. *Crit Rev Oncol Hematol* 96, 308-318.
- Rueda A, Olmos D, Vicioso L, Quero C, Gallego E, Pajares-Hachero BI, Mendiola M, Casanova M, Alvarez M, Provencio M, Alba E (2015). Role of vascular endothelial growth factor C in classical c. *Leuk Lymphoma* 56, 1286-1294.

AWARDS AND RECOGNITION

- 1st Prize for Young Researchers in Oncology, AstraZeneca Foundation, Spain.
- Expert Panel Member, 1st Advanced Prostate Cancer Consensus Conference (APCCC), Switzerland.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

“Since its creation a decade ago, in 2005, MDU has helped 271 haematologists, pathologists and oncologists, active in 98 NHS hospitals. During this period of time, MDU has carried out more than 4,273 tests on samples from 2,178 patients with cancer.”

OVERVIEW

The main duty of the Molecular Diagnostics Unit (MDU) is to provide support to medical professionals of the National Health System (NHS) and the CNIO Clinical Research Units, through the provision of a wide variety of sensitive and specific molecular tests, with the aim of determining alterations in DNA sequences or changes in expression levels of key genes that are involved in cancer. These assays enable the improvement of early diagnosis, the detection of minimal residual disease in patients showing clinical remission, the monitoring of the response to therapy, as well as facilitate decision making amongst different treatment options. MDU is also entrusted with the development, implementation, standardisation and supply of the very latest technologies and methodologies in the field of molecular diagnostics, in order to improve cost, reliability and flexibility. Finally, we are very much committed to disseminating knowledge pertaining to the field of molecular oncology diagnostics by hosting and mentoring biomedical students.

RESEARCH HIGHLIGHTS

Expanding our support

In 2015, we developed a new assay based on the detection of mutations by Sanger sequencing in exons 5-8 of the *TP53* tumour suppressor gene. This sequence encodes the DNA binding domain of the protein and contains approximately 90% of the detected mutations (FIGURE). *TP53* is the most commonly mutated gene in human cancer, especially in pancreatic, skin, oesophagus, head/neck, and colorectal cancers where more than a third of the patients carry mutations in *TP53*. Recently, mutations in *TP53* have become of particular interest as there is evidence that the mutated gene can elicit gain-of-function effects by acquiring oncogenic properties that alter the expression of several other genes and thus, favour the progression and dissemination of cancer. The TP53 mutation status is therefore a helpful prognostic marker and a potential predictor of response to therapy.

Additionally, in August 2015, MDU started a collaboration with the CNIO Gastrointestinal Cancer Clinical Research Unit in a sub project of a national clinical trial – FRAGANCE (Phase I/II Study to Assess the Efficacy and Safety of Nab-paclitaxel in Combination With Gemcitabine for the Treatment of Fragile Patients With Advanced or Metastatic Pancreatic Cancer) – geared towards personalised medicine. This study aims to investigate alternative drug therapy regimes in patients with advanced pancreatic cancer by using the chemo-sensitivity expression profiles of total RNA extracted from their Circulating Tumour Invasive Cells (CTICs). Data from whole gene expression experiments will be analysed using a proprietary algorithm that is able to generate a list of potentially sensitive and resistant drugs for each patient, which can then be used to guide anti-cancer therapies in these patients.

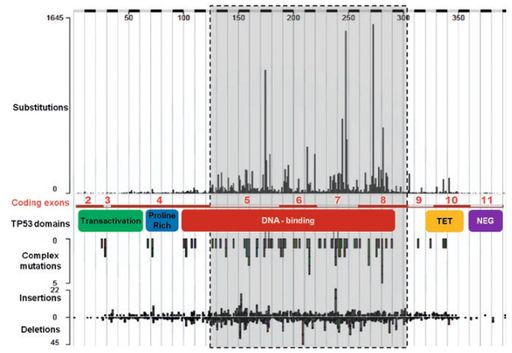

Figure The detection of mutations in *TP53* will enable the prognosis and improve the treatment of patients affected by several cancers (TET: Tetramerisation domain; NEG: Negative regulators domain).

Tutoring

MDU has remained committed towards its policy regarding training and educational programmes; in 2015, our Unit welcomed a medical resident and 3 undergraduate students. ■

PUBLICATION

- Griffiths M, Patton SJ, Grossi A, Clark J, Paz MF, Labourier E; Labceutics International BCR-ABL1 Standardization Study Group (2015). Conversion, correction, and International Scale standardization: results From a Multicenter External Quality Assessment Study for BCR-ABL1 testing. *Arch Pathol Lab Med* 139, 522-529.

AWARDS AND RECOGNITION

- Associate professor, *Universidad Politécnica de Madrid*.

TRANSLATIONAL BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Post-Doctoral Fellow
Hector Tejero

OVERVIEW

The genomics medicine revolution brings new hope in the fight against cancer. Thousands of tumours from different cancer types have been sequenced and the genomes characterised, confirming the complexity of cancer genomes and providing a new perspective in tackling cancer. Computational approaches have the ability to decipher cancer genome marks, but still there are many challenges to be faced prior to translating cancer genome discoveries into clinical medicine.

The Translational Bioinformatics Unit uses computational methodologies to achieve genome analyses of cancer patients' data, in order to identify new biomarkers and mechanisms of drug response. Our main goal is to translate this knowledge into effective treatments for cancer patients.

"We have developed PanDrugs to guide the selection of therapies from amongst the results of genome-wide studies. Pandrugs takes into account the biological relevance of the genes that can be therapeutically targeted and the clinical use of the drugs."

Graduate Student
Javier Perales

Technicians
Elena Piñeiro (TS)*, Kevin Troulé (since December) (PEJ-L)**

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

RESEARCH HIGHLIGHTS

The paradigm of personalised medicine is the identification of the appropriate drug for the right patient, using molecular profiles. The success of personalised treatment depends on each individual molecular profile, which can *a priori* be considered as being very heterogeneous. High-throughput technologies are being used to dissect the genetic heterogeneity of tumours, and in parallel, bioinformatics has emerged as a critical discipline to transform the huge amount of genomic data into comprehensive models. However, these analyses have resulted in the identification of hundreds (or thousands) of mutations and other alterations in the same tumour; therefore, we need new approaches to establish the relevance of these changes, and more importantly, to prioritise those that could be of clinical use for cancer therapy.

Our main research objective is to gain a better understanding of the impact of cancer genomics on making clinical decisions by developing a new computational pipeline. This pipeline is to relate and prioritise drug therapies, based on the wide range of actionable genomic alterations for each individual patient instead of the population average data.

This novel computational approach (PanDrugs, http://pandrugs.bioinfo.cnio.es) is based on the analysis and integration of genomic data (mutations, copy number variations or gene expression levels), with functional data (protein essentiality) and pharmacological data (sensitivity or resistance to antitumour drugs). PanDrugs integrates several public pharmacological resources with a curated target-drug resource that includes single gene-drug and pathway-drug associations and an extensive classification of these drugs based on their status (approved, clinical candidates or experimental probes and drug/target family). We have validated the approach by applying it to publicly available data (ICGC and TCGA cancer genome projects). ■

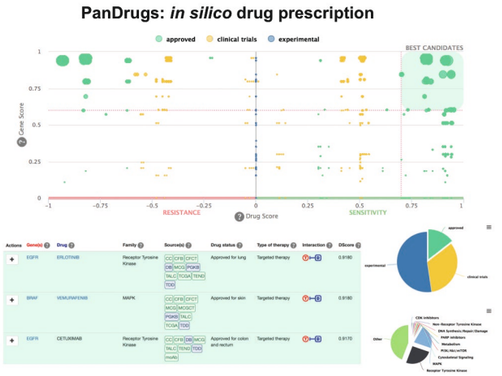

Figure The figure shows the results of the Pandrugs web tool (http://pandrugs.bioinfo.cnio.es). PanDrugs calculates drug-gene scores combining both the biological and clinical relevance of this interaction, and prioritising drugs by their susceptibility to respond and status classification (FDA approved, clinical trial or experimental inhibitors).

PUBLICATIONS

- Soucheray M, Capelletti M, Pulido I, Kuang Y, Paweletz CP, Becker JH, Kikuchi E, Xu C, Patel TB, Al-Shahrour F, Carretero J, Wong KK, Jänne PA, Shapiro GI, Shimamura T (2015). Intratumoral Heterogeneity in EGFR-Mutant NSCLC Results in Divergent Resistance Mechanisms in Response to EGFR Tyrosine Kinase Inhibition. *Cancer Res* 75, 4372-4383.
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H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

"We contribute towards redefining the response criteria for Multiple Myeloma. This year we have described a new molecular alteration that can lead to leukaemia, opening up new avenues for the treatment of leukaemia."

Staff Scientists
Rosa Ayala (since December), Inmaculada Rapado, Beatriz Sánchez-Vega (since February)

Post-Doctoral Fellows
María Linares (since May), Ricardo Sánchez

Graduate Students
Alicia Arenas, Alejandra Leivas, Esther Onecha, Yanira Ruíz

Technician
Alba García

OVERVIEW

The Haematological Malignancies Clinical Research Unit focuses on three 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 a treatment, for example by studying minimal residual disease; and iii) study immune mechanisms of cancer control, with a special focus on NK cells.

→ *In vitro research*: i) establish the effects of new anticancer molecules in *in vitro* models of the disease; ii) determine the mechanisms of resistance to anticancer drugs.

→ *Clinical research*: translate preclinical findings to benefit patients through a phase I clinical trials unit.

RESEARCH HIGHLIGHTS

The most relevant achievements of our group in 2015 are:

→ We characterised, in collaboration with the MD Anderson Cancer Center, heterogeneous nuclear ribonucleoprotein K (hnRNP K) as a haploinsufficient tumour suppressor that regulates proliferation and differentiation programmes in haematological malignancies.

→ We published, in collaboration with the Spanish Myeloma Group, that both the sequential and alternating administration of VMP (Bortezomib plus melphalan and prednisone) and Rd (lenalidomide plus low-dose dexamethasone) in elderly patients with newly diagnosed multiple myeloma yields impressive results, even in high-risk patients.

→ Finally, we have redefined the role of stringent complete response in multiple myeloma. ■

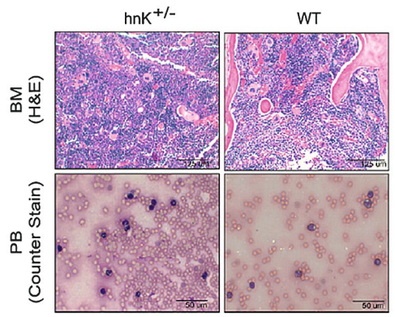

Figure *Hnrnpk*$^{+/-}$ Mice Have Reduced Survival and Are Tumour Prone. H&E staining of paraffin-embedded bone marrow sections and peripheral blood (PB) smears from wild-type *Hnrnpk*$^{+/-}$ mice diagnosed with myeloid hyperplasias. The scale bar for H&E staining represents 125 µm and 50 µm for PB smears. BM, bone marrow.

PUBLICATIONS AT OTHER INSTITUTIONS

- Gallardo M *et al.* (incl. Martinez-Lopez J) (2015). hnRNP K Is a Haploinsufficient Tumor Suppressor that Regulates Proliferation and Differentiation Programs in Hematologic Malignancies. *Cancer Cell* 28, 486-499.
- Ziv E *et al.* (incl. Martinez-Lopez J) (2015). Genome-wide association study identifies variants at 16p13 associated with survival in multiple myeloma patients. *Nat Commun* 6,7539.
- Mateos MV *et al.* (incl. Martínez-Lopez J) (2015). Sequential versus alternating administration of VMP and Rd in elderly patients with newly diagnosed MM. *Blood*. PMID: 26500339.
- Martínez-López J *et al.* (2015) Critical analysis of the stringent complete response in multiple myeloma: contribution of sFLC and bone marrow clonality. *Blood* 126, 858-862.
- Mateos MV *et al.* (incl. Martínez-López J) (2015). Bendamustine, bortezomib and prednisone for the treatment of newly diagnosed multiple myeloma patients: results of a prospective phase 2 Spanish/Pethema trial. *Haematologica* 100, 1096-1102.
- San Miguel JF *et al.* (incl. Martinez-Lopez J) (2015). Impact of prior treatment and depth of response on survival in MM-003, a randomized phase 3 study comparing pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone in relapsed/refractory multiple myeloma. *Haematologica* 100, 1334-1334.
- Mateos MV *et al.* (incl. Martinez-Lopez J) (2015). Treatment for patients with newly diagnosed multiple myeloma in 2015. *Blood Rev* 29, 387-403.
- Campa D *et al.* (incl. Martinez-Lopez J) (2015). Risk of multiple myeloma is associated with polymorphisms within telomerase genes and telomere length. *Int J Cancer* 136, E351-E358.
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- Castro N *et al.* (incl. Martinez-Lopez J) (2015). CALR mutations screening should not be studied in splanchnic vein thrombosis. *Br J Haematol* 170, 588-589.

AWARDS AND RECOGNITION

- "Premio Dr J. Font 2015" Award for the best medical publication by a Spanish Physician, *Mutual Médica*, Spain.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
Clinical Research Unit Head

Staff Scientists
Daniel E. Castellano, Rocio García, Lara C. Iglesias, Santiago Ponce, Jon Zugazagoitia

Post-Doctoral Fellows
Teresa Agullo, Carmen V. Díaz, Irene Ferrer, Sonia Molina

Graduate Students
Ángela Marrugal, Laura Ojeda, Álvaro Quintanal

Technicians
Laura García, Clara López (until November), Rocío Suárez

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 are focused 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 non-small-cell lung cancer xenograft (PDX) platform for testing new drugs/targets. We are also developing PDXs from 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 immuno-oncology.

"Our Group has significantly contributed to the discovery of biomarkers and the early development of new drugs tailored to 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. In the case of lung cancer, we have actively participated in successful genomically-driven phase I trials in the setting of EGFR acquired resistance, in particular in the development of new T790M selective third-generation EGFR inhibitors. Moreover, our Centre has made a notable contribution in the development of an immune checkpoint blockade therapy (PD-1 and CTLA4) for small-cell lung cancer; this treatment regimen has shown encouraging clinical results against this deadly disease. As far as colorectal cancer is concerned, we have conducted a first-in-human phase I trial with a novel anti-EFGR monoclonal antibody showing treatment responses in cetuximab/panitumumab refractory patients. Finally, new drugs targeting TGF, PI3KCA, MEK1/2 have been tested in histology-agnostic early phase I trials conducted by our group leaders.

Conducting practice changing randomised controlled trials

Probably one of our Group's major clinical research achievements has been the co-leadership of 3 of the most important NSCLC and renal-cell carcinoma phase III trials conducted during the last decade. Programmed death-1 ligand 1 (PD-1/L1) blockers have been demonstrated to improve overall survival – compared to standard second-line therapy – in pulmonary adenocarcinomas, squamous cell carcinomas and renal-cell carcinomas, changing treatment paradigms for these diseases. Drugs targeting these immune checkpoints are currently being approved for use in lung and renal-cell cancers and these research findings are rapidly being translated to the first-line and adjuvant settings. Our Group is leading some of these important pivotal trials. In the case of colorectal cancer, a significant contribution was made with an oral agent (TAS-102) that has been shown to increase overall survival in standard treatment refractory patients. Finally, important contributions have also been made in establishing new standards for prostate cancer.

Novel biomarker development and translation

PD-L1 expression, assessed by immunohistochemistry, was investigated as a potential predictive marker for evaluating the benefit of anti-PD-1 therapies in 2 pivotal phase III lung cancer trials. Our Group has presented the first data suggesting a potential predictive effect of PD-L1 expression in non-squamous non-small-cell lung carcinoma. This is the first-time ever that PD-L1 expression appears to be useful for selecting candidates for treatment with these therapies in solid tumours. However, the issue of predictive markers for evaluating the benefit of immune checkpoint inhibitors remains an unresolved area of debate, and intensive research, including that of our Group, is currently ongoing in this field. Research from our laboratory has also validated PD-L1 expression assessed by immunohistochemistry as a potential prognostic factor in malignant pleural mesothelioma. This may provide a good rationale to test anti-PD-1/L1 drugs for this disease. In addition, our Group has validated, for the first time, a transcriptomic gene signature that is predictive for chemotherapy benefit in metastatic colorectal cancer patients. If further confirmed in independent cohorts, this could have potential clinical implications, as it may help to optimise chemotherapy delivery and selection in these subsets. ■

PUBLICATION

- Paz-Ares L *et al*; INSPIRE investigators. Necitumumab plus pemetrexed and cisplatin as first-line therapy in patients with stage IV non-squamous non-small-cell lung cancer (INSPIRE): an open-label, randomised, controlled phase 3 study. *Lancet Oncol* 16,328-37.

PUBLICATIONS AT OTHER INSTITUTIONS

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- Brahmer J *et al.* (incl. Paz-Ares L) (2015). Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *New Engl J Med* 373, 123-135.
- Thatcher N *et al.* (incl. Paz-Ares L) (2015). Necitumumab plus gemcitabine and cisplatin versus gemcitabine and cisplatin alone as first-line therapy in patients with stage IV squamous non-small-cell lung cancer (SQUIRE): an open-label, randomised, controlled phase 3 trial. *Lancet Oncol* 16, 763-774.
- Estevez-Garcia P *et al.* (incl. Paz-Ares L) (2015). Gene expression profile predictive of response to chemotherapy in metastatic colorectal cancer. *Oncotarget* 6, 6151-6159.
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AWARDS AND RECOGNITION

- Irene Ferrer has been awarded the 2015 Grant for Oncology Researchers from the Spanish Association Against Cancer (*AECC*).

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, as defined by the Spanish Law 14/2007 on Biomedical Research and the Royal Decree RD 1716/2011, a "Biobank for biomedical research purposes". 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 it hosts other collections of biological samples for different purposes. Samples and their associated information are collected 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 Community of 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.
→ Institutional registry of all human samples residing in the Centre for research purposes.

Other services

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

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 National Institute of Health Carlos III (ISCIII).

During 2015, the CNIO-Biobank has processed 19 tissue requests from 11 scientific research projects. Additionally, as the Spanish National Biobank Network Coordination Office, we have managed 31 scientific research projects of high complexity.

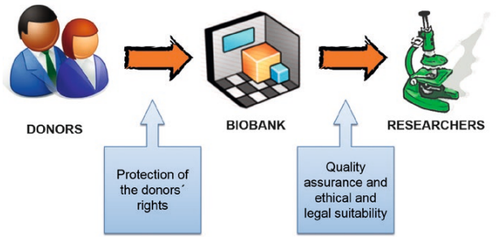

Figure The main objective of the CNIO Biobank is to facilitate the access of human biological samples for researchers at the CNIO and other collaborating institutions, and to ensure that they meet the scientific and ethical requirements in compliance with Spanish legislation.

The mean impact factor of the 14 publications published in 2015, for which our Unit provided support was 6.583. We also provided sample and/or documental support for the familial cancer activities of the CNIO Human Cancer Genetics Programme.

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), *Plan de Cáncer Familiar de la Comunidad de Madrid*, and the *Sociedad Española de Anatomía Patológica* (*SEAP*). ■

PUBLICATIONS

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- Zapatero A, Morente M, de Vidales CM, Adrados M, Lopez C, Nieto S, González MJ, Arellano R, Conde AC, Vicente FG (2015). HIF1A expression in localized prostate cancer treated with dose escalation radiation therapy. *Cancer Biomark* 1, 41-46.

AWARDS AND RECOGNITION

- Member, Canada Foundation for Innovation Evaluation Committee.
- Director, University Masters on "Biobanking and use of human samples in research", *Universidad Católica de Valencia*, 3rd Edition (2014-2015) and 4th Edition (2015-2016).

Direction of Innovation

ANABEL SANZ
Director of Technology Transfer

“Harnessing the innovative power of CNIO’s scientists and collaborators as a driver for drug discovery; time to set up our efforts.”

Research results should eventually lead to innovations and practical applications that can be implemented within the life sciences sector, and, in our case, particularly, within the Spanish National Health System.

The CNIO establishes strategic industrial collaborations in order to gain access to broader expertise, greater resources and to contribute to a faster application of research results in society. The CNIO maintains a successful network of industrial collaborators ranging from big pharmaceutical to smaller biotechnology companies, including Eli Lilly, Roche, Merck Serono, Boehringer Ingelheim, Daiichi Sankyo, Celgene and MEI pharma. Significantly, in 2015, CNIO renewed its collaboration with the Roche ‘Extending the Innovation Network (EIN) Programme’ for 2 more years to support innovative research approaches. Furthermore, the CNIO entered into a new collaboration with Pfizer. As a result of this 3 year collaboration, we expect to generate knowledge and tools to improve the efficacy and safety of the therapeutic interventions that could also be applied to personalised medicine.

Additionally, the CNIO collaborates with patient focused stakeholders such as the Paradifference Foundation that supports research on rare tumours at the CNIO, with the aim of developing more efficient treatments and essentially finding a cure for the disease.

It’s now been 4 years since the Innovation strategy was launched at the CNIO with an emphasis on collaborative drug discovery. Harnessing the innovative power of CNIO’s scientists through the fostering of *intra* collaborations has resulted in a significant increase in the number and diversity of projects in the early drug discovery pipeline developed at ETP-CNIO (Experimental Therapeutics Programme). Currently, ETP-CNIO is working on several projects in various stages of development, ranging from HTS Assay Definition to Lead Optimisation. External collaborations with leading institutes such as VIB (Flemish Institute for Biotechnology) further contribute to enrich the portfolio.

The CNIO maintains a leading position within the academic sector when it comes to the commercialisation of Monoclonal Antibodies. CNIO’s 2015 antibody catalogue contained antibodies against 20 mouse and 69 human protein antigens.

Fostering an innovation culture is key for maximising the innovation potential of a Centre like ours. A number of intra and extramural initiatives have been organised. A debate about ‘Innovation as a Bridge Between Science and Society’ was held at the CNIO; it highlighted how researchers are working towards pursuing their dreams to unravel discoveries that can become useful for patients.

In 2015, with the support of the *Banco Santander* Foundation, 3 students from the CNIO were granted the opportunity to attend the 4th edition of the ‘Management Fundamentals for Scientists and Researchers’ course hosted at the reputed *IE Business School*. This course will enable them to transfer their scientific knowledge and research to the marketplace in the form of a spin-off company or a transfer accord.

In 2015, in recognition of its innovation strategy, the CNIO was awarded with the Innovation Recognition Award by the Spanish Association of Innovative Companies (*Foro de Empresas Innovadoras*, *FEI*), for its accomplishments in fostering and developing innovative technologies for the oncology sector.

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 that are 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 from the CNIO Research Groups, they also provide support and collaborate with groups from other institutions, both public and private.

2015 has been a year of consolidation for the Programme's activities. Our resources have been kept essentially stable; however, towards the end of the year, the Programme was significantly reinforced by 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.* We are convinced that this inflow of young people undergoing this training step in their professional careers will be refreshing for the Core Units, and will also help enhance their training activities and capabilities, in addition to providing additional resources to face future challenges and respond to the demands from the users.

As an indication of our high level of commitment towards training and education, the Programme has been involved in the organisation of courses, workshops and specialised meetings, such as the Course on Flow Cytometry, the Workshop on New Applications and Technologies in Confocal Microscopy, and the Course on Animal Experimentation. Moreover, members of our staff have participated in an increasing number of Masters and other training activities hosted at the CNIO and elsewhere. Finally, the number of PhD and Master's students in the Programme has reached a peak this year.

This year, the Core Units were particularly active in attracting funding from external sources through activities related to innovation. As many as six contracts and agreements have been signed with private companies and public institutions, based on the technologies mastered by several of our Core Units. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit have grown by about 40% compared to the previous year, thus reaching a historical maximum.

"It is not possible to stay at the forefront of cancer research without the support provided by first-class Core Units mastering their technologies and disciplines. Thus, CNIO's excellence is mirrored by the outstanding achievements of the Biotechnology Programme's Core Units."

Last but not least, 2015 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 about 30 publications, including several papers in top journals.

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Guadalupe Luengo, Jorge Monsech, Ana Belén Moreno (since December), Ángeles Rubio

"The Genomics Unit helps CNIO scientists to understand the molecular processes underlying cancer by providing them with a toolbox for DNA and RNA analyses that is dedicated to an array of applications, either at the single locus or at more global genomic levels."

OVERVIEW

Genomics is the discipline that uncovers the life of the genome, its structural features, regulation and expression. The genome is the blueprint of life, the ensemble of the genetic material that keeps the detailed assembly instructions of the species. Any given cell of an individual keeps a copy of the genome deep in its nucleus. Chemically made of linear DNA macromolecules and distributed into chromosomes, the genome is packed with and interpreted by a myriad of protein cohorts. It is expressed into RNA transcripts that constitute the intermediate step between the genetic material and the functional proteins that run the cell. While less than a 2% fraction of a mammal's genome codes for protein, a vast majority of the genome (80%) has been found to participate in some biochemical event or another. The genome is not immutable; it suffers damage and alterations. Cancer derives from the accumulation of such alterations. Cells with a damaged genome can transform and escape control, and develop into a tumour. The field of Genomics sheds light on this complexity.

RESEARCH HIGHLIGHTS

Each cancer genome is different. Even an individual tumour harbours a number of subclonal genomes, each bearing some unique alteration. By employing a distinct set of powerful methodologies, Genomics reveals the genetic diversity of cancer genomes and helps to dissect transformation 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 can detect modifications at a structural level: mutations, binding of protein factors, variations in chromatin folding. Others are capable of examining functional choreographies: changes in the transcriptome in response to treatments, which may uncover therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of coverage. 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 and small RNAseq, or genome-wide location of interacting protein factors on chromosomal DNA by ChIPseq. These applications are based on the use of the sequencing-by-synthesis technology from Illumina. On the other hand, gene expression or transcriptome and detection of chromosomal copy number anomalies can be addressed with Agilent DNA microarrays. 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 mutations in candidate genes as well as for the verification of cloned genes or inserts. A cDNA clone repository from the IMAGE-MGC consortium provides scientists with reagents to transfect genes or to express a given protein of interest. The Unit also provides a transgenic mouse genotyping service, based on allele-specific quantitative PCR for a quick and efficient turnaround time. ■

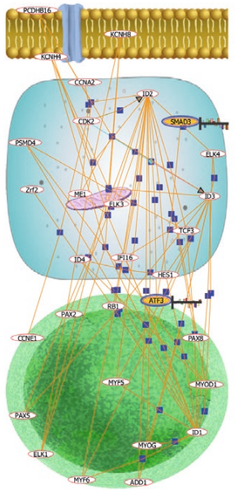

Figure Data obtained from high-throughput genomic technologies can readily be projected into cellular networks to pinpoint or represent functional physiological states. This figure shows protein factor interactions that are found altered under given experimental conditions according to a genomic survey of the mRNA transcriptome.

PUBLICATIONS

- Palla AR, Piazzolla D, Alcazar N, Cañamero M, Graña O, Gómez-López G, Domínguez O, Dueñas M, Paramio JM, Serrano M (2015). The pluripotency factor NANOG promotes the formation of squamous cell carcinomas. *Sci Rep* 5, 10205.
- Foronda M, Morgado-Palacin L, Gómez-López G, Domínguez O, Pisano DG, Blasco MA (2015). Profiling of Sox4-dependent transcriptome in skin links tumour suppression and adult stem cell activation. *Genomics Data* 6, 21-24.

TRANSGENIC MICE CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Student
Aleida Pujol

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

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

OVERVIEW

Genetically modified 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 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 2015, the Unit has contributed to 6 peer review articles, in collaboration with CNIO and external groups, including projects related to the characterisation of cell cycle regulator functions *in vivo* and in tumour development, as well as of new mechanisms of lymphatic system development."

RESEARCH HIGHLIGHTS

During 2015, the Transgenic Mice Unit has focused on the establishment of 2 new technologies: the use of mouse haploid embryonic stem cells (hESCs) for cancer-related genetic screenings, and the incorporation of the CRISPR/Cas9 system for genome editing in mice.

The first mouse hESC lines were established in 2011, by Anton Wutz and Joseph Penninger, from wild type parthenogenetic embryos. Since then, they have been shown to be a powerful tool for genomewide forward- and reverse- genetic screenings in mice using transposons or gene-trap lentiviruses for genomewide mutagenesis. The haploid karyotype enables direct phenotypic selection of recessive mutations that would be silent in a diploid context. We are interested in exploiting the potential of mouse hESCs for cancer related screenings by generating parthenogenetic hESCs from cancer mouse models and mutant mice available at the CNIO. For this purpose we are creating a collection of mutant hESCs, called HaploESCancer collection, derived from CNIO mice. This year we have established haploid mouse embryonic stem cell lines from p53 KO, Brca1_lox and ATR_lox mice by $SrCl_2$ activation of mouse oocytes. These cells will be mutagenised using PiggyBac transposition – ENU or CRISPR/Cas9 – in order to obtain mutant clones covering nearly the whole set of protein/RNA coding loci in the mouse genome.

The CRISPR/Cas9 system, imported from bacterial and archaeal genomes, has expanded the currently available set of mammalian genome engineering tools, providing an easy, efficient, flexible and versatile method of introducing targeted mutations in mammalian genes. The CRISPR/Cas9 system has also been used to generate knockout and knockin mice by introducing the guide CRISPR RNA and the Cas9 RNA directly into mouse zygotes. We have succesfully used this system directly in mouse zygotes for generating deletions of regulatory regions and knockout alleles by open reading frame alteration. We are now interested in exploring the advantages of this system for precise genome editing with respect to gene targeting in mouse embryonic stem cells (FIGURE). ■

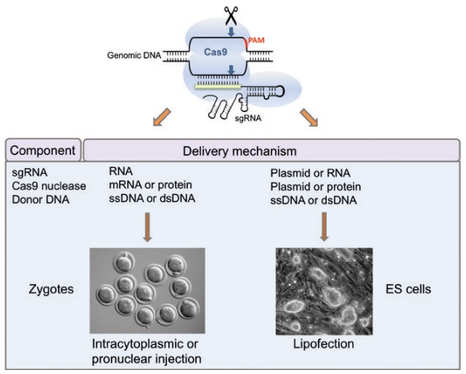

Figure Use of the CRISPR/Cas9 system for genome editing in the mouse. The figure illustrates the different strategies and delivery mechanisms used to target any given sequence of genomic DNA in the germ line of the mouse using zygotes or mouse embryonic stem cells (ES cells).

PUBLICATIONS

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- Viera A, Alsheimer M, Gómez R, Berenguer I, Ortega S, Symonds CE, Santamaría D, Benavente R, Suja JA (2015). CDK2 regulates nuclear envelope protein dynamics and telomere attachment in mouse meiotic prophase. *J Cell Sci* 128, 88-99.
- Búa S, Sotiropoulou P, Sgarlata C, Borlado, LR, Eguren M, Domínguez O, Ortega S, Malumbres M, Blanpain C, Méndez J (2015). Deregulated expression of Cdc6 in the skin facilitates papilloma formation and affects the hair growth cycle. *Cell Cycle* 14, 3897-3907.

MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

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

**Titulado Superior* (Advanced Degree)

"The Unit produces novel and high-quality mAbs that are used in basic research in order to gain new insights into the human cancer development process. We are also highly specialised in mAbs characterisation and provide CNIO researchers with reliable and well-validated reagents that add value to their research projects."

OVERVIEW

The development of hybridoma technology in 1975 has led to the generation of large panels of highly specific reagents that have had a tremendous impact in basic and applied research. The availability of monoclonal antibodies (mAbs) has enabled investigators to ask new questions and to develop new insights and applications that will benefit the diagnosis and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an *à la carte* generation of mAbs, which can then be used as tools to characterise new pathways involved in cancer development. We are highly specialised in the production of mouse and rat monoclonal antibodies. The Unit also offers mAb production in gene-inactivated mice, mAb characterisation and validation, medium-scale mAb production and a *Mycoplasma* testing service for the cell culture facility.

RESEARCH HIGHLIGHTS

During the last 15 years, the Monoclonal Antibodies Unit has generated a large number of mAbs (against more than 120 different antigens), mostly targeting molecules for which mAbs are not commercially available. Many of these mAbs have been licensed to external companies, in turn generating revenues from royalties that represent an important source of funding for the CNIO.

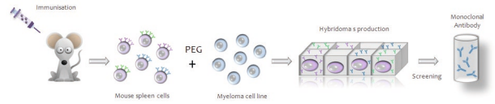

Figure Scheme of the procedure for the production of monoclonal antibodies.

Each year we prepare and update a detailed catalogue of CNIO mAbs that contains the datasheets of more than 70 thoroughly validated, high-quality mAbs (accessible at http://www.cnio.es/ing/servicios/anticuerpos/default.aspx).

In 2008, in collaboration with Oxford University, we founded EuroMAbNet (www.euromabnet.com), the first European non-profit organisation of academic laboratories with an internationally recognised reputation for generating and using validated mAbs. This international network is essential as an arena in which mAb laboratories can exchange knowledge, share cutting-edge methodology and create common strategies in order to standardise and improve the production of these important resources.

The number of mAbs generated as research tools by the research community has exponentially increased. Although this wealth of reagents has exciting scientific potential, a substantial number of mAbs fail even the most fundamental tests of activity or specificity. Furthermore, many research laboratories are unaware of this problem or lack the skills to test these reagents themselves. The lack of technical standards for mAbs often makes their selection a hopeless endeavour, wasting both time and valuable research funds. The use of poorly characterised reagents is of major concern to the scientific community, as perpetuation of serious scientific misconceptions inevitably compromises the advancement of science.

To help address mAb unreliability, EuroMAbNet has published a position paper (Roncador et al., 2015) and we have posted some easy to follow guidelines on our website (http://www.euromabnet.com/guidelines); these guidelines provide a set of criteria and recommendations that will help researchers to select the most effective mAbs from amongst those available in the market, as well as to provide the strategic guidance needed to perform antibody validation. ■

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Elena Andrés, Juan A. Cámara (TS)*, Cristina Penalba (since December) (PEJ-L)**, Silvia Sánchez, Gloria Visdominé

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

"The future of molecular imaging is indeed very promising. The continued advancements in the field are likely to lead to major breakthroughs in our understanding of *in vivo* biology, as well as result in improvements in the clinical detection of disease and patient management."

OVERVIEW

Molecular imaging is revolutionising the way we study the inner workings of the human body, diagnose diseases, approach drug design, and assess therapies, clinically as well as preclinically. The field as a whole is helping to enable the real-time visualisation of complex biochemical processes involved in normal physiology and disease states in living cells, tissues, and intact subjects. Generally speaking, 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 pathology regions, and provide insight regarding the mechanisms of disease. Molecular imaging shows enormous promise in the areas of diagnostics, therapy monitoring, drug discovery and development, and can contribute towards our understanding of nanoscale reactions such as protein-protein interactions and enzymatic conversion.

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, to develop and update protocols and techniques to optimise the visualisation of tumours in both the preclinical and clinical fields, as well as to assess and advise researchers on the best-suited imaging modality for their research projects.

This year, in collaboration with the Seve-Ballesteros Foundation Brain Tumour Group and the Proteomics Unit at the CNIO, we have identified Membrane-type 1 matrix metalloproteinase (MT1-MMP) as an attractive biomarker for Glioblastoma (GBM) imaging. This is due to the fact that this protein is actively involved in tumour growth and progression, correlates with tumour grade and is closely associated with poor prognosis in GBM patients. We reported the development of a new tracer (^{89}Z-LEM2/15) for the efficient detection of MT1-MMP in preclinical GBM models (FIGURE). 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, 3D tool for GBM diagnosis and follow-up.

The Molecular Imaging Unit also started a collaboration with the CNIO Gastrointestinal (GI) Cancer Clinical Research Unit in order to develop new tracers based on the Immuno-PET technology for pancreatic carcinoma.

In 2015, we continued our grant project with the Massachusetts Institute of Technology (MIT), titled 'Improved Molecular Imaging by Multi-tracer PET', which focuses on the use of dual isotopes to simultaneously assess different biological changes. We also provided imaging support in clinical trials conducted under CNIO's Clinical Research Programme.

Furthermore, we continued our active participation in the international consortium focused on imaging, *M+Visión*, led by the MIT. ■

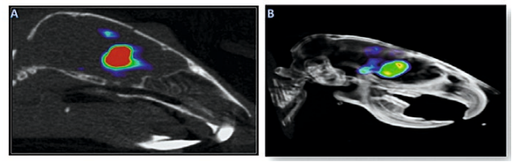

Figure PET-CT imaging with radiolabelled ^{89}Zr-LEM2/15 in mice bearing orthotopic xenografts containing patient-derived neurospheres. (**A**) Sagittal projection showing the specific tumour uptake. (**B**) 3D rendering of the same mouse.

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AWARDS AND RECOGNITION

- Scientific Advisory Board Chair and Faculty, the Madrid-MIT *M+Visión* Consortium, Spain.
- Project Evaluator of the *Fondo de Investigaciones Sanitarias* (FIS), *Agencia Nacional de Promoción Científica y Tecnológica, Ministerio de Ciencia, Tecnología e Innovación Productiva de Argentina*.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Ultan P. Cronin (TS)*, Elena Garrido (TS)*, Tania López (since December) (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 allows multiparametric analyses for the identification, quantification and isolation of defined subpopulations of cells, based on the expression levels of fluorescent markers and their relation to each other.

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

We count with 4 analysers and 2 high-speed cell sorters with different configurations of lasers and detectors, which enable us to cater for 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 the appropriate training, whereas cell sorters are operated by the Unit's staff. Our sorters can separate up to 4 defined populations at a time, as well as perform single cell cloning. We can accept human samples to sort according to Biosafety regulations.

"Initiating cancer cells are too elusive to isolate for further characterisation of their expression patterns due to their low numbers. In collaboration with other European groups, we have developed a method to assess maximum recovery (*Rmax*) in cell sorting."

RESEARCH HIGHLIGHTS

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

- → Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- → Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, 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.

We have developed several new multicolour panels for the detection of different cellular subtypes (progenitors, T, B and inflammatory cells) from different sample types, such as haematopoietic tissues, pancreas, skin, liver, lung, etc., and have combined these panels with the detection of proliferation and cell death.

In collaboration with the CNIO Tumour Suppression Group, we used some of these multicolour panels for the detection of erythroid progenitors, and combined them with cell death and cell proliferation assays in order to characterise a Diamond-Blackfan anaemia mouse model based on the heterozygous deficiency of the ribosomal gene *Rpl11*.

Furthermore, the Unit participated in the development of a method to assess the instrument performance of cell sorters, based on the centre stream catch, and calculation of the maximum recovery for a particular sorting condition; Rmax. This method will be very useful for assessing instrument performance during the sorting of rare populations.

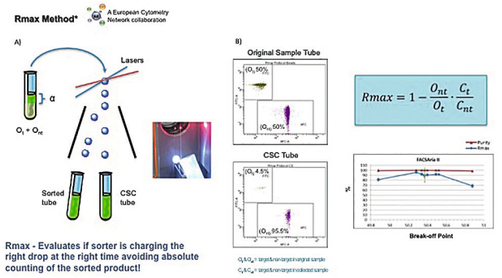

Figure (**A**) Rmax is based on the collection and the inspection of the central stream catch (CSC) tube so that no precious sample is wasted. (**B**) Representative plots of the "ideal bead sample" and the inspection of the CSC to calculate Rmax according to the formula shown, where the purity is assumed to be close to 100% (which is the usual). The right bottom panel is a representative plot of the purity and Rmax values, while screening the break-off point (BOP) of the sorter. As shown, the purity is not much affected by being slightly off the BOP but the recovery is indeed affected.

The Unit also continues its commitment to developing comprehensive flow cytometry training for different applications at the CNIO. This year we delivered a basic flow cytometry course, as well as a specific course focused on multicolour immunophenotyping and another specific course geared towards cell proliferation and cell death modules. We also launched a 2-day hands-on cell sorting course that was especially designed for operators. All courses were well attended and received excellent feedback. ■

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Book chapter

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AWARDS AND RECOGNITION

- Elected Member of the Directive Board "Sociedad Ibérica de Citometría" (SIC), Spain and Portugal.

CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Technicians
Jesús Gómez (since December) (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 the CNIO Research Groups with all the standard methodologies as well as the latest advances in microscopy. The Unit offers access to state-of-the-art equipment and software packages related to confocal microscopy, as well as 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 transferring advanced microscopy methodologies in cancer research to society, always with the aim of increasing our understanding of the cell biology and the disorders of cells that cause cancer."

RESEARCH HIGHLIGHTS

The Confocal Microscopy Unit is equipped with 3 laser scanning confocal systems (Leica SP2 and SP5) that incorporate UV and multiphoton excitation, as well as a white light laser and a Hybrid Detector, and 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 multiwell 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 multiwell plates but also in tissue sections.

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

During 2015, the Confocal Microscopy Unit contributed to the microscopy field in several aspects: it published a new tool, named iMSRC, that implements the latest advances in intelligent screening; in its portfolio of techniques, it included new microfluidics devices for live-cell assays in perfusion chambers; and it also continued to establish scientific collaborations with CNIO researchers, covering several aspects of cancer studies such as multiphoton intravital imaging in mice. Moreover, the Confocal Microscopy Unit is dedicating a significant effort towards the development and implementation of High-Content Screening technology at the CNIO; for example, during this last year, we helped to run screenings aimed at testing compounds that could modify mitotic checkpoints, integrity of nucleoli, DNA Damage, BrdU, cell proliferation, etc. ■

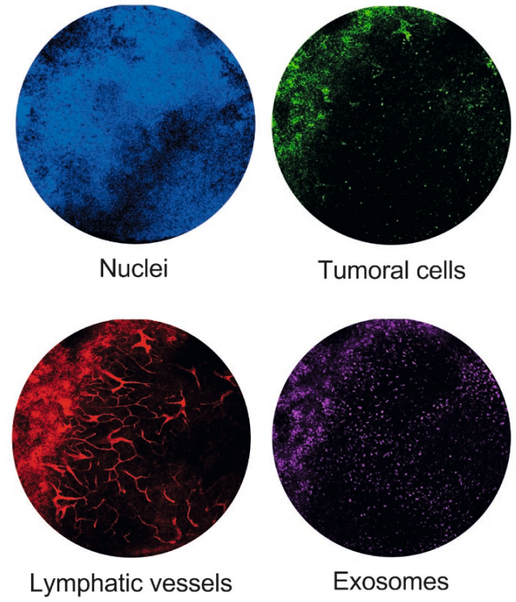

Figure Intravital imaging of the mouse popliteal lymph node labelled for nuclei (blue), tumour cells (green), lymphatic vessels (red) and tumour-secreted exosomes (magenta).

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- Carro A, Perez-Martinez M, Soriano J, Pisano DG, Megias D (2015). iMSRC: converting a standard automated microscope into an intelligent screening platform. *Sci Rep* 5, 10502.

PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Staff Scientist
Jorge L. Martínez

OVERVIEW

The proteome is a system in which highly interconnected proteins are dynamically modified, through physical interactions and/or post-translational modifications, in order to carry out specific cellular functions. Recent developments in sample preparation, liquid chromatography, mass spectrometry (MS) and data analysis have enabled researchers to investigate diverse facets of proteomics 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 complex cellular processes at the protein level. This vast amount of data is providing new insights into the molecular mechanisms underlying diverse human pathologies such as cancer.

"Our main goal is to implement and provide MS-based proteomic technologies to research groups in order to better understand the molecular causes of cancer. The Unit also provides support for recombinant protein expression, offering access to state-of-the-art technologies in the field."

Graduate Students
Silvia L. Gomes, Ana Martínez (since March)

Technicians
Fernando García (TS)*, Nuria Ibarz (TS)*, Jaime Martínez (TS)*, Álvaro Otero (since December) (PEJ-L)**, M. Isabel Ruppen (TS)*, Pilar Ximénez de Embún (TS)*, Eduardo Zarzuela (since December) (PEJ-L)**

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

RESEARCH HIGHLIGHTS

Throughout 2015, the Unit has implemented and optimised several aspects of the quantitative proteomics workflow. We have introduced an orthogonal separation technique (high-pH reversed-phase chromatography) to pre-fractionate samples in order to increase proteome coverage (FIGURE). Likewise, precursor isolation efficiency was optimised in the 2 MS platforms to improve accuracy and precision. Finally, we introduced a statistical model for data obtained from relative protein expression, which allows us to identify truly significant changes with higher sensitivity. All these approaches are being implemented in different projects that are currently ongoing at the Unit, such as the profiling of exosomes (in collaboration with the CNIO Microenvironment and Metastasis Group) and secretomes (in collaboration with the CNIO Melanoma Group) as molecular markers of metastasis. We are also investigating the role of transcriptional regulators in the development of pancreatic diseases (in collaboration with the CNIO Epithelial Carcinogenesis Group). Together with the CNIO Genomic Instability Group, we are using proteomic expression profiling to understand haploidy. Also, in close collaboration with the CNIO Tumour Suppression Group, we are conducting several proteomic analyses to study the foundations of ground state pluripotency.

Pertaining to the other section activities, the Unit has mainly focused on the production of cancer-related proteins for structural studies using X-ray crystallography and Small-angle X-ray scattering, in collaboration with the CNIO Crystallography Core Unit and the CNIO Experimental Therapeutics Programme. The obtained data results will be used to rationally guide the development of small molecule inhibitors and novel bioactive compounds as potential therapeutic agents. Finally, the Unit has continued collaborating with the CIEMAT and the CNIO Molecular Imaging Unit, in setting-up a platform to radiolabel engineered antibodies against either validated or novel tumour targets for positron emission tomography imaging (immunoPET). The aim is to develop noninvasive methods to obtain information about the *in vivo* status of the specific target for diagnostic and prognostic purposes. ■

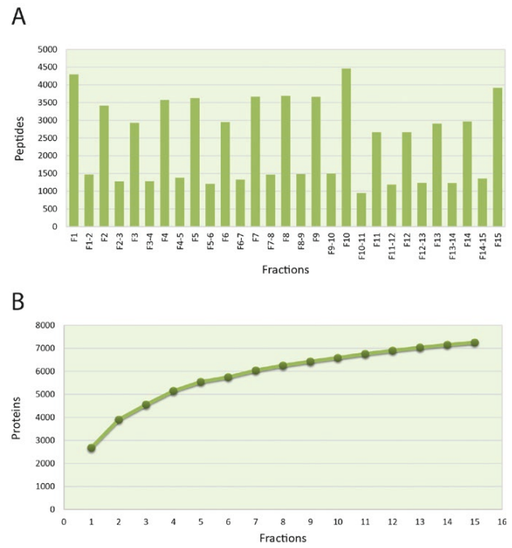

Figure Pre-fractionation of a complex proteome using high-pH RP chromatography. (**A**) Unique peptides identified in each fraction and peptides identified in 2 consecutive fractions. This low overlap indicates the high resolution of this technique. (**B**) More than 7,000 proteins are identified in 15 fractions.

PUBLICATIONS

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- Lovera S, Morando M, Pucheta-Martinez E, Martinez-Torrecuadrada JL, Saladino G, Gervasio FL (2015). Towards a Molecular Understanding of the Link Between Imatinib Resistance and Kinase Conformational Dynamics. *PLoS Comput Biol*. PMID: 26606374.
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HISTOPATHOLOGY CORE UNIT

Alba De Martino
Core Unit Head

Technicians
María Gómez, Patricia González, Gabino Hernández (since May), María Lozano, Verónica Neva (since December), Maria Udriste, Zaira Vega, Nuria Cabrera

"The Histopathology Core Unit participates in several External Quality Assessment Schemes, such as NordiQC and UKNEQAS, which independently evaluate the quality of the stains performed at the Unit. In 2015, 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, *in situ* hybridisation (ISH) (microRNAs and ALU sequences), and TUNEL in FFPE tissues. Furthermore, the Unit offers laser-capture microdissection, slide digitalisation, image analysis and quantification services. 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

Throughout 2015, the Unit incorporated new equipment such as the Axio Scan.Z1 Slide Scanner for brightfield, fluorescence and polarised light; and the Ventana Discovery Ultra IHC research instrument, as the solution for non-conventional IHC and ISH research protocols including multi-labelling and immunofluorescence. These instruments, along with the new Definiens Tissue Studio software, will enable us to obtain comprehensive and consistent data from tissue-based assays for biomarker and morphological assessment in research and drug discovery.

All the developed techniques follow a standardised validation process. In 2015, the Unit has added several new antibodies to its portfolio, which includes more than 1,000 currently available antibodies that have been optimised for both human and mouse tissue samples. The antibody validation process follows rigorous testing 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.

Aware 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 stains performed at the Unit and in which more than 800 laboratories participate worldwide. In 2015, our Unit scored very high in evaluated techniques, and several protocols developed by the Unit were incorporated into the 'Best Methods section' of the UKNEQAS Cellular Pathology Technique website. ■

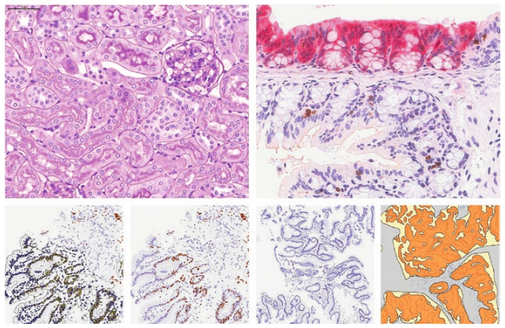

Figure Techniques developed at the Histopathology Core Unit and routinely used by CNIO researchers. PAS staining, Kidney. Selected as *Best Method* by the UKNEQAS quality assessment service (top left). GFP (red) and CD3 (brown) double labelling immunohistochemistry in mouse intestine (top right). Ki67 immunohistochemistry and quantification of positive and negative nuclei; haematoxylin stained human oesophagus and tissue area quantification (orange) both assessed by Definiens Tissue Studio software (bottom panels).

PUBLICATION

González-Loyola A, Fernández-Miranda G, Trakala M, Partida D, Samejima K, Ogawa H, Cañamero M, de Martino A, Martínez-Ramírez Á, de Cárcer G, Pérez de Castro I, Earnshaw WC, Malumbres M (2015). Aurora B overexpression causes aneuploidy and p21Cip1 repression during tumor development. *Mol Cell Biol* 35, 3566-3578.

ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services

"Mouse models are essential in cancer research, and as such are used extensively by most CNIO Research Groups. As another indicator of excellence, at the CNIO, we put all our efforts into ensuring a rational use of animal models, in compliance with all the legal and ethical requirements."

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 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 Research Groups, Sections and Units from all our Research Programmes, except the Structural Biology and Biocomputing Programme.

Our Animal Facility has the capacity to house 19,000 type IIL cages (each with an average capacity for 3.5 mice). 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. Bedding, water, and cages are sterilised by autoclaving, and the feed is irradiated. 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 housing xenograft models. To maintain clean air in the premises, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are 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 generate the highest productivity possible 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 currently harbours more than 1,500 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying more than 300 gene targeted alleles and close to 200 transgenic integrations. More than 100 gene targeted alleles and 50 transgenic mouse strains of cancer-related genes have been generated by the Research Groups at the CNIO, and approximately 200 genetically modified lines have been imported from other research centres. The Facility also provides access to more than 70 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, 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. 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. ■

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

The main focus of the Programme is the development of *Early-Drug Discovery* projects. Over time, we have progressed to reach *in vivo proof-of-concept* (PoC) and /or have entered into licensing agreements with our advanced lead compounds. As already known, the ataxia telangiectasia and *Rad3*-related protein (ATR) inhibitors discovered at the CNIO have been licensed to Merck Serono for further clinical development, where one of the ETP-CNIO inhibitors continues its progression in the company pipeline towards its characterisation as a potential candidate for clinical trials.

We have also made progress with our CDK8 project. Our Medicinal Chemistry Group has successfully generated and optimised novel CDK8 inhibitors, yielding highly potent, selective and orally bioavailable compounds with intellectual property rights. The use of these compounds has allowed us to identify cell lines that are sensitive to CDK8 inhibition. Selected cell lines will be used to establish *in vivo* models for PoC studies.

The telomeric repeat binding factor 1 (TRF1) inhibition project, carried out in collaboration with the Telomeres and Telomerase Group (Maria Blasco), has advanced during 2015. A first chemical series of TRF1 inhibitors identified during a preliminary screening campaign, in particular compound ETP-037, has been used by CNIO's researchers to demonstrate that TRF1 inhibition leads to significant antitumour effects in a mouse model of one of the most aggressive types of lung cancer (KrasG12V p53-/- NSCLC). The deconvolution studies using series 1 compounds and related inhibitors have allowed us to identify PI3K inhibition as a novel mechanism for TRF1 modulation. Interestingly, we have identified a second and unrelated chemical series of TRF1 inhibitors; this series is currently being explored with the aim of increasing the TRF1 inhibition potency, as well as to design and prepare chemical affinity probes for the investigation of the molecular target responsible for their TRF1 inhibition profile, including TRF1 itself. Other screening activities and combinations of TRF1 modulating agents are planned for the next stage.

We have continued the development of *Kinase X** inhibitors. The ETP has generated highly potent compounds that have demonstrated good general selectivity. Only 3 main off-targets, among more than 480, have been identified and characterised at the cellular level. Some representative inhibitors have displayed excellent Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties, as well as oral bioavailability. These candidates will be used in target validation studies, including *in vivo* PoC.

* Kinase x (confidential), B. Lambrecht, VIB Inflammation Research Centre, University of Gent (Belgium).

"The Experimental Therapeutics Programme (ETP) is a well-established early-Drug Discovery Group (e-DD) at the CNIO. The integration of e-DD activities, alongside the Centre's excellence in basic research, is contributing towards CNIO's transformation into a more Comprehensive Cancer Research Centre that endeavours to help bring new therapies to the patients."

In 2015, ETP participated in several *Exploratory Projects*. The collaboration with Manuel Serrano's Group (CNIO Tumour Suppression Group) in the field of Gluconeogenesis and Cancer Stem Cell (CSC) therapeutics deserves special attention. Under this framework, we have identified interesting hit compounds for both indications. In particular, Cpd-1 and Cpd-2 have demonstrated their ability to selectively kill CSCs at submicromolar concentrations. Importantly, these hits inhibit the tumour-initiating capacity of pancreatic CSCs after the inoculation of pretreated cells in mice, in comparison with control untreated cells. Currently, we are investigating the target profile of these hits in order to identify the mechanism of action behind this effect. We expect to decipher the mechanism of action of the identified hits for both projects during the next stage of the project. The identification of the molecular targets involved will pave the way for the future development of targeted therapeutic agents. Other *Exploratory Projects* include collaborations with CNIO researchers Marcos Malumbres (Mastl and Haspin kinases) and Daniel Lietha (FAK inhibitors).

Finally, we have made progress in our second collaborative project with VIB (*target X***). The ETP-Biology Group has completed the assay development activities and has carried out a screening campaign with more than 5K compounds. Several hits have been identified and they are currently under characterisation.

** Target x (confidential), P. Carmeliet, VIB Department of Transgene Technology and Gene Therapy, KU Leuven (Belgium).

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OVERVIEW

The process of generating advanced compounds that demonstrate their *in vivo* proof-of-concept in different early drug discovery projects begins with the discovery or generation of 'hits'; initial molecules that exhibit a desirable effect at the biochemical or cellular assay level on a particular oncological target. In the following phases – Hit-to-Lead and Lead Optimisation – each hit series will be modified systematically and in a multifactorial manner in order to establish, on the one hand, Structure-Activity-Relationships (SAR), the key modifications to improve potency and selectivity; and on the other hand, to confer drug-like properties to the molecules that will improve how molecules behave in the organism. Structural information about the target, obtained through structure-based drug design techniques using molecular modelling and methodologies such as X-ray crystallography, can sometimes be applied to develop the SAR in a faster and more focused manner. At the end of the process, we will obtain more potent and selective compounds with acceptable pharmacokinetic (PK) properties that can be tested in several *in vivo* models.

"We were involved in the generation of a proprietary chemical series of CDK8 inhibitors with *in vivo* properties that can be used in mechanistic studies (PoC), as well as in the identification and exploration of tool compounds in several target-validation oncology projects, e.g. TRF1 inhibition."

RESEARCH HIGHLIGHTS

During 2015, our Section was involved in several projects:

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

During this year, we focused our chemical efforts on the multifactorial optimisation of compounds in our main chemical series. We have synthesised more than 50 compounds and have obtained very potent compounds with a low nanomolar biochemical activity range and cellular β-catenin modulation at low micromolar concentrations. This chemical series consists of CDK8 and CDK19 inhibitors that have been profiled in terms of other cyclin kinases showing high selectivity versus CDK1, CDK2, CDK4, CDK5, CDK7 and CDK9. During the chemical exploration, we identified ETP-27 as an advanced compound (CDK8 IC_{50}=0.5 nM; β-catenin IC_{50}= 310 nM), which was profiled against a panel of 468 kinases (KinomeScan prolife/DiscoveRx) showing Selectivity Scores of S(35)=0.069 and S(10)=0.017. The Selectivity Score or S-score is a quantitative measure of compound selectivity and it is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested, excluding mutants. This compound showed acceptable Clearance (< 30 % hepatic blood flow) and good oral levels in pharmacokinetic studies. Additional *in vitro* and *in vivo* characterisation of ETP-27 is planned.

Kinase X* inhibitors

The project Kinase x inhibitors is undertaken in collaboration with VIB (Belgium). This project focuses on the generation of high-quality chemical probes that can be used in further target validation studies. We have focused our chemical exploration on the optimisation of 2 main chemical series of Kinase x inhibitors that belong to a novel chemical space. We have identified very potent compounds with binding and kinase biochemical activities in the low nanomolar range. During the chemical exploration, our main goal was to improve the selectivity of these series, which presented different off-target activities; the selectivity profile of the compounds was followed using the KINOMEscan screening platform. So far, we have been able to significantly improve the selectivity by exploration of the gatekeeper area with several chemical fragments. The *in vivo* pharmacokinetic profile of some representative compounds from one of the chemical series showed good oral levels and acceptable clearance. This aspect is very important for the use of these compounds, not only *in vitro* but also for *in vivo* target validation studies. The second chemical series requires additional fine optimisation in order to improve certain metabolic stability issues associated with a particular fragment of the molecule. During the first 10 months of the year, 310 compounds were synthesised.

Kinase Y* inhibitors

The project Kinase Y inhibitors is another collaborative undertaking with VIB. Complementary to the High-Throughput Screening (HTS) campaign carried out in the Biology Section of the Programme, we have also been involved in the synthesis of several reference compounds, as well as in the generation of potential hits by applying rational design strategies. The biological activity of the synthesised compound will be followed using biochemical and cellular assays.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This is a collaborative project with the CNIO Telomeres and Telomerase Group, in which several hits have been identified in a screening assay that measures the removal of TRF1 from telomeres. During this period, we have assisted in the validation of the hits, providing a more advanced compound, ETP-037, with an optimised profile for *in vivo* use. The compound effectively impaired the progression of already formed lung carcinomas and decreased the association of TRF1 to telomeric DNA in mice. We have conducted deconvolution studies, using the identified hits to determine the molecular target behind this effect. The results point to PI3K inhibition as the mechanism of action for TRF1 modulation. Additionally, we are exploring a second chemical series and 30 compounds have been synthesised to date. We have identified compounds that displayed a significant TRF1 downregulation in *Kras*^{G12V}*p53*$^{-/-}$ NSCLC cells when treated at concentrations of 10 micromolar for 48h. Affinity probes will be designed and prepared to determine the molecular mechanism of action of this class of TRF1 inhibitors.

Collaboration in Exploratory Projects

The Experimental Therapeutics Programme is collaborating with several CNIO researchers in the initial phases of drug discovery, for example, with the Groups of Manuel Serrano in the fields of Gluconeogenesis and Cancer Stem Cells, Daniel Lietha in the synthesis of allosteric FAK inhibitors, and Marcos Malumbres in the Mastl and Haspin kinase field. ■

* confidential.

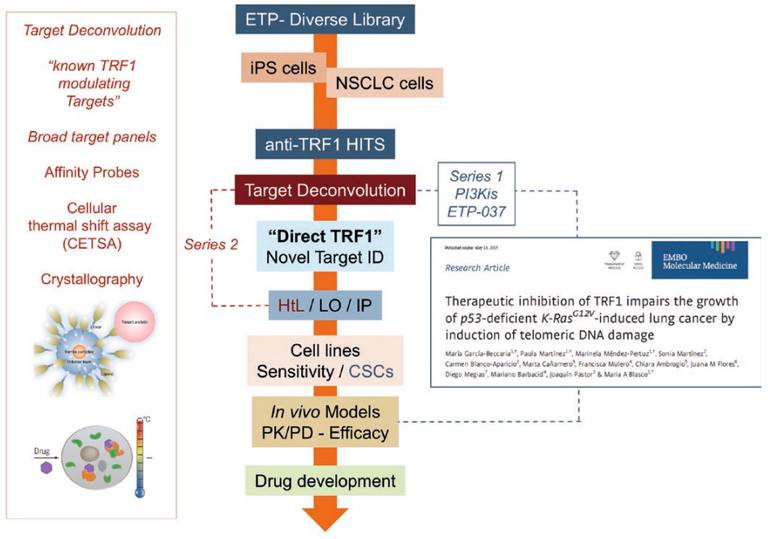

Figure 1 TRF1 Platform. Hits from 2 different chemical series were identified in a preliminary screening campaign (iPS cells, NSCLC cells). Among them, hit ETP-037 (Series 1), with *in vivo* properties, showed significant antitumour efficacy in a lung cancer mouse model (KrasG12V p53-/- NSCLC). Deconvolution studies with series 1 led to the identification of PI3K inhibition as a novel mechanism for TRF1 modulation. Series 2 is currently being explored in order to increase TRF1 inhibition and to design/prepare chemical affinity probes for the deconvolution of the molecular target responsible for their TRF1 inhibition profile, including TRF1 itself.

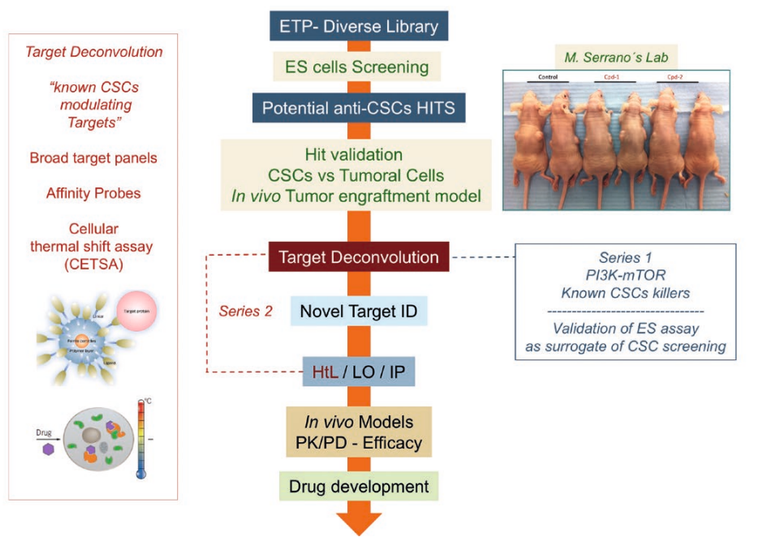

Figure 2 Cancer Stem Cell (CSC) Platform. A preliminary screening campaign carried out in ES cells, led to the identification of several hits with different profiles. Known CSC killers were identified in chemical Series 1; in this case PI3K-mTOR inhibitors. Hit compounds cpds-1 and -2, demonstrated the ability to selectively kill CSCs at sub-micromolar concentrations; these represent chemical Series 2. These hits inhibited the tumour initiating capacity of pancreatic CSCs after inoculation of pretreated cells in mice, in comparison with control untreated cells. Deconvolution studies to identify their target profile are currently being implemented in order to identify the mechanism of action behind this effect.

PUBLICATIONS

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- García-Beccaria M, Martínez P, Méndez-Pertuz M, Martínez S, Blanco-Aparicio C, Cañamero M, Mulero F, Ambrogio C, Flores JM, Megias D, Barbacid M, Pastor J, Blasco MA (2015). Therapeutic inhibition of TRF1 impairs the growth of p53-deficient K-RasG12V-induced lung cancer by induction of telomeric DNA damage. *EMBO Mol Med 7,* 930- 949.
- Alonso-Curbelo D, Osterloh L, Cañón E, Calvo TG, Martínez-Herranz R, Karras P, Martínez S, Riveiro-Falkenbach E, Romero PO, Rodríguez-Peralto JL, Pastor J, Soengas MS (2015). RAB7 counteracts PI3K-driven macropinocytosis activated at early stages of melanoma development. *Oncotarget 6,* 11848-11862.

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**Titulado Superior* (Advanced Degree)

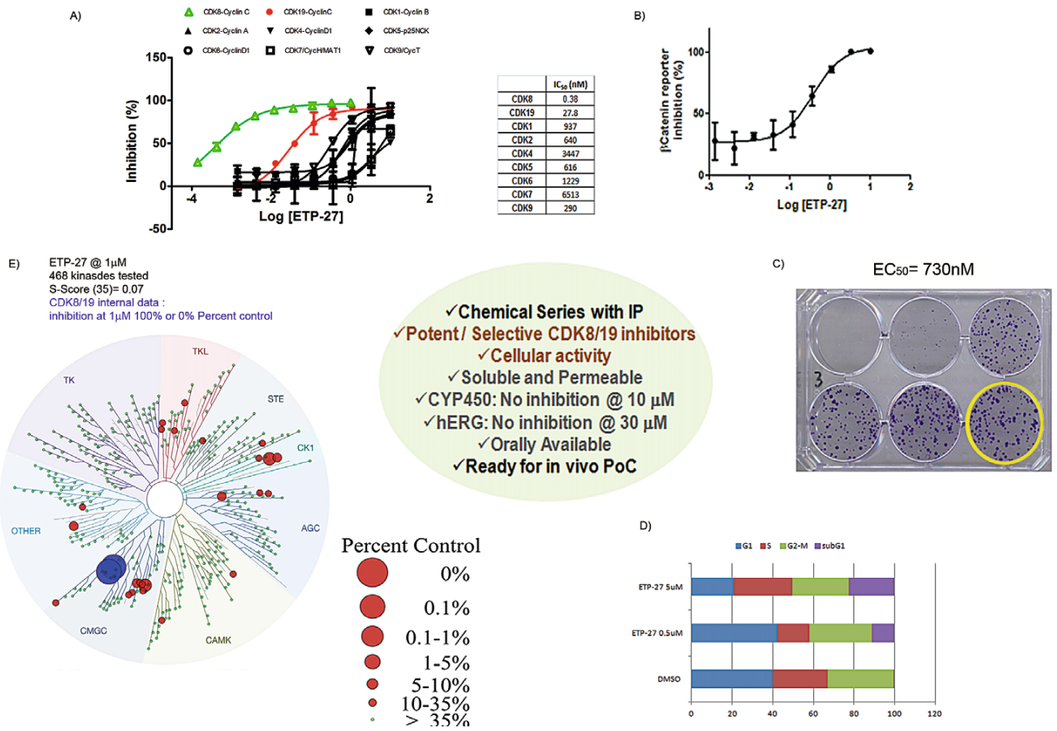
OVERVIEW

The Biology Section is devoted to the biochemical, cellular, and *in vitro/in vivo* pharmacological characterisation of the compounds synthesised within the Experimental Therapeutics Programme (ETP), with the aim of obtaining novel anticancer agents with optimised profiles that demonstrate *in vivo* proof-of-concept in animal models of disease.

The molecular pharmacological characterisation of novel agents is very important for establishing the tumour types and/or molecular backgrounds in which these new therapies could show efficacy as a single agent or in combination settings. Our experimental approach is based on the evaluation of the inhibition of proliferation in a minimum panel of 40 tumour cells from different origins. Subsequently, taking into account the growth inhibition data (GI_{50}), we evaluate 3 different doses of the new molecule in combination with a library of 100 approved, or still in clinical trials, therapeutic agents, covering both chemotherapy and targeted therapies. The final aim is to identify sensitive tumours and potential therapeutic combinations.

"We purified human MASTL full length protein, which allowed us to set up a novel biochemical assay in order to run a screening, and also carried out the biochemical, cellular and pharmacological characterisation of proprietary chemical compounds such as CDK8 inhibitors."

RESEARCH HIGHLIGHTS



	IC_{50} (nM)
CDK8	0.38
CDK19	27.8
CDK1	937
CDK2	640
CDK4	3447
CDK5	616
CDK6	1229
CDK7	6513
CDK9	290

Figure *In vitro* characterisation of ETP-27. (**A**) *In vitro* kinase activity profile. Dose-dependent inhibition of CDK8-CCNC in a panel of CDKs described in the graph. (**B**) Cellular inhibition of CDK8 activity measured by β-catenin reporter assay in the HCT116 colon cell line. (**C**) Dose-dependent inhibition of colony formation in HCT116 cells (the well with the circle corresponds to DMSO-treated cells). (**D**) Cell cycle analysis of HCT116 cells treated with 0.5 and 5 μM for 48h. (**E**) TREE*spot*™ representation of KINOME*scan*® results for ETP-27 tested at 1μM. CDKs data are not included in the representation as they are tested without cyclins, instead, internal data is represented.

During 2015, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

The CDK8 and its paralog CDK19 kinases are components of a multi-protein Mediator complex involved in transcription control. Several studies have indicated that high overexpression and activity of CDK8 could be drivers of malignant progression in colorectal cancer.

We seek to obtain selective CDK8 inhibitors against other CDKs, either transcriptional or cell cycle regulator CDKs. For this purpose, we have tested 54 newly prepared compounds in a binding assay for CDK8; 28 of them were also tested in a panel of CDKs and subsequently in a functional cellular assay, and were profiled for their Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties. Thus, we have identified selective CDK8/CDK19 inhibitors against other CDKs with controlled ADMET properties. We have also performed mouse pharmacokinetic studies on 5 compounds; in general the molecules have a controlled clearance and are orally bioavailable. Among these, we have deeply characterised ETP-27, a very potent CDK8/CDK19 inhibitor with good cellular inhibition of the β-catenin reporter and a strong inhibition of colony formation in the HCT116 colon cell line; the inhibitor induces cell death in a dose-dependent manner (FIGURE 1). Our next steps will focus on the testing of this compound *in vivo*.

Kinase X

This project is undertaken in collaboration with VIB (Belgium). Newly synthesised compounds from 2 different chemical series have been characterised at the biochemical, cellular and ADMET level. We have tested 410 compounds in the primary biochemical assay; 64 of them were screened in selectivity panels looking for off-target activities and profiled in ADMET assays. The target has 3 different isoforms and the 22 more promising compounds were evaluated in all the isoforms; our compounds behave as pan–isoform inhibitors. The determination of the solubility and permeability of the compounds has enabled a better interpretation of our cellular data. Pharmacokinetic studies of the 6 more promising compounds were performed, showing that the compounds reached sufficient levels to be tested in mouse tumour models.

Kinase Y

In order to evaluate the feasibility of initiating an early-drug discovery process for this undisclosed target, we performed a biochemical screening of our ETP-5K library in collaboration with VIB. We set up and validated the High-Throughput Screening (HTS) assay. In a single point screening assay with cut-off values set at 50% inhibition, we achieved a hit rate of 0.77. Hits were confirmed in a dose-response assay and we obtained inhibitors in the high nanomolar or low micromolar activity range; we will evaluate a number of analogues of the identified hits in order to obtain better starting points for the Hit to Lead (HtL) exploration.

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We have purified active human MASTL full protein from insect cells. We have set up a biochemical assay with the obtained protein for the validation of hits previously identified in a phenotypic cell-based screening. Some of the hits are inhibitors of hMASTL at the micromolar level. We plan to run a biochemical screening to identify more potent hMASTL inhibitors as starting points for the HTL phase.

Telomeric repeat binding factor 1 (TRF1)

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres, developed by Maria Blasco's Group, has been transferred to ETP. During a previous screening, we identified PI3K inhibitors as modulators of TRF1 binding to telomeres that might have therapeutic implications. We have contributed towards the validation of this initial finding by testing other known structurally unrelated PI3K inhibitors, and correlating TRF1 dissociation with the inhibition of AKT phosphorylation. We have also tested around 110 analogues from a second hit that were identified in the initial screening; some of these have shown an improved activity as TRF1 inhibitors.

Systematic identification of new therapies for the Avatar mouse models

In collaboration with the CNIO Gastrointestinal Cancer (GIC) Clinical Research Unit, we have started a project to accelerate the identification of potential new therapies that could be translated from Avatar mouse models to patients. For this purpose, we treat freshly disaggregated cells from already established Avatar mouse models with a single point library of 80 approved or in clinical trials drugs. We have determined the growth inhibition data (GI_{50}) for the more promising drugs and the results have been transferred to the GIC Group. The active drugs will be tested in the Avatar mouse model.

Cancer Stem Cells (CSCs) and Gluconeogenesis

The CNIO Tumour Suppression Group, led by Manuel Serrano, has developed 2 assays to search for specific killers of CSCs and for gluconeogenesis inhibitors. ETP-Biology has provided support in running the experiments and to interpret the screening results obtained with the diverse ETP-library 640. The identified hits are under further characterisation.

Support to other CNIO Groups

We have given support to the CNIO Breast Cancer Clinical Research Group by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), the levels of several standards of care drugs in tumour and host-mouse plasma samples from different mouse models of cancer. ■

PUBLICATION

García-Beccaria M, Martínez P, Méndez-Pertuz M, Martínez S, Blanco-Aparicio C, Cañamero M, Mulero F, Ambrogio C, Flores JM, Megias D, Barbacid M, Pastor J, Blasco MA (2015). Therapeutic inhibition of TRF1 impairs the growth of p53-deficient K-RasG12V-induced lung cancer by induction of telomeric DNA damage. *EMBO Mol Med* 7,930-949.

CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

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**Titulado Superior* (Advanced Degree)

"We are using a combination of *in vitro* and *in vivo* approaches to obtain a complete understanding of the metabolic status of tumours."

SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer metabolism. 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, or by acting synergistically with other anti-tumour agents. A combination of *in vitro* and *in vivo* approaches are being used 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. 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 and mouse models developed at the CNIO; the process includes the use of non-invasive *in vivo* imaging technologies, and immunohistochemical characterisation of tumours based on different metabolic and tumour markers (FIGURE).

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 use glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels; a phenomenon known as the 'Warburg effect' (Warburg 1956). Warburg argued that this altered metabolic state was the underlying cause for cancer.

The molecular mechanisms that drive an altered tumour metabolism have only recently begun to be understood as a result of large-scale genomic sequencing and advances in metabolomic profiling technologies. Recent studies have shown that many oncogenes, including Myc and Ras, impart an altered metabolic phenotype in cancer cells by regulating 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 (FIGURE, A); 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 (FIGURE, B). This situation may leave tumour cells in a frail position when exposed to certain treatments or under certain circumstances, while normal cells may be able to compensate. Furthermore, the high requirements of nutrients and other soluble factors, together with the hypoxic conditions found in tumours, creates a 'non-friendly' microenvironment for the anti-tumour immune surveillance, while facilitating the growth of other tumour-promoting cells such as stroma and myeloid cells (FIGURE C). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes in this process. ■

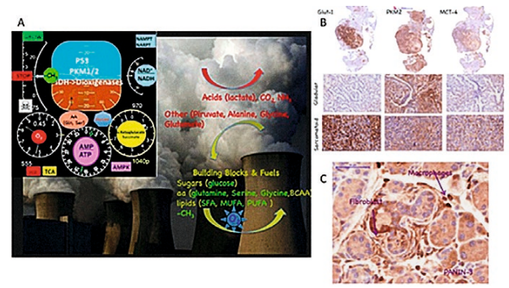

Figure (**A**) Tumours are cellular factories that need an abundant supply of nutrients and fuels, and require clean up systems and a central control station to survive and interact with their surroundings. (**B**) Immunohistochemical analysis showing the expression of glycolytic markers in a pancreatic adenocarcinoma *(PDAC)* derived from a Elas-tTA/tetO-Cre; K-Ras(+/LSLG12Vgeo);p53(lox/lox): *PDAC* Mouse model (Carmen Guerra and Mariano Barbacid, from the CNIO Experimental Oncology Group), with 2 well-defined differentiated cell types (sarcomatoid and glandular), each type using a different metabolic programme. (**C**) Immunohistochemical analysis for PKM2, showing the tumour microenvironment in a PANIN-3 from the same *PDAC* model. In addition to the tumour cells, there are fibroblasts and myeloid cells that may be facilitating tumour growth.

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 are being validated *in vitro* and *in vivo* using animal models developed at the CNIO (FIGURE). 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 do not only occur in the DNA sequence, but also occur 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 last 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. Thus, contrary to genetic mutations, epigenetic aberrations can be reversed through the targeting of 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 therapeutics. ■

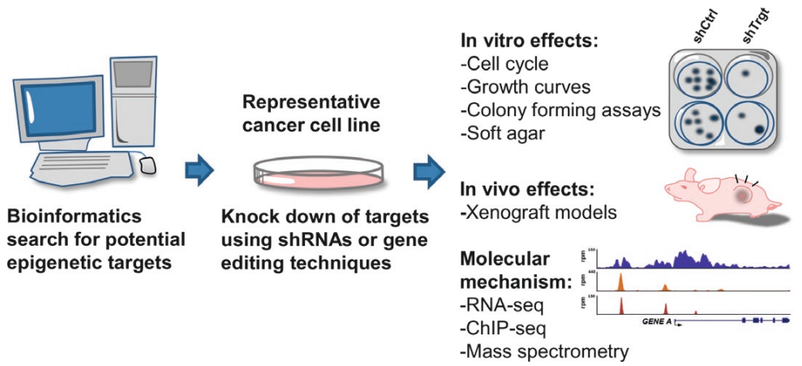

Figure *In vivo* and *in vitro* strategies for target validation.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

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

Technololy Transfer Manager M. Cruz Marín

The CNIO continues to consolidate its leading position among cancer research centres with the production of scientific knowledge through the publication of papers in peer-reviewed journals. The Technology Transfer and Valorisation Office is dedicated to creating world-class impact through world class-research.

The Technology Transfer Team supports the activities of the CNIO Innovation Department to help bridge the gap between research and innovation. In order to channel knowledge towards applications, the Office advocates and boosts collaborative research, contract research, consultancy, spin off companies, licensing, and patenting.

The CNIO implements a rational intellectual property protection strategy based on technical and valorisation dossiers that address major issues regarding the patentability of the results and their commercial viability. The patent portfolio is composed of 23 live patent families that cover numerous territories. In 2015, new patent applications have been filed for 4 new inventions derived from the work of CNIO scientists and collaborators. The CNIO portfolio of patents is particularly strong in small molecules.

In 2015, the CNIO entered into a number of important partnerships. To name just a few, a new collaboration with Pfizer was established whereby 4 research groups at the CNIO will coordinate their efforts to better understand the biological processes underlying major tumours, such as those in breast, lung or pancreatic cancer, with the aim to design new diagnostic and therapeutic interventions. Other important collaborations include those with Boehringer Ingelheim, Eli Lilly, Daiichi Sankyo, Roche and Merck Serono. Additionally, a number of agreements were also signed with smaller biotech companies. Through these collaborations, CNIO scientists will explore new therapeutic indications of commercial or experimental drugs, and take part in the discovery and validation of novel anti-cancer targets, the use of 'avatar' mouse models in personalised medicine, as well as the generation of ground-breaking knowledge and technologies with the potential to revolutionise disease therapy. In 2015, 20 such contracts were negotiated, thereby securing future revenues for collaborative research that amount to 4.5 million euros; this amount represents more than 10% of the total annual budget of the CNIO.

In addition, 144 non-disclosure, material transfer and other IPR agreements were signed with both public and private entities. Of these agreements, 75% (108 out of 144) were with foreign organisations; this number provides a good indication of the internationalisation of the CNIO's scientific activity.

"The Technology Transfer and Valorisation Office is dedicated to creating world-class impact through world class-research."

We continue to monitor our licensing agreements. In 2015, our partners have validated preclinical data in order to advance towards the clinical development of 2 of our previously licensed programmes. We expect that our collaborator Merck Serono will soon complete the preclinical development of ATR inhibitors, thereby accelerating the translation of CNIO's research into new potential treatment options for cancer patients. Our monoclonal antibody commercial pipeline has also been strengthened, and now counts 24 reagents in commercialisation.

The successful licensing of these technologies provides significant financial support for CNIO's activities as well as for the inventors themselves; the revenues are distributed according to the CNIO royalty distribution policy and regulations. Altogether, the income generated from Intellectual Property Rights (IPR) in 2015 amounts to about 800,000 €, thanks to the efforts of more than 40 CNIO Inventors who have contributed to this IPR. This income will contribute to the economic sustainability of the CNIO, as well as support its research activities.

Once again this year, the technology transfer potential of the CNIO was successfully recognised by renowned stakeholders such as the *Botín* Foundation who nominated one of our scientists, Maria Blasco, as a 2015 '*Botin Researcher*'; a distinction that only a handful of prestigious researches hold. Through this award the *Botín* Foundation will extend its support to the technology transfer activities related to the research of the Telomeres and Telomerase Group at the CNIO for 5 more years.

A world-class research institute such as the CNIO cannot thrive without driven individuals who are passionate about pushing the frontiers of present-day knowledge and who have the ambition to share new-found knowledge with others. CNIO is proactive when it comes to fostering the entrepreneurial activities of its researches and, therefore, in collaboration with the *IE Business School*, it provides business training for scientists who are interested in translating their findings into solutions that will benefit cancer patients and society in general.

PRIVATE SPONSORS

"We would like to thank all our sponsors and donors for the generous support that we received from them in 2015. 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 the CNIO. This acclaimed 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.

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 the CNIO Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos, since 2012. This year, they have also further extended their support to 3 CNIO researchers: Manuel Hidalgo, Head of the GI Cancer CRU and Director of the Clinical Research Programme; Miguel Quintela, Head of the Breast Cancer CRU; and Joaquín Martínez-López, Head of the H12O-CNIO Haematological Malignancies CRU. These Groups focus on translating 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. One young scientist, Ana Ortega, who came from the Sloan Kettering Institute for Cancer Research in New York, was the recipient of a Santander Foundation-CNIO Fellowship in 2015. 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 scientific programmes. These 2 well-recognised organisations collaborate with the CNIO in this regard by supporting 3 research groups: Manuel Serrano, Head of the Tumour Suppression Group; Maria A. Blasco, Head of the Telomeres and Telomerase Group; and Óscar Fernández-Capetillo, Head of the Genomic Instability Group.

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 recipients of the *Jesús Serra* Foundation's Visiting Scientist Award in 2015 were: Chaitanya R. Divgi from Columbia University in New York (USA), Marcin Nowotny from the International Institute of Molecular and Cell Biology in Warsaw (Poland), Eva Nogales from the University of California in Berkeley (USA), and Patrick Sung from Yale University School of Medicine in New Haven (USA).

AVON, funds the Breast Cancer Clinical Research Unit, led by Miguel Quintela, since 2010. The Research Project 'Avon-CNIO' on breast cancer research has the main goal of advancing personalised treatment for breast cancer patients.

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

OTHER SPONSORS

The Centre also benefits from generous donations from other companies and foundations, as well as from local associations that are equally dedicated to the battle against cancer. During 2015, our activities and seminars were also supported by: ***the French Embassy,*** **Fressia Group,** ***the Fundación Antoni Serra, and the Fundación Banco Sabadell.***

We would also like to express our gratitude to all the '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 2015.

Communication

COMMUNICATIONS

NURIA NORIEGA Head of Communications

Communications Officer **Vanessa Pombo**

In 2015, the presence of our Centre in the media kept its rising trend: over 2,600 mentions were tracked in the national and international press, representing an increase of 15% compared to the already remarkable figures of 2014. Our presence on radio/TV programmes and in news bulletins has also experienced a significant increase. Figures for radio and TV hits almost tripled those of 2014; these include shots during prime-time newscasts and magazine programmes broadcasted by public as well as private media channels.

In 2015, the CNIO submitted 24 press releases to the global news service EurekAlert! Throughout the year, these stories received over 83,000 hits from around the world, surpassing the grand total of 300,000 visits.

Two CNIO discoveries in particular caught the attention of the media and society: in May, research on TRF1 and cancer immortality published under the title 'Therapeutic inhibition of TRF1 impairs the growth of p53-deficient K-RasG12V-induced lung cancer by induction of telomeric DNA damage' hit the front pages of the newspapers, among other media outlets; in October, the international research on metastasis titled 'Tumour exosome integrins determine organotropic metastasis', in which the CNIO participated, had an outstanding media impact and was also covered by the main TV and radio stations in Spain.

An exceptional achievement in 2015 was the agreement CNIO established with the TV & Media Group *Atresmedia*, one of the leading media groups in Spain. Through this agreement, 'Constantes y Vitales' – the social responsibility initiative of the TV channel *laSexta* and the AXA Foundation – launched the engaging action #CadaPasoEsVital, to support the research conducted by the Microenvironment and Metastasis Group led by Héctor Peinado at the CNIO.

Our social networks are also consolidating their communities. By December 2015, our Twitter channel had over 8,700 followers, with whom we keep up an ongoing and valuable dialogue via the platform. Furthermore, in February, we announced the launch of the 'CNIO Friends' social networks. In only 10 months, our Facebook account reached over 26,000 followers – who constantly send us words of encouragement, affection and congratulations – and reached more than 44.6 million people as a whole.

"A year of growth together with media and society."

The 'CNIO Friends' initiative was greatly successful during its first year of existence. Both Marcos Argumosa's 10 consecutive marathons in April, and the video homage in honour of our donors, produced by the visual artist Amparo Garrido in June, resulted in almost 120 media hits, including digital and print media outlets, radio and TV broadcast. The video received almost 23,500 views on Youtube as well.

To strengthen our bonds with our donors, we launched the 'CNIO Friends' newsletter, via which we keep them informed about our Centre's latest news every other month. And thanks to an agreement with the Spanish railway operator RENFE, our video homage in their honour was aired in the Spanish high-speed long-distance trains in December and at Christmas time, helping us to spread the word about our cancer research during one of the most public-spirited times of the year.

CNIO APPEARANCES IN PRINT AND DIGITAL MEDIA

2012 | 2013 | 2014 | 2015

JAN-MAR	
98	
195	
387	
612	
APR-JUNE	
250	
378	
547	
630	
JULY-SEPT	
220	
342	
603	
682	
OCT-DEC	
193	
438	
760	
746	
TOTAL	
761	
1353	
2297	
2670	

CNIO APPEARANCES ON RADIO AND TV

2013 | 2014 | 2015

RADIO	
31	
34	
116	
TV	
28	
35	
69	
TOTAL	
59	
69	
185	

PRESS CLIPPINGS

1 'El Cultural', *El Mundo*, January 2, 2015
2 *Gaceta Médica*, January 19, 2015
3 *El País*, March 22, 2015
4 *ABC*, March 27, 2015
5 *SINC*, March 30, 2015
6 *Los Desayunos de TVE*, *TVE*, April 8, 2015
7 *El País Semanal*, April 12, 2015
8 *Diario Médico*, April 20, 2015

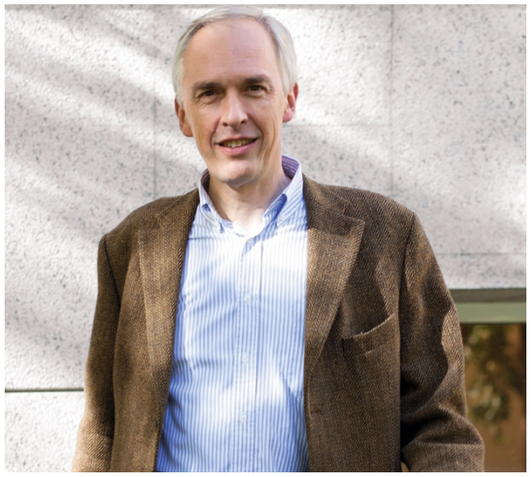
9 *El Mundo*, April 25, 2015
10 *La Aventura del Saber*, La 2, April 28, 2015
11 *La Razón* (front page), May 14, 2015
12 *ABC,* June 13, 2015
13 *Saber Vivir*, *TVE*, June 18, 2015
14 *ConCiencia*, *Telemadrid*, July 4, 2015
15 *Diario Médico* (front page), July 6, 2015
16 *La Rioja* (front page), September 1, 2015

17 *La Opinión*, September 22, 2015
18 *ABC*, September 26, 2015
19 *El País*, October 2, 2015
20 *Gaceta Médica*, (front page) October 5, 2015
21 *Más Vale Tarde*, *La Sexta*, October 19, 2015
22 *La Vanguardia*, October 26, 2015
23 *La Razón*, October 29, 2015
24 *Diario Médico* (front page), November 16, 2015

INVITED GUEST SPEAKERS (Distinguished Seminar Series)

Mª José García Borge, January 16, 2015

Ignacio Cirac, January 23, 2015



Hughes de Thé, October 30, 2015

Carlos Caldas, December 4, 2015

SOCIAL EVENTS

On World Cancer Day, the CNIO organised an open public debate on cancer research and clinical practice with CNIO researchers, the Spanish Association Against Cancer (*AECC*), and the San Carlos Clinical University Hospital. The event was sponsored by Bristol-Myers Squibb. February 4, 2015.

Carmen Vela, Secretariat of State for Research, Development and Innovation, talked about her professional career from a gender perspective, for the seminar series of the CNIO Women in Science Office (WISE). March 10, 2015.

Over 200 participants enjoyed the European Researchers' Night at the CNIO; this unique event aims to encourage scientific careers and foster an entrepreneurial spirit in young people. September 25, 2015.

The event '*Innovation: Bridge between Science and Society*' hosted a dialogue between the former Minister of Education, Ángel Gabilondo, and CNIO's Director, Maria A. Blasco. The event was organised by the CNIO, the *Banco Santander* Foundation and the *Instituto de Empresa* Business School. October 19, 2015.

Throughout the year, Benefactors and Sponsors of 'CNIO Friends' could visit the Centre in order to meet its Director Maria A. Blasco, as well as to enjoy a guided tour of its facilities.

CNIO Offices

DEAN'S OFFICE

MARÍA S. SOENGAS
Dean for Academic Affairs

Participants
Mónica Álvarez, Ana F. Batalha, Hugo Bernard, Jasminka Boskovic, Donatello Castellana, Daniela Cerezo, Almudena Chaves, Guillermo de Cárcer, Eleonora Lapi, Ana Losada, Lola Martínez, Raúl Martínez, Francisca Mulero, David Olmeda, Lisa Osterloh, Catherine E. Symonds, Álvaro Ucero, Özge Uluckan

The CNIO is recognised for the relevance and international projection of its scientific groups. 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 active medical centres, to ultimately bridge the gap between academic and clinical environments.

Importantly, the CNIO Student Association (CNIOSA) and Postdoc Association (CNIOPDA) are active organisers of talks and seminars coordinated by the Dean's Office. Examples of topics that we covered last year include *Effective Job Hunting and Interviewing*, as well as *Negotiation, Leadership and Confidence Workshops* conducted by the expert scientific coach Rob Thompson. These events are performed in concert with CNIO's Training Programmes and the Innovation and Communication Offices, which are deeply committed to providing the best environment for our personnel. We are most grateful to the *Fundación Jesús Serra* for their generous donation that helps us 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 numerous participants of all ages.

A particularly inspirational event this year was our Annual *CNIO Lab Day*. We were fortunate to host John Diffley (The Francis Crick Institute, UK) who spoke about his personal experience in setting up his laboratory, emphasising the value of perseverance, risk-taking and independent thinking. It was also exciting (and encouraging) to learn about the success stories of alumni from CNIO laboratories who now have productive careers in academia (Eva González Suarez, IDIBELL, Barcelona), non-profit organisations (Marta Puyol, AECC, Madrid) and industry (Sara Álvarez, NIMGenetics, Madrid). We also had six outstanding talks given by CNIO trainees that covered exciting discoveries in the fields of stem cell biology, epidemiology, proteomics, tumour metastasis and drug development. Progress made in other basic and translational aspects of cancer were discussed in over fifty posters, which together emphasised the breadth of research covered by our different Scientific Programmes.

"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."

Finally, we also had the pleasure of announcing the establishment of the '*Director's List*', an initiative promoted and endorsed by CNIO's leadership as a formal platform to recognise and give specific visibility to 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 in regards to training, scientific divulgation and outreach. After a motivating review by our Director Maria Blasco on how the CNIO has consolidated and expanded our basic and clinical Scientific Programmes, solidified innovation and drug development activities and in general, reinforced our international recognition, it was with great satisfaction that we could present the first edition of our Director's List:

1. Awards to Excellence in Research by Predoctoral Fellows
Elena Domènech (for an outstanding publication in *Nat Cell Biol*), Elena López-Guardamillas (*Cell Metab*), Julia Specks (*Genes & Dev*), Silvia Álvarez (*Nat Commun*) and Laia Richart (*Nat Commun*).

2. *Award to Excellence in Research by Postdoctoral/Staff Investigators*
Sergio Ruiz, for outstanding contributions in the fields of genomic instability and stem cell pluripotency (*Nat Commun*).

3. Outstanding Contribution to Outreach and Awareness
Lisa Osterloh, for her tireless and altruistic efforts in the organisation and supervision of the European Researcher's Night, visits by high- and middle-schools, as well as the multiple talks and seminars given on career development.

In summary, we are as proud as ever for all that this vibrant community of young investigators at the CNIO has achieved, while mentored by a committed faculty at the frontline of cancer research.

CNIO WOMEN IN SCIENCE OFFICE

Lola Martínez
Coordinator

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

Maria A. Blasco, Nicole Dölker, Pablo Fernández, Raquel García-Medina, Diego Megías, Fernando Peláez, Alejandra Tavera

"No struggle can ever succeed without women participating side by side with men."

Muhammad A. Jinnah, 1940.

The recent data published by the main scientific organisation in Spain (CSIC) in their report "*Informe Mujeres y Ciencia* 2015" speak for itself, and continue to show during 2014, the consistent classical 'scissors' pattern displayed in the comparative career paths of male and female professionals. Although about 50% of women are represented in the pre-doctoral and post-doctoral stages of the scientific career, those percentages go down significantly as we move up the scientific career ladder: a meagre 25% of women are represented at the Principal Investigator level versus 75% of men, and this representation continues to shrink as we move up to the levels of department directors and beyond.

Similar scenarios can be found all over Europe, and this global picture is also true even in countries such as the United States of America where policies to address those imbalances were implemented many years ago. Therefore, there is still a lot of work to be done in order to achieve gender equality in science.

At the CNIO Women in Science Office (WISE), we believe there is still a real need for actions to be undertaken to ensure gender equality in the research career. At the end of 2012, our office was created with the aim to promote awareness about these important aspects and to help correct the observed imbalances in the career ladder at the CNIO community. We are convinced that everyone within the CNIO community needs to work together towards a common goal: to help outstanding female researchers to reach the top as they have lots of fresh ideas to offer to science and they can most certainly contribute towards the CNIO's scientific productivity.

The Office has two main working groups:

- → Work/Life Balance: their aim is to promote and support initiatives to help improve the delicate balance between the professional and personal life, one of the main challenges faced by researchers at the CNIO.
- → Seminars and Events: their aim is to raise awareness about gender issues in the scientific field (and others) and point out the difficulties that female researchers may experience in their quest to reach the top, as well as to provide networking opportunities to all CNIO researchers.

"Science still has many struggles to overcome for its full advancement, including the gender gap. Women have a lot to contribute, so let's make the CNIO an equal opportunities Centre."

In 2015, the WISE office managed to invite several top female leaders from different areas to come and tell us about their experience with gender issues, thereby giving our young scientists ideas and advice on how to best overcome some of the hurdles that they may face during their careers. These seminars are aimed to stimulate institutional 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. Some of the talks given during 2015 include:

- → Sex, Science and Society: a triangle that matters? Flora de Pablo. Professor, *Centro de Investigaciones Biológicas*, CSIC. Madrid, Spain. February 17.
- → A professional career with a gender perspective. Carmen Vela Olmo. Secretariat of State of Research, Development and Innovation, Ministry of Economy and Competitiveness. Madrid, Spain. March 10.
- → My life with phage ø29. Margarita Salas. Honorary Professor, *Centro de Biología Molecular "Severo Ochoa"*, CSIC-UAM. Madrid, Spain. April 14.
- → Women at the top: making it happen. María del Mar Martínez. Director at McKinsey's Madrid Office. Madrid, Spain. June 9.
- → Women in business and science: two parallel paths. Natalia González-Valdés. Director of Communications and Corporative Social Responsibility (CSR) for L'Oreal Spain. Spain. November 24.

In addition, the Office proposed several initiatives to the CNIO Direction in relation to work/life balance issues. As an example, a proposal was elaborated and put forward to the General Secretary of the ISCIII for the organisation of an urban camp during non-school days for children of CNIO employees; this was done in collaboration with members of the ISCIII. ■

Facts & Figures

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 2015, researchers at the CNIO were involved in 143 projects that received extramural funding.

The CNIO actively participates in a total of 66 collaborative projects: 31 are international collaborative projects (for 4 of them the CNIO acts as project coordinator) and 35 are collaborative projects at the national level (8 of which are coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7th Framework Programme and Horizon 2020, the US National Institutes of Health (NIH), the United States Department of Defense (DoD), the Melanoma Research Alliance, the Paradifference Foundation and the AXA Research Fund.

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2015, 19 of these projects received international funding and 58 received national funding. The international individual projects are also funded by the European Commission (through the European Research Council (ERC) Advanced, Consolidator and Starting grants, and the Marie Curie Actions), the Worldwide Cancer Research (WCR, formerly AICR), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

AXA RESEARCH FUND

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A. (coordinator) Serrano, Manuel	Identification and manipulation of molecular pathways relevant for age-dependent tissue regeneration

EUROPEAN COMMISSION

7 FRAMEWORK PROGRAMME (2007-2013)

COST ACTION

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

EURATOM

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Serrano, Manuel	RISK-IR: Risk, stem cells and tissue kinetics-ionising radiation

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
Valencia, Alfonso	Open PHACTS: An open, integrated and sustainable chemistry, biology and pharmacology knowledge resource for drug discovery

INTEGRATED PROJECT

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MitoSys: Systems biology of mitosis
Valencia, Alfonso	BLUEPRINT: A BLUEPRINT of haematopoietic epigenomes
Valencia, Alfonso	ASSET: Analysing and striking the sensitivities of embryonal tumours
Valencia, Alfonso	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research

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

NETWORKS OF EXCELLENCE (NOE)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	EUROCANPLATFORM: A European platform for translational cancer research

SMALL OR MEDIUM-SCALE FOCUSED RESEARCH PROJECTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	LUNGTARGET: New approaches for the targeted therapy of non-small cell lung cancer
Malats, Núria	TransBioBC: Translation of novel Biomarkers for Bladder Cancer for clinical outcome prediction
Robledo, Mercedes	ENS@T- CANCER: European network for the study of adrenal tumours-structuring clinical research on adrenal cancers in adults

ERA-NET ON TRANSLATIONAL CANCER RESEARCH (TRANSCAN)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	Bio-PaC: Biomarkers of tumor recurrence in pancreatic cancer ERA-NET on European Funding for Neuroscience Research (NEURON II)

ERA-NET ON EUROPEAN FUNDING FOR NEUROSCIENCE RESEARCH (NEURON II)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human

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
Valencia, Alfonso	OpenMinTeD: Mining INfrastructure for TExt and Data

MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

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

SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	BRIDGES: Breast cancer risk after diagnostic gene sequencing

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (MIT)

M+VISION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Pérez Moreno, Mirna Alicia	Team Leuko: Home-based neutrophil blood testing to tailor chemotherapy regimens to personal toxicity limits

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor Soengas, María S. (coordinator)	Imaging and therapeutic targeting of lymphangiogenesis in melanoma

PARADIFFERENCE FOUNDATION

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

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Radiolabeled exosomes for the early detection of metastases and to predict breast cancer premetastatic niche
Peinado, Héctor	Organ-tropic metastatic secretomes and exosomes in breast cancer
Peinado, Héctor	Exosomes in development and therapy of malignant mesothelioma

US NATIONAL INSTITUTES OF HEALTH (NIH)

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

VOLKSWAGEN FOUNDATION / *FUNDACIÓN VOLKSWAGEN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Lietha, Daniel	Nanoapertures loaded with individual molecules

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

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION

7 FRAMEWORK PROGRAMME (2007-2013)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERC Advanced Grant RAS AHEAD: Ras genes in health and disease
Fernández-Capetillo, Óscar	ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health
Heeschen, Christopher	ERC Advanced Grant Pa-CSC: Molecular characterization and targeted elimination of metastatic pancreatic cancer stem cells
Serrano, Manuel	ERC Advanced Grant CANCER&AGEING: Common mechanisms underlying cancer and ageing
Wagner, Erwin F.	ERC Advanced Grant AP-1-FUN: AP-1(Fos/Jun) functions in physiology and disease

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	PERSMEDOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy
Squatrito, Massimo	GLIDD: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas
Ramón-Maiques, Santiago Moreno, María	COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target

HORIZON 2020 (2014-2020)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Hidalgo, Manuel	ERC Advanced Grant AVATAR: Integrating genomics and avatar mouse models to personalize pancreatic cancer treatment
Serrano, Manuel	ERC Advanced Grant CELLPASTICITY: New Frontiers in Cellular Reprogramming: Exploiting Cellular Plasticity

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

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	Prognostic and therapeutic impact of lymphovascular niches in melanoma

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
Lietha, Daniel	Targeting regulatory mechanisms for allosteric cancer drug discovery
Malumbres, Marcos	New therapeutic strategies by inhibiting Mastl in breast tumors
Pérez Moreno, Mirna Alicia	Defining the role of macrophage-derived Wnts in squamous cell carcinoma
Soengas, María S.	Harnessing endo/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system
Wagner, Erwin F.	Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis

NATIONAL GRANTS COLLABORATIVE PROJECTS

COMMUNITY OF MADRID / *COMUNIDAD AUTÓNOMA DE MADRID (CAM)*

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*
Blasco, Maria A. Serrano, Manuel (coordinator)	*Programa ReCaRe: Reprogramación en cáncer y regeneración*
Campos-Olivas, Ramón Lietha, Daniel	*Programa BIPEDD 2: Plataforma integrada de bioinformática para el descubrimiento de nuevos fármacos basado en la estructura del receptor*
González-Neira, Anna	*Programa VISIONANIMAL: Modelos animales para el estudio de enfermedades de la visión*
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*
Montoya, Guillermo	*Programa INTERACTOMICS: Interactomics del centrosoma*
Real, Francisco X.	*Programa CEL-DD: Linajes y competición celular en el desarrollo y la enfermedad*
Robledo, Mercedes	*Programa TIRONET: Fisiopatología tiroidea: Mecanismos implicados en cáncer, autoinmunidad y mecanismo de acción de hormonas tiroideas*
Soengas, María S.	*Programa NANODENMED: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas*

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 EN SALUD (RETICS)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	*Red Temática de Investigación Cooperativa en Cáncer* (RTICC) (Group RD06/0020/1060)
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) (RD12/0036/0050)
Real, Francisco X.	*Red Temática de Investigación Cooperativa en Cáncer* (RTICC) (Group RD12/0036/0034)
Valencia, Alfonso	*Red Temática de Investigación Cooperativa en Biomedicina Computacional* (COMBIOMED) (Group RD07/0067/0014)

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*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2* (PT13/0001/0005)
Morente, Manuel M. (coordinator)	*Plataforma de Biobancos* (Coordination node and group PT13/0010/0001)
Muñoz Peralta, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2* (Group PT13/0001/0010)
Valencia, Alfonso	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2* (Group PT13/0001/0030)

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Gómez, Carlos Jesús Hidalgo, Manuel (starting in June)	Chemosensitivity profiles for the personalized therapy of advanced colorectal cancer
Hidalgo, Manuel	Personalized treatment for pancreatic cancer patients
Hidalgo, Manuel	Personalized treatment for pancreatic cancer patients II

MINISTRY OF ECONOMY AND COMPETITIVENESS / *MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD (MINECO)*

NATIONAL R&D&I PLAN 2008-2011

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	*Programa CONSOLIDER RNAREG: Una aproximación integrada a la regulación post-transcripcional de la expresión génica y su papel en enfermedad*

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, Oscar Malumbres, Marcos Real, Francisco X. Serrano, Manuel	ONCObio: *Biología del Cáncer*
Malumbres, Marcos (Coordinator)	CellSYS: Functional and Systems Biology of Cell Proliferation
Peinado, Héctor	REDiEX: *Red de Excelencia en Investigación e Innovación en Exosomas*
Serrano, Manuel (Coordinator)	SENESTHERAPY: Cell senescence in cancer therapy

CHALLENGES-COLLABORATION/*RETOS-COLABORACIÓN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	*Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores*

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

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Cellular aging in first episode early-onset psychosis

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	Cancer and immunodeficiency in children
Malats, Núria Real, Francisco X. (coordinator)	Invasive bladder cancer: towards precision medicine
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

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
Fernández-Capetillo, Óscar	Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment
Soengas, María S.	Role of RNA binding proteins in melanoma progression: searching for new diagnostic markers and therapeutic targets

MADRI+D* FOUNDATION / *FUNDACIÓN MADRI+D

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Dean's Office for Academic Affairs	*Ven a conocer a los científicos, ¡conviértete en un científico!* European Researchers' Night 2014, organized by Madri+d Foundation and founded by European Commission on the framework of H2020 Programme

NATIONAL GRANTS INDIVIDUAL PROJECTS

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

RESEARCH PROJECTS IN HEALTH

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
Cascón, Alberto	Exome sequencing of trios, mother-father-proband, in pediatric patients with multiple pheochromocytomas/paragangliomas
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
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
González-Neira, Anna	Personalizing breast cancer treatment: prediction model construction for taxanes and anthracyclines efficacy thought the integration of different genomic approaches
Hidalgo, Manuel	Targeting Pancreatic Cancer Stroma
Malats, Núria	Aetiology of pancreas cancer: Application of "omics" technologies in the assessment of risk factors
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
Olmos, David	Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer
Pérez de Castro, Ignacio	An integrative Study of Chromosomal Instability and Cancer: looking for prognostic markers and therapeutic opportunities
Quintela, Miguel Angel	From systems biology to clinical trials: high-throughput studies and definition of predictive factors and resistance mechanisms against breast cancer drugs
Robledo, Mercedes	Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility
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
Squatrito, Massimo	Investigating the role of Fra1 and Fra2 in glioma tumor formation and treatment response
Urioste, Miguel	PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene

MINISTRY OF ECONOMY AND COMPETITIVENESS / *MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD (MINECO)*

NATIONAL R&D&I PLAN 2008-2011

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MitoSYS: Physiological and therapeutic relevance of mitotic kinases and phosphatases
Montoya, Guillermo	Macromachines: Structural biology of macromolecular machines involved in chromosome dynamics
Pérez Moreno, Mirna Alicia	CrosSkin: Intercellular crosstalk in skin physiology and disease
Real, Francisco X.	Transcriptional control of acinar cells differentiation and pancreatic cancer
Rodríguez, Cristina	Identification of markers predictive of paclitaxel severe neurotoxicity using genome-wide platforms
Uluçkan, Özge	PsorTACEmiR21: Investigating the role of microRNA21/TIMP-3/TACE in psoriasis - evaluating the potential therapeutic implications
Valencia, Alfonso	Development of biocomputing systems and subjacent computational methods for the analysis of oncologic personalised therapies
Wagner, Erwin F.	HepAP-1: From liver physiology to hepatitis and hepatocellular carcinoma (HCC): role of AP-1 (Fos/Jun) proteins

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	*Acreditación del CNIO como Centro de Excelencia* "Severo Ochoa"

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

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Méndez, Juan	REPLICON: Molecular mechanisms that control eukaryotic DNA replication
Ramón-Maiques, Santiago	CADstructure: Structural determination of the architecture of CAD, an antitumoral target that controls the biosynthesis of pyrimidines
Ruiz, Sergio	RSHIPS: Replicative stress during somatic cell reprogramming

CHALLENGES-RESEARCH/*RETOS-INVESTIGACIÓN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	PANTHER: A three prong strategy to fight pancreatic ductal adenocarcinoma
Blasco, Maria A.	TeloHealth: Telomeres, telomerase and disease
Djouder, Nabil	MILC: Metabolic inflammation in liver cancer
Fernández-Capetillo, Óscar	BREAKINGRAD: Exploring the limits of radioresistance in mammals
Losada, Ana	COHESIN: Cohesin function and regulation: a multidisciplinary approach
Muñoz, Daniel	REMODEL: Cellular senescence as an active player in tissue remodelling
Muñoz, Javier	steMS: Understanding ground state pluripotency of embryonic stem cells through mass spectrometry-based proteomics
Ortega, Sagrario	HaploEScancer: Haploid ES cells for cancer research
Osorio, Ana	IPAGEN: Exploring the mechanism of action of PARP inhibitors in breast and ovarian cancer patients. Identification of new genetic predictors of response
Pastor, Joaquín	CDK8eDD: CDK8 a novel target in cancer therapy. Relevance of CDK8 kinase activity, discovery and optimization of selective orally bioavailable CDK8 inhibitor
Peinado, Héctor	METASTAXOMEs: Role of tumor-secreted exosomes in lymph node microenvironment reprogramming during metastasis
Serrano, Manuel	CANCERAGE: Cancer and ageing-associated diseases: new frontiers and new strategies
Soengas, María S.	MEL-STOP: Vesicular trafficking in melanoma progression and treatment response
Valiente, Manuel	ReACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis

ACQUISITION OF SCIENTIFIC AND TECHNICAL EQUIPMENT*/ADQUISICIÓN DE EQUIPAMIENTO CIENTÍFICO-TÉCNICO*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González, David	Scientific data storage infrastructure

ACTION TO PROMOTE THE COMMUNICATION OF SCIENTIFIC AND TECHNICAL RESULTS OR INNOVATION IN HIGH-LEVEL INTERNATIONAL CONFERENCES */ ACCIÓN DE DINAMIZACIÓN DE LA COMUNICACIÓN DE RESULTADOS CIENTÍFICO-TÉCNICOS O DE LA INNOVACIÓN EN CONGRESOS INTERNACIONALES DE ALTO NIVEL*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Metastasis Initiation: Mechanistic and Therapeutic Opportunities

EXCELLENCE-EUROPE / *EUROPA EXCELENCIA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Cristina	ANGIOMARKER: Predicting antiangiogenic drug response in cancer: markers and mechanisms
Valiente, Manuel	BrainMET: Deconstructing metastatic disease in the brain Research-Europe / *Europa Investigación*

RESEARCH-EUROPE / *EUROPA INVESTIGACIÓN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	CancerCureAdvisor: An open bioinformatics platform for personalized treatment of cancer

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 Young Researchers Program/ Programa *Jóvenes Investigadores*

YOUNG RESEARCHERS PROGRAM/ *PROGRAMA JÓVENES INVESTIGADORES*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez, Mónica	GPGenCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer
Lecona, Emilio	UBQREP: Modulation of DNA Replication by ubiquitination of chromatin proteins

ASTRAZENECA FOUNDATION */ FUNDACIÓN 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*

BBVA FOUNDATION / *FUNDACIÓN BBVA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Jiménez, Alberto	*Desarrollo de nuevas herramientas diagnósticas no invasivas por imagen para el diagnóstico del glioblastoma multiforme, el tumor cerebral más maligno*

FERO FOUNDATION / *FUNDACIÓN FERO*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Predictive biomarkers for brain metastasis in small cell lung cancer

SPANISH ONCOLOGY GENITOURINARY GROUP/*GRUPO ESPAÑOL DE TUMORES GENITOURINARIOS (SOGUG)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	*Validación multi-institucional de perfiles de expresión génica en sangre periférica en pacientes con cáncer de próstata resistente a la castración*

SPANISH GROUP OF NEUROENDOCRINE TUMOURS/*GRUPO ESPAÑOL DE TUMORES NEUROENDOCRINOS (GETNE)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Robledo, Mercedes	ParafeOMICS*: Identificación de marcadores diagnósticos y pronósticos en feocromocitomas y paragangliomas a través de la integración de cuatro plataformas de análisis masivo*

MUTUA MADRILEÑA* FOUNDATION / *FUNDACIÓN MUTUA MADRILEÑA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Jiménez, María	*JunB/AP-1, supresor tumoral en la piel. Mecanismos moleculares e interacción funcional con p53*

RAMON ARECES* FOUNDATION / *FUNDACIÓN RAMÓN ARECES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Montoya, Guillermo	*Desarrollo de bisturíes moleculares para la reparación de genes implicados en enfermedades monogénicas*
Serrano, Manuel	*Reprogramación nuclear in vivo e interrelación funcional entre p27 y Sox2*

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

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*

EDUCATION & TRAINING PROGRAMMES

One of CNIO's commitments is to increase its training capacity in order to give students and professionals the opportunity to successfully advance their careers in the healthcare sector. During 2015, the CNIO signed several new agreements with Spanish Universities and other institutions such as Ministries and secondary schools; namely, with the *Universidad Politécnica de Madrid, Universidad Católica de Murcia, Universidad de Castilla La Mancha, Universidad Autónoma de Barcelona, Universidad Complutense de Madrid, Universidad de A Coruña, Universidad de Sevilla, Ministerio de Educación, Cultura y Deporte, IES Moratalaz* (Madrid), *IES Benjamín Rua* (Móstoles, Madrid), *IES Jaime Ferrán Clúa* (San Fernando de Henares, Madrid), *IES Ramón y Cajal* (Valladolid), *Centro de Formación Rozona* (Avilés, Asturias) and *Centro Educativo OPESA* (Madrid). These agreements formalise the procedures that are to be followed in order to enable students from the aforementioned institutions to perform laboratory practices.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2011	**2012**	**2013**	**2014**	**2015**
Training of PhD students	123	121	116	108	**105**
Post-doctoral training	83	81	67	55	**48**
Training for MDs	20	16	21	14	**25**
Laboratory training for MSc/BSc students	46	42	36	73	**80**
Laboratory training for technicians	26	26	19	21	**27**
Master's Degree in Molecular Oncology (graduated)	37	37	37	34	**29**

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 (303.5 hours). During this time the students actively participate in research projects in one of the CNIO groups. During 2015, 9 students from 5 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, 73 students participated in these programmes, of which 3 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, 15 students obtained their PhD degrees in 2015 and 26 more joined the CNIO in that same year. One third of the 105 students working at the CNIO in 2015 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 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 2015, 2 pre-doctoral students received one of these recognised fellowships.

The distribution of students across the CNIO's Research Programmes in 2015 was as follows: 54.3% of students worked in the Molecular Oncology Programme, 13.3% in the BBVA Foundation- CNIO Cancer Cell Biology Programme, 9.5% in the Structural Biology and Biocomputing Programme, 12.4% in the Human Cancer Genetics Programme, 6.7% in the Clinical Research Programme, 2.9% in the Biotechnology Programme and the remaining 1.0% in the Experimental Therapeutics.

FUNDING OF PHD TRAINING	NO.
SPANISH ENTITIES	83
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (Pre-doctoral Fellowships)	37
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (I+D Projects)	6
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte* (Pre-doctoral Fellowships)	5
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (I+D Projects)	2
"la Caixa" Foundation / *Fundación "la Caixa"* (Pre-doctoral Fellowships)	29
Spanish Association Against Cancer (AECC) / *Fundación Científica de la AECC* (I+D Project)	1
FEDER Telethon Foundation/*Fundación Teletón FEDER para la investigación de Enfermedades Raras*	1
CIBERER	1
CNIO "Severo Ochoa"	1
INTERNATIONAL ENTITIES	22
European Commission Framework Programme	5
Marie Skłodowska-Curie actions of the European Commission (Pre-doctoral Fellowship)	1
European Research Council	1
Fullbright Spain / Fulbright España (Pre-doctoral Fellowship)	1
Fundação do Ministério de Ciência e Tecnologia de Portugal (FCT) (Pre-doctoral Fellowship)	1
UK National Institute of Health	1
Melanoma Research Alliance	1
Prostate Cancer Foundation Young Investigator Award	1
AXA	1
Boehringer Ingelheim Fonds	1
Boehringer Ingelheim International GMBH	1
Pfizer	1
Santander Universidades (Pre-doctoral Fellowship)	1
TOTAL	**105**

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 attractive research projects at the forefront of cancer research.

In 2015, 48 postdoctoral fellows worked at the CNIO. Notably, 42% of these fellows were from outside of Spain, many coming from very prestigious international institutions.

Once again in 2015, the *Fundación Banco Santander* agreement with the CNIO resumed 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 Memorial Sloan Kettering Institute of Cancer Research (New York), was awarded a Santander Foundation-CNIO Fellowship in 2015.

FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS	NO.
SPANISH ENTITIES	25
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (Post-doctoral Fellowships)	3
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (I+D Projects)	3
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (I+D Projects)	2
Community of Madrid / *Comunidad Autónoma de Madrid* (I+D Projects)	2
Madrid-MIT M+Visión (Post-doctoral Fellowships)	2
Spanish Association Against Cancer (AECC) / *Fundación Científica de la AECC* (Post-doctoral Fellowships)	5
La Marató TV3 Foundation / *Fundació La Marató TV3*	1
Botín Foundation / *Fundación Botín*	1
Banco Santander Foundation / *Fundación Banco Santander* (Post-doctoral Fellowship)	1
Cris Foundation / *Fundación Cris*	3
CIBERER	1
CNIO	1
INTERNATIONAL ENTITIES	23
European Commission Framework Programme	7
European Research Council	3
Association for International Cancer Research	5
AXA	2
Daiichi Sankyo Agreement	1
Federation of the Societies of Biochemistry and Molecular Biology (Post-doctoral Fellowship)	1
Hoffmann-La Roche	2
Leukemia and Lymphoma Society (Post-doctoral Fellowship)	1
Pfizer	1
TOTAL	**48**

POSTGRADUATE PROGRAMMES

In addition, the CNIO – in collaboration with 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, Master's in Molecular and Cell Biology, Master's in Molecular Biomedicine, and Master's 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

Manuel Hidalgo, CNIO's Vice-Director of Translational Research codirects – in collaboration with the CEU-San Pablo University in Madrid (USP-CEU) – 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 *Alcala 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 600 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 16 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 (704-712 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student of Analytical Assays and Quality Control for 11 weeks (370 hours); to 2 students of Clinical Diagnosis for 14 weeks (380 hours); and to 3 students of Medical Archiving and Recording for 14 weeks (440 hours). Of the 27 students who participated in this programme in 2015, 5 were hired by the CNIO.

TRAINING FOR MDS

In line with CNIO's commitment to bridge the 'bench to bedside' gap, the Centre offers excellent training opportunities in molecular diagnostics and familial cancer genetics to MDs and other health care professionals; this initiative is a collaborative effort with the Spanish Ministry of Health (current *Ministerio de Sanidad, Servicios Sociales e Igualdad*). Training usually consists of a 3-month period during residency. In 2015, 25 medical residents from 14 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2015, 11 scientists were supported by the Ramón y Cajal Programme. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (now Spanish Ministry of Economy 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. Twenty other scientists were funded by similar programmes, including the Miguel Servet (3 contracts), Sara Borrell (1 contract) and Río Hortega (2 contracts) programmes, Juan de la Cierva programme (Spanish Ministry of Economy and Competitiveness, 3 contracts) and the Spanish Association Against Cancer (*AECC*, 8 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 2015, Eva Nogales from the University of California in Berkeley (USA), Chaitanya Divgi from the Columbia University in New York (USA), Marcin Nowotny from the Institute of Molecular and Cell Biology in Warsaw (Poland) and Patrik Sung from the Yale University in New Haven (USA) were beneficiaries of the Jesús Serra Foundation's Visiting Researcher Programme.

'SCIENCE BY WOMEN' PROGRAMME

The Women for Africa Foundation launched the 'Science by Women' programme aimed at promoting access for African women to science and technology by inviting them to "Severo Ochoa" Centres of Excellence to benefit from sabbatical stays in Spain. CNIO will host 3 of them

SCIENTIFIC EVENTS

CNIO FRONTIERS MEETINGS

CNIO Frontiers Meetings are the main international conferences that are organised by the CNIO. 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.

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT
23-25 MARCH, 2015

ORGANISERS

- **Manuel Hidalgo**, CNIO, Madrid, Spain
- **Alberto Bardelli**, IRCC, Torino, Italy
- **Lillian Siu**, Princess Margaret Cancer Centre, Toronto, Canada
- **Josep Tabernero**, VHIO, Barcelona, Spain

SESSIONS

- New Targets-Pathways in Clinical Development (1)
- Innovative Approaches in Drug Development
- New Targets-Pathways in Clinical Development (2)
- Immunotherapy Approaches for Cancer Treatment
- Personalizing Cancer Treatment

SPEAKERS

- **Gerhardt Attard**, ICR, London, UK
- **Mariano Barbacid**, CNIO, Madrid, Spain
- **Alberto Bardelli**, University of Turin - Candiolo Cancer Institute IRCCS, Italy
- **Maria Blasco,** CNIO, Madrid, Spain
- **Hilary Calvert**, UCL, London, UK
- **Luis Diaz**, Johns Hopkins University, Baltimore, US
- **Jeffrey Engelman**, Massachusetts General Hospital Cancer Center, US
- **Manel Esteller**, IDIBELL, Barcelona, Spain
- **Oscar Fernández Capetillo**, CNIO, Madrid, Spain
- **Manuel Hidalgo**, CNIO, Madrid, Spain
- **Sunil R Hingorani**, Fred Hutchinson Cancer Research Center, Seattle, US
- **Timothy Hoey**, OncoMed Pharmaceuticals, Redwood City, US
- **Tak W. Mak**, Princess Margaret Cancer Centre, University of Toronto, Canada

- **Ignacio Melero**, CIMA & Clinica Universidad de Navarra, Pamplona, Spain
- **Drew Pardoll**, Johns Hopkins University, Baltimore, US
- **William Pao**, Roche Pharma Research & Early Development, Basel, Switzerland
- **Ingrid Sasson**, Sanofi Oncology, Paris, France
- **David J. Shields**, Pfizer Inc., NY, US
- **Lillian Siu**, Princess Margaret Cancer Centre, Toronto, Canada
- **Mario Sznol**, Yale University, New Heaven, US
- **Josep Tabernero**, VHIO, Barcelona, Spain
- **Christophe Le Tourneau**, Curie Institute, Paris, France
- **Jaap Verweij**, Erasmus University Medical Center, Rotterdam, The Netherlands
- **Elisabeth G. de Vries**, University Medical Center, Groningen, The Netherlands
- **Ann L. White**, University of Southampton, UK

In addition, 5 short talks were selected among participants' contributions and 9 posters were presented.

METASTASIS INITIATION: MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES
28-30 SEPTEMBER, 2015

ORGANISERS

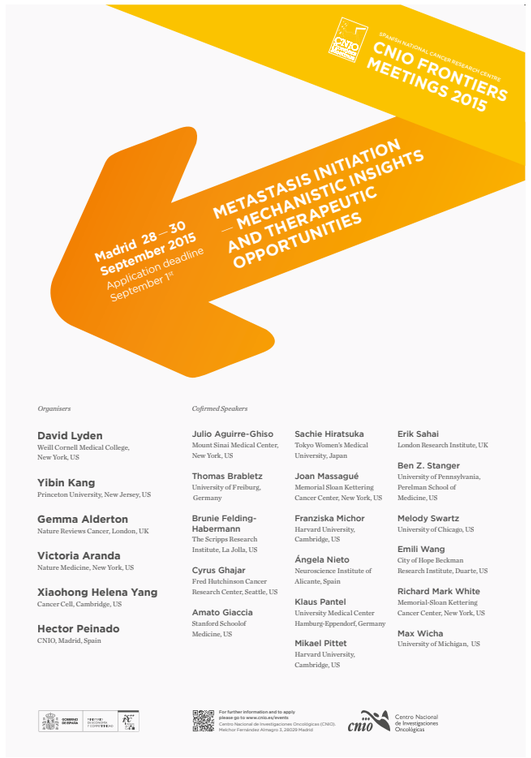
- **David Lyden,** Weill Cornell Medical College, New York, US
- **Yibin Kang**, Princeton University, New Jersey, US
- **Gemma Alderton**, Nature Reviews Cancer, London, UK
- **Victoria Aranda**, Nature Medicine, New York, US
- **Li-Kuo Su**, Cancer Cell, Cambridge, US
- **Héctor Peinado**, CNIO, Madrid, Spain

SESSIONS

- Cell Fate Regulation, Stem Cells and Metastasis
- Epithelial-to-Mesenchymal Transition
- Circulating Factors: Microvesicles and Exosomes
- Circulating Factors: Circulating Tumor Cells/ Platelets/ Circulating DNA and RNA
- Pre-Metastatic Niche
- Disseminated, Dormant and Metastasis-Initiating Tumor Cells
- Organ-Specific Metastasis and Micrometastatic Disease
- Imaging Early Metastatic Events
- Targeting Metastasis
- Modeling Metastasis

SPEAKERS

- **Julio Aguirre-Ghiso**, Mount Sinai Medical Center, New York, US
- **Salvador Aznar Benitah**, Institute for Research in Biomedicine, Barcelona, Spain
- **Thomas Brabletz**, University of Erlangen- Nuremberg, Germany
- **Janine Erler**, Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Denmark
- **Brunhilde H. Felding**, The Scripps Research Institute, La Jolla, US
- **Cyrus Ghajar**, Fred Hutchinson Cancer Research Center, Seattle, US
- **Amato Giaccia**, Stanford School of Medicine, US
- **Kent W. Hunter**, Center for Cancer Research National Cancer Institute, Bethesda, US
- **Joan Massagué**, Memorial Sloan Kettering Cancer Center, New York, US



- **Ángela Nieto**, Neuroscience Institute of Alicante, Spain
- **Klaus Pantel**, University Medical Center Hamburg-Eppendorf, Germany
- **Mikael Pittet**, Harvard University, Cambridge, US
- **Erik Sahai**, London Research Institute, UK
- **María S. Soengas**, Spanish National Cancer Research Centre, Madrid, Spain
- **Ben Z. Stanger**, University of Pennsylvania, Perelman School of Medicine, US
- **Melody Swartz**, University of Chicago, US
- **Emily Wang**, City of Hope Beckman Research Institute, Duarte, US
- **Richard Mark White**, Memorial-Sloan Kettering Cancer Center, New York, US
- **Max Wicha**, University of Michigan, US

In addition, 12 short talks were selected among participants' contributions and 42 posters were presented.

OTHER MEETINGS & CONFERENCES

The CNIO annually hosts various international meetings and conferences. Within this category, the 5 national and international events held in 2015 focused on recent advances in the areas of Biobanks, Sarcoma, Macromolecular Structures, Bioinformatics, Pharmacogenetics and Pharmacogenomics.

PAPEL DE LA FARMACOGENÉTICA Y FARMACOGENÓMICA EN LA MEDICINA DEL SIGLO XXI: ESTADO ACTUAL Y NUEVOS RETOS
20-21 APRIL, 2015

ORGANISERS
- **Luis López-Fernández**, IiSGM, Madrid
- **Anna González-Neira**, CNIO, Madrid
- **Cristina Rodríguez-Antona**, CNIO, Madrid

SPEAKERS
- **Angel Carracedo**, Galician Public Foundation of Genomic Medicine, Santiago University, Spain
- **Arcadi Navarro**, PompeuFabra University, Spain
- **Roderic Guigó**, CRG, PompeuFabra University, Spain
- **Mario Fraga**, IUOPA, HUCA, Oviedo University, Spain
- **Munir Pirmohamed**, Institute of Translational Medicine, UK
- **Gerard Siest,** European Society of Pharmacogenomics and Personalized Therapy
- **Adrián Llerena**, University of Extremadura, Spain
- **Mª Jesús Arranz**, Santa Creu i Sant Pau Hospital, Spain
- **Cristina Rodríguez-Antona**, CNIO, Spain
- **Joaquín Dopazo,** *Príncipe Felipe* Research Center, Spain
- **Carlos Lopez-Otín**, Oviedo University, Spain
- **Miguel Martín,** *Gregorio Marañon* University Hospital, Spain
- **Miquel Tarón,** Amadix, Spain
- **Manuel Hidalgo**, CNIO, Spain
- **Salvador Martín Algarra,** The Clinic of the University of Navarre, Spain
- **David Páez,** *Santa Creu i Sant Pau* Hospital, Spain
- **Howard McLeod,** UNC School of Medicine, US
- **Luis López-Fernández,** IiSGM, Spain
- **Mercé Brunet,** IDIBAPS, Barcelona University, Spain
- **Jesús García-Foncillas,** *Jiménez Díaz* Foundation, Autonomous University of Madrid, Spain

III DIA NACIONAL DEL SARCOMA
25 SEPTEMBER, 2015

- **Mª Angeles Díaz**, Sarcoma Patients Spanish Association
- **Javier Martín Broto**, Spanish Group for Sarcoma Research
- **Miguel Urioste**, Spanish National Cancer Research Centre
- **Javier Martinez Gutiérrez**, Mª Paz Jiménez Casado Foundation

BIOINFORMATICS AS A DRIVER OF INNOVATION
12 NOVEMBER, 2015

ORGANISERS
- **National Bioinformatics Institute (INB); Intel and Atos**

SPEAKERS
- **Craig Rhodes**, Intel Corporation
- **Natalia Jiménez**, Atos
- **Andy Smith**, ELIXIR
- **Alfonso Valencia**, ELIXIR and CNIO
- **Fátima Al-Shahrour**, CNIO
- **Ángela Del Pozo**, INGEMM, La Paz University Hospital
- **Rafael Navajo**, GMV
- **Jacques Beckmann**, Swiss Institute for Bioinformatic
- **Manuel Cendagorta-Galarza**, Institute for Technology and Renewable Energy
- **Carlos Flores**, *Nuestra Señora de la Candelaria* University Hospital
- **Robert Sugar**, Intel
- **Marisol Quintero**, Bioncotech Therapeutics
- **Manuel Pérez**, Institute of Genomic Medicine in Valencia

VI CONGRESO NACIONAL DE BIOBANCOS
18-19-20 NOVEMBER, 2015

ORGANISERS
- **IRBLleida and Biobanks Platform**, CNIO

SPEAKERS
- **Federico Rojo**, *investigador del Grupo Español de Investigación en Cáncer de Mama* (GEICAM)
- **Pilar Garrido**, *jefe de sección de Oncología Médica, Hospital Universitario Ramón y Cajal*
- **Eva López**, *Directora Médica de Oncología en Novartis*
- **Pilar Nicolás**, *Cátedra Interuniversitaria de Derecho y Genoma Humano. Universidad de Deusto*, Bilbao
- **Miriam Cuatrecases**, *coordinadora de la Red Catalana de Bancos de Tumores (XBTC)*
- **Alberto Villanueva**, *investigador responsable del Grupo de Resistencia Farmacológica y Xenografts del ICOIDIBELL*
- **Eva Colas**, *investigadora del grupo de patología oncológica del Instituto de investigación Biomédica de Lleida, IRBLleida*
- **Miguel Abal**, *investigador principal del laboratorio de Oncología Médica Traslacional, Complejo hospitalario universitario de Santiago/SERGAS*

- **Hartmut Juhl**, *fundador y Director Ejecutivo de INDIVUMED*
- **Daniel Gil**, *responsable del Área Social, Departamento de Comunicación, Farmaindustria. Academia Europea de Pacientes (EUPATI)*
- **Natacha Bolaños**, *responsable de relaciones internacionales y especialista en la rehabilitación de pacientes con cáncer. Grupo Español de Pacientes con Cáncer (GEPAC)*
- **Marta Puyol**, *responsable de Investigaciones Biomédicas, Fundación Científica Asociación Española Contra el Cáncer (AECC)*
- **Emilio Vargas**, *coordinador de la Plataforma de Ensayos Clínicos del Instituto de Salud Carlos III*
- **Alberto Rábano**, *responsable de Neuropatología y Banco de Tejidos de la Fundación Cien*
- **Julia del Amo Valero**, *red de investigación en SIDA, Red Coris, Centro Nacional de Epidemiología, Instituto de Salud Carlos III*
- **Ramon Maspons**, *coordinador de Innovación en la Agencia de Calidad y Evaluación Sanitarias de Cataluña" (AQuAS)*
- **Blanca Miranda**, *Directora del Biobanco del Sistema Sanitario Público de Andalucía*
- **Rosario Mata**, *Coordinadora Médica y de Asuntos Regulatorios de IATA (Iniciativa Andaluza en Terapias Avanzadas)*
- **Javier Montero**, *Director de la Oficina de Transferencia de Tecnología del Sistema Sanitario Público de Andalucía*
- **José Ramón Fernández**, *Director Gerente del Instituto de Investigación Biosanitaria de Granada*
- **Pilar Najarro**, *Directora de Operaciones de Life Length*
- **Julio Font**, *Director General de HistoCell*
- **Javier Campión**, *Director Científico de Making Genetics*

2ND MEETING OF THE MADRID MACROMOLECULAR STRUCTURAL CLUB
25 NOVEMBER, 2015

ORGANISERS
- **Santiago Ramón-Maiques, Daniel Lietha, Jasminka Boskovic**, CNIO, Madrid, Spain

SPEAKERS
- **Joahnne Le Coq**, CNIO, Madrid, Spain
- **Armando Albert**, IQFR-CSIC, Madrid, Spain
- **José R. Castón**, CNB-CSIC, Madrid, Spain

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, 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 technologists.

FLOW CYTOMETRY COURSE
9-10-11 FEBRUARY, 2015

SPEAKERS/ORGANISERS
- **Rui Gardner,** Institute Gulbenkian of Science, Portugal
- **Lola Martínez,** CNIO, Spain

CELL SORTING COURSE
12-13 FEBRUARY, 2015

SPEAKERS/ORGANISERS
- **Rui Gardner,** Institute Gulbenkian of Science, Portugal
- **Lola Martínez**, CNIO, Spain

HANDS-ON INTRODUCTION TO R 2015
1 JULY, 2015

ORGANISER
- **CNIO Bioinformatics**

SPEAKER
- **Ramón Díaz Uriarte,** Institute of Biomedical Research *Alberto Sols,* Spain

ACCES TO ENCODE DATA THROUGH THE UCSC GENOME BROWSER
4 NOVEMBER, 2015

ORGANISER
- **CNIO Bioinformatics**

SPEAKERS
- **Osvaldo Graña and David G. Pisano,** CNIO, Spain

INTRODUCTION TO FUNCTIONAL ANALYSIS OF GENE EXPRESSION EXPERIMENTS
25 NOVEMBER, 2015

ORGANISER
- **CNIO Bioinformatics**

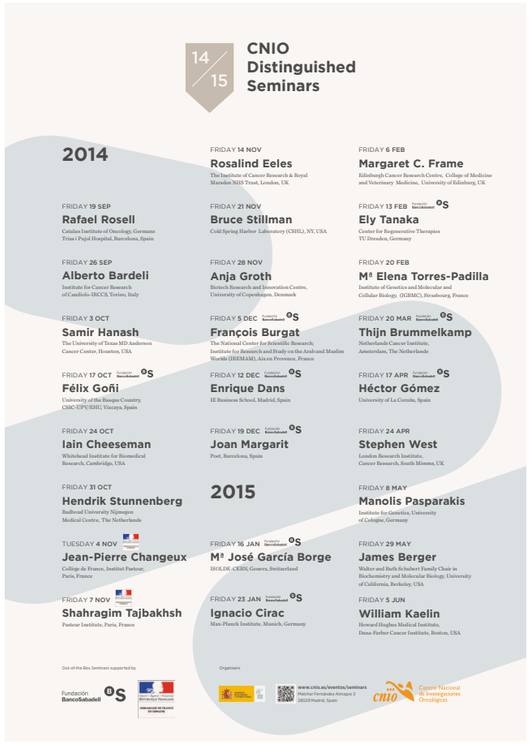
SPEAKERS
- **Gonzalo Gómez and Daniel Rico,** CNIO, Spain

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. This year, the French Embassy sponsored one of these seminars.

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 'outside-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 21 distinguished speakers in 2015.



DATE	SPEAKER	ORGANISATION	TITLE	
JANUARY				
16/01/2015	**María José García Borge**	ISOLDE, CERN, Geneva, Switzerland	The Nucleus: a trip to the heart of the matter, and its societal applications	Fundación BancoSabadell
23/01/2015	**Ignacio Cirac**	The Max Planck Institute, Munich, Germany	Quantum Physics: From the Schrödinger cat to the most powerful computers	Fundación BancoSabadell
FEBRUARY				
06/02/2015	**Margaret C Frame**	Edinburgh Cancer Research Centre, College of Medicine and Veterinary Medicine, University of Edinburg, UK	Imaging and targeting cancer processes	Fundación BancoSabadell
20/02/2015	**Maria Elena Torres Padilla**	Institute of Genetics and Molecular and Cellular Biology, (IGBMC), Illkirch France	Epigenetic mechanisms in early mammalian development	
MARCH				
20/03/2015	**Thijn Brummelkamp**	Netherland Cancer Institute, Amsterdam, The Netherlands	Haploid genetic screens in human cells to study disease-relevant processes	Fundación BancoSabadell
APRIL				
24/04/2015	**Stephen West**	London Research Institute, Cancer Research UK	Regulatory control of DNA strand break repair and links to human disease	
MAY				
08/05/2015	**Manolis Pasparakis**	Institute for Genetics, University of Cologne, Germany	RIP kinases in cell death, inflammation and cancer	
22/05/2015	**Luigi Fontana**	Washington University School of Medicine, USA	Promoting Health and Longevity through Diet: Metabolic and Molecular Mechanisms	
29/05/2015	**James Berger**	Johns Hopkins University School of Medicine, Baltimore, USA	Running rings (and spirals) around DNA: molecular mechanisms for initiating replication	
JUNE				
19/06/2015	**Elly Tanaka**	Center for Regenerative Therapies Dresden - CRTD, Germany	Proliferation to patterning during vertebrate limb regeneration	Fundación BancoSabadell
SEPTEMBER				
04/09/2015	**James Hurley**	University of California, Berkeley, USA	From HIV pathogenesis to coated vesicles, and back again	
11/09/2015	**Roger Williams**	MRC Laboratory of Molecular Biology, Cambridge, UK	Structures and dynamics of phosphoinositide 3-kinase complexes in cellular signalling and sorting	
18/09/2015	**Siamon Gordon**	University of Oxford, UK	Macrophage receptors and immune interactions	
25/09/2015	**Megan C. King**	Yale University, New Haven, USA	The cell biology of DNA repair	

OCTOBER				
02/10/2015	**William C. Hahn**	Dana-Farber Cancer Institute, Boston, USA	Integrated functional approaches to identify cancer targets	
09/10/2015	**Eduard Batlle**	Institute for Research in Biomedicine (IRB Barcelona), Spain	Connecting Intestinal Stem Cells to Colorectal Cancer	
30/10/2015	**Hugues de Thé**	University Institute for Haematology, Paris, France	Curing APL through therapy-induced PML/RARA degradation	
NOVEMBER				
20/11/2015	**Lee Zou**	Harvard Medical School, The Jim & Ann Orr MGH Research Scholar, Boston, US	Sensing and Signaling DNA Damage by Checkpoint Pathways	
27/11/2015	**Angel Lanas Arbeola**	Research Health Institute of Aragon, Zaragoza, Spain	Aspirin and colorectal cancer: Clinical impact and mechanisms of action	
DECEMBER				
04/12/2015	**Carlos Caldas**	University of Cambridge, UK	Characterizing and modelling breast cancer heterogeneity	
18/12/2015	**Robert Schwabe**	Columbia University, New York, USA	How Liver Injury Leads to Fibrosis and Cancer	

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 58 *ad-hoc* seminars were organised by CNIO researchers in 2015.

DATE	SPEAKER	ORGANISATION	TITLE	
JANUARY				
13/01/2015	**Fabio Rinaldi**	Institute of Computational Linguistics, University of Zurich, Switzerland	Large-scale biomedical text mining for knowledge discovery	
27/01/2015	**Tyler Alioto**	Genome Assembly and Annotation Team Leader. CNAG. Barcelona, Spain	A comprehensive assessment of somatic mutation calling in cancer genomes	
29/01/2015	**Monika Hegi**	University of Lausanne, Switzerland	Epigenetic deregulation in glioma, biomarkers and new opportunities	
30/01/2015	**Tony Mok**	The Chinese University of Hong Kong, China	Blood based genomic biomarkers for lung cancer	
30/01/2015	**Joan Vila Domenech**	REGICOR, Barcelona, Spain	*Generación de informes reproducibles utilizando R y LaTeX*	
FEBRUARY				
16/02/2015	**Ignacio I. Wistuba**	Anderson Clinical Faculty Chair for Cancer Treatment and Research - Department of translational Molecular Pathology - UT MD Anderson Cancer Center, Houston, USA	Molecular pathogenesis of lung cancer	
17/02/2015	**Flora de Pablo**	CSIC, Madrid, Spain	Sex, Science and Society: a triangle that matters?	WISE SEMINAR
24/02/2015	**Guillaume Filion**	CRG, Barcelona, Spain	Promoters interpret the chromatin context in different ways	

MARCH				
10/03/2015	**Maria Carmen Vela Olmo**	Secretariat of State of Research, Development and Innovation, Ministry of Economy and Competitiveness, Madrid, Spain	A professional career with a gender perspective	WISE SEMINAR
26/03/2015	**Manuel Fernando Garavito**	University of the Andes, Bogotá, Colombia	Targeting the pyrimidine metabolism in a devastating plant pathogen	
27/03/2015	**Nuria Flames**	Biomedical Institute of Valencia, (IBV-CSIC), Valencia, Spain	Regulatory logic of serotonergic neuron terminal differentiation in *C.elegans*	
APRIL				
10/04/2015	**Roel Verhaak**	Department of Genomic Medicine, and the Department of Bioinformatics and Computational Biology at the UT MD Anderson Cancer Center in Houston, USA	Genomic characterization of disease evolution in glioma	
10/04/2015	**Alan Clarke**	Cardiff University, UK	PI3k and Wnt pathway driven neoplasia modelled in the mouse	
13/04/2015	**Curtis Harris**	National Cancer Institute, Bethesda, USA	Interweaving the threads of p53, microRNA, DNA methylation and inflammation networks into the tapestry of cancer and aging	
14/04/2015	**Margarita Salas Falgueras**	CBMSO, Madrid, Spain	My life with phage ø29	WISE SEMINAR
16/04/2015	**Liset Menendez de la Prida**	Instituto Cajal-CSIC, Madrid, Spain	Electrophysiological biomarkers of epileptogenesis	
16/04/2015	**Alejo Efeyan**	MIT, Cambridge, USA	Physiology of nutrient sensing by mTOR	
23/04/2015	**Jean Pierre David**	Universitäsklinikum Hamburg, Germany	From teeth to toes, from arthritis to tumor, in the skeleton, Rsk2 makes it all	
MAY				
05/05/2015	**Robert Loewe**	Medical University of Vienna, Department of Dermatology, Austria	Identification of a chemokine profile associated with melanoma progression	
06/05/2015	**Katherine Hoadley**	Lineberger Comprehensive Cancer Centre, UNC Chapel Hill, USA	TCGA pan-cancer subtype analysis	
14/05/2015	**Jesús Rojo**	The Marie Skłodowska-Curie Action (MSCA) Spanish National Contact Point. Fundación para el Conocimiento madri+d	2015 call from the EU-funded Horizon 2020 programme, Marie Skłodowska-Curie Actions (MSCA) – Individual Fellow (IF)	
14/05/2015	**Caroline Beckett & Joanne Worobec**	Sigma-Aldrich Corporation, St. Louis, USA	Targeted genome editing using CRISPR technology	
21/05/2015	**Carme Caelles**	Biochemistry & Molecular Biology. School of Pharmacy. University of Barcelona, Spain	Regulation of insulin signaling by JNK: consequences on systemic insulin resistance	
27/05/2015	**Erica Sloan**	Monash Institute of Pharmaceutical Sciences, Monash University Faculty of Pharmacy, Victoria, Australia	Beta-blockade of cancer: repurposing old drugs to block metastasis	

JUNE				
08/06/2015	**Josh Denham**	ACRISP Research Associate, PhD student, Accredited Exercise Physiologist (ESSA) Federation University Australia, Faculty of Science, Victoria, Australia	The influence of exercise training on leukocyte telomere length and DNA methylation in humans	
09/06/2015	**María del Mar Martínez**	Director at McKinsey's Madrid Office, Spain	Women at the top: making it happen	WISE SEMINAR
11/06/2015	**Bruno Amati**	Italian Institute of Technology (IIT) and European Institute of Oncology (IEO), Milan, Italy	Myc-driven transcriptional programs in tumor development: toward new therapeutic opportunities	
17/06/2015	**Sergey Grivennikov**	Fox Chase Cancer Center, Philadelphia, USA	Microbial and cytokine drivers of tumor elicited inflammation in colorectal cancer	
26/06/2015	**Fernando Moreno-Herrero**	Spanish National Biotechnology Centre (CSIC), Madrid, Spain	Deconstructing molecular machines using single molecule methods	
JULY				
09/07/2015	**John Harrington & Ángel López**	IBM-Watson	IBM Watson Oncology: "A rapid way to accelerate human expertise in oncology"	
10/07/2015	**Rudolf Zechner**	Institute of Molecular Biosciences. Karl Franzens Universität Graz, Graz, Austria	Lipolysis: intersection between metabolism and signaling	
22/07/2015	**Pau Rué**	University of Cambridge. Department of Genetics. Cambridge. UK	Cell fate decision-making by the numbers: development and differentiation	
23/07/2015	**Laura Soucek**	Vall D'Hebron Institute of Oncology, Barcelona, Spain	How to target the "undruggable": inhibiting Myc in cancer	
24/07/2015	**Chaitanya R. Divgi**	Columbia University Medical Centre, New York, USA	Molecular imaging of the cancer phenotype	
31/07/2015	**Mª Dolores Alonso Guirado**	Bioinformatics and Biostatistics Service, Biological Research Center, CSIC, Madrid, Spain	Genome assembly of a filamentous fungus from Illumina sequencing reads using different approaches	
AUGUST				
21/08/2015	**Ana Barat**	Dublin City University, Ireland	Bioinformatics approaches for chromatin, development and cancer research	
SEPTEMBER				
03/09/2015	**Daniel Gianola**	Department of Animal Sciences, Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, USA	Prediction of complex traits	
21/09/2015	**Biola María Javierre**	Babraham Institute, Nuclear Dynamics Programme, Cambridge, UK	Genomic regulatory architecture in human haematopoiesis links disease-associated variants with their target genes	
24/09/2015	**Lene Uhrbom**	Uppsala University, Sweden	A cell of origin-based strategy to decipher glioblastoma biology	
24/09/2015	**Esteban Gurzov**	The University of Melbourne, Australia	Protein tyrosine phosphatases: molecular switches in pathology	

OCTOBER				
13/10/2015	**Sandra Rodríguez Perales**	Molecular Cytogenetics Unit, Human Cancer Genetics Programme	Human genome engineering tools and other new technologies available at the CNIO Cytogenetics laboratory	
14/10/2015	**Ralf Paus**	Centre for Dermatology Research, University of Manchester, UK	Translational immunology of the hair follicle: excursions into terra incognita	
16/10/2015	**Joan Seoane**	Vall d'Hebron Institute of Oncology, Barcelona, Spain	Intratumor Heterogeneity in Glioblastoma	
23/10/2015	**Patrick Sung**	Yale University School of Medicine, New Haven, Connecticut, USA	Role of the BRCA2-DSS1 Complex in Homologous Recombination	
NOVEMBER				
03/11/2015	**Sven Pettersson**	Karolinska Institutet, Stockholm, Sweden	Decoding of microbiome mediated mechanisms that tune mammalian host biology	
04/11/2015	**Theresa Guise**	Indiana University, Indianapolis, USA	Molecular mechanisms of muscle weakness associated with bone metastases	
10/11/2015	**Liliana Mellor**	North Carolina State University, Raleigh, USA	Cartilage degeneration and regeneration: Potential use of adipose-derived stem cells in osteochondral tissue engineering	
16/11/2015	**Pierre Savatier**	Stem Cell and Brain Research Institute, Bron, France	Naive pluripotent stem cells and chimeric competency in human and non-human primates	
23/11/2015	**Dimitrios Morikis**	University of California, Riverside, US	Evolution of electrostatics in a link between innate and adaptive immunity	
23/11/2015	**Ludmila Prokunina-Olsson**	National Institutes of Health (NIH), Rockville, USA	Exploring the bladder cancer GWAS signals: decoding the message and translating it into molecular mechanisms and clinical applications	
24/11/2015	**Natalia González-Valdés**	Corporate Communications and CSR (Corporate Social Responsibility) Director of L'Oreal Spain	Women in Business and Science: two parallel paths	WISE SEMINAR
26/11/2015	**Roberto Buccione**	EMBO Molecular Medicine, Heidelberg, Germany	Data reproducibility, research integrity and the EMBO Press transparent editorial process	
DECEMBER				
01/12/2015	**Graham Robertson**	School of Molecular Biosciences, University of Sydney, Australia	Tumorkines promote dysregulated metabolism in fat and liver in cancer cachexia	
14/12/2015	**Dimitrios Morikis**	BioMoDeL; University of California, US	Discovery of Complement System Biomarkers using Virtual Screening	
15/12/2015	**Prem Premsrirut**	Mirimus, Inc., a spin-off company of Cold Spring Harbor Laboratory and Stony Brook University School of Medicine, NY, US	RNAi and CRISPR/Cas9-based advanced *in vivo* models for drug discovery	
21/12/2015	**Paula Gutiérrez-Martínez**	Children's Hospital Boston, Harvard Medical School, US	Diminished apoptotic priming underlies increased survival of aged hematopoietic stem cells in response to DNA damage	
21/12/2015	**Samuel Peña Llopis**	The University of Texas Southwestern Medical Center, Texas, US	Molecular genetic classification of renal cell carcinoma and beyond	
22/12/2015	**Silvia Vega-Rubín de Celis**	The University of Texas Southwestern Medical Center, Texas, US	Tumorigenesis regulation by autophagy	

SCIENTIFIC DIVULGATION EVENTS

RESEARCHERS' NIGHT
25 SEPTEMBER, 2015

This year, the CNIO participated in 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 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 25, 2015) and learn about cancer research. The activities were entirely organised by voluntary contributions from 30 young researchers, and 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
2-15 NOVEMBER, 2015

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 (XV *Semana de la Ciencia*, November 2–15, 2015). In 2015, 50 people participated in guided visits to the Centre's research facilities.

GEPAC

This year, the CNIO participated for a third time in the annual meeting of the Spanish Group of Patients with Cancer (GEPAC), whose membership includes foundations such as the CRIS Foundation against cancer, AEAL, AECAT and the Sandra Ibarra Foundation – all supporters of the CNIO. This large congress, held in Madrid, is open to the members of the general public who are affected by or interested in cancer. Various societies, interest groups and pharmaceutical companies affiliated with oncology also participate in this event. It was a privilege for us to participate with our stand for the third year running. The idea was to be present so that we could answer people's questions about cancer research and the latest developments.

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 2015, more than 498 people participated in such guided visits; most of them were ESO and *Bachillerato* student groups, but also professionals in the health sector.

JORNADA DÍA MUNDIAL DEL CÁNCER
4 FEBRUARY, 2015

On the 4th of February, the CNIO celebrated World Cancer Day by hosting an open-doors day; the event, sponsored by Bristol-Myers Squibb, welcomed patients, associations, relatives and anyone with an interest in learning more about recent advances in cancer research.

The CNIO Director, Maria Blasco, opened the event by introducing the Centre and its lines of research. Afterwards, a first debate entitled entitled 'Cancer research: prevention and treatment' was held by with Óscar Fernández-Capetillo, Head of the Chromosome Instability Group, and the oncologist Marta Blanco, from the Spanish Association Against Cancer (AECC). A second talk, entitled 'The future of cancer research and clinical oncology', was led by Manuel Hidalgo, the Director of CNIO's Clinical Research Programme, and Eduardo Díaz-Rubio, the Director of the Medical Oncology Service at the San Carlos Clinical University Hospital. After the talks, the registered attendees had the opportunity to visit the Centre's facilities.

BCNMOMENTS
23 JULY, 2015

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, the students had the chance to visit the labs guided by young scientists.

INNOVACIÓN PUENTE ENTRE CIENCIA Y SOCIEDAD
19 OCTOBER, 2015

On the 19th of October, we hosted the 'Innovation: Bridge between Science and Society' event at the CNIO, together with the Banco Santander Foundation and the *Instituto de Empresa* Business School. The event consisted of a dialogue between the Autonomous University of Madrid professor and former Minister of Education, Ángel Gabilondo, and the CNIO Director, Maria Blasco; they highlighted the CNIO's commitment to innovation and the translation of scientific knowledge for the benefit of society

ADMINISTRATION

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Director General de la Fundación Bancaria Caixa d' Estalvis i Pensions de Barcelona, "la Caixa"

· **Rafael Pardo Avellaneda**
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Director de la Fundación BBVA

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Presidente del Grupo PRISA

· **Pío Díaz de Tuesta Vázquez**
Legal Adviser, *Caja Madrid* Foundation
Aseor Jurídico de la Fundación Caja Madrid

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· **Margarita Blázquez Herranz**
Deputy Director General for Networks and Cooperative Research Centres, 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, 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 213, 353 Euros. This amount was received as base salary, seniority, small bonuses.
- Members of the CNIO Board of Trustees are not remunerated.

SCIENTIFIC ADVISORY BOARD

- **Mariann Bienz**, PhD, FRS, FMedSci (Chair)
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  Division of Protein and Nucleic Acid Chemistry
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  Max Planck Institute for Molecular Physiology
  Dortmund, Germany

MANAGEMENT

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	SCIENTIFIC EVENTS	**Moro, Mercedes** Head	
	SCIENTIFIC PUBLISHING	**Cerdá, Sonia** Head	
	LIBRARY & ARCHIVES	**López, Victoria** Head	
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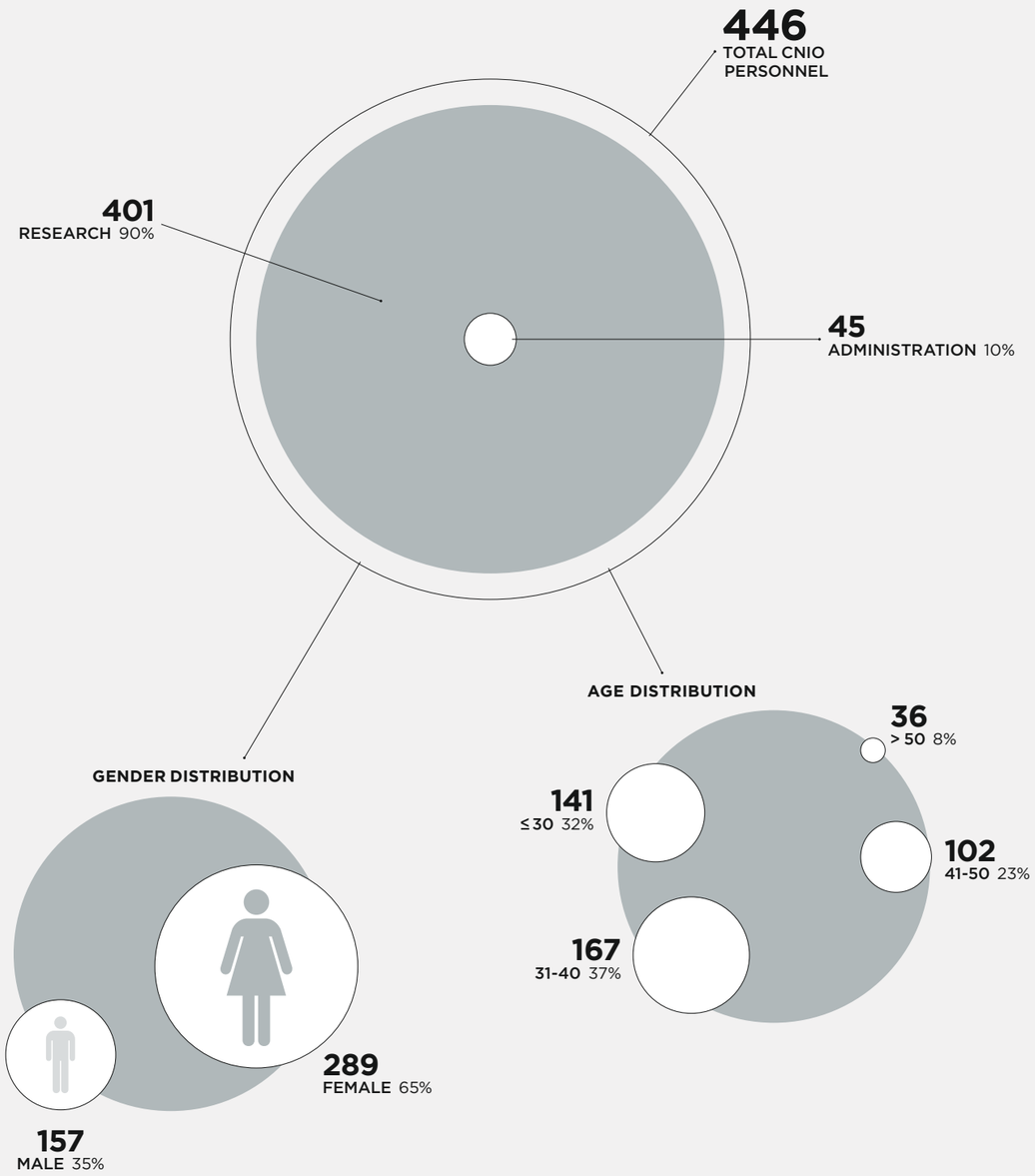
	HUMAN RESOURCES	**Pérez, José Lorenzo** Head **Bardají, Paz**	**Carbonel, David** **Martín, Francisco**
	ECONOMIC MANAGEMENT	**Salido, M. Isabel** Head **Galindo, José Antonio** **García, Juan J.**	**Rodríguez, M. José** **Fernández, Rut** (since December)*
	AUDIT	**García-Risco, Silvia** **Hernando, M. Elena**	**Doyagüez, Laura** (since December)*
INFRASTRUCTURE MANAGEMENT	**de Dios, Luis Javier** Director		
	MAINTENANCE	**Vicente, Miguel** (Head, until May) **Garrido, Fernando** (Head, since December)	
	PREVENTION & BIOSECURITY	**Cespón, Constantino** Head	**Bertol, Narciso**
	INFORMATION TECHNOLOGIES	**Fernández, José Luis** Head **de Miguel, Marcos**	**Echeverría, Rebeca** (since December)*
EXTRAMURAL CLINICAL RESEARCH	**López, Antonio** Director		

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

CNIO PERSONNEL 2015



SCIENTIFIC PERSONNEL 2015

TOTAL SCIENTIFIC PERSONNEL 100% **401**

DISTRIBUTION BY PROGRAMMES

STRUCTURAL BIOLOGY AND BIOCOMPUTING 11%	44
BIOTECHNOLOGY 12%	48
CANCER CELL BIOLOGY 10%	40
HUMAN CANCER GENETICS 12%	47
CLINICAL RESEARCH 17%	68
MOLECULAR ONCOLOGY 29%	118
EXPERIMENTAL THERAPEUTICS 9%	36

DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 11%	44
GRADUATE STUDENTS 23%	94
STAFF SCIENTISTS 17%	69
PRINCIPAL INVESTIGATORS 13%	51
TECHNICIANS 36%	143

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS	FEMALE	61%	**27**
	MALE	39%	**17**
GRADUATE STUDENTS	FEMALE	70%	**66**
	MALE	30%	**28**
STAFF SCIENTISTS	FEMALE	65%	**45**
	MALE	35%	**24**
PRINCIPAL INVESTIGATORS	FEMALE	37%	**19**
	MALE	63%	**32**
TECHNICIANS	FEMALE	73%	**104**
	MALE	27%	**39**
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**261**
	MALE		**140**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL 100% **401**

POST-DOCTORAL FELLOWS 13%	27
GRADUATE STUDENTS 33%	67
STAFF SCIENTISTS 18%	37
PRINCIPAL INVESTIGATORS 11%	23
TECHNICIANS 24%	48
TOTAL 100%	202

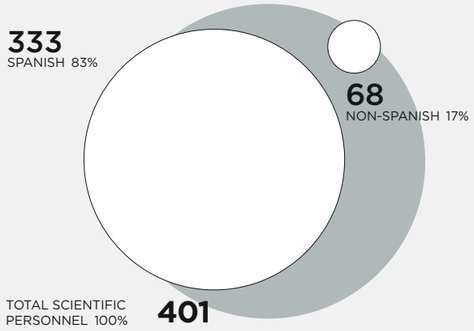
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

POST-DOCTORAL FELLOWS 14%	16
GRADUATE STUDENTS 20%	23
STAFF SCIENTISTS 17%	20
PRINCIPAL INVESTIGATORS 12%	14
TECHNICIANS 37%	42
TOTAL 100%	115

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

POST-DOCTORAL FELLOWS 1%	1
GRADUATE STUDENTS 5%	4
STAFF SCIENTISTS 14%	12
PRINCIPAL INVESTIGATORS 17%	14
TECHNICIANS 63%	53
TOTAL 100%	84

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

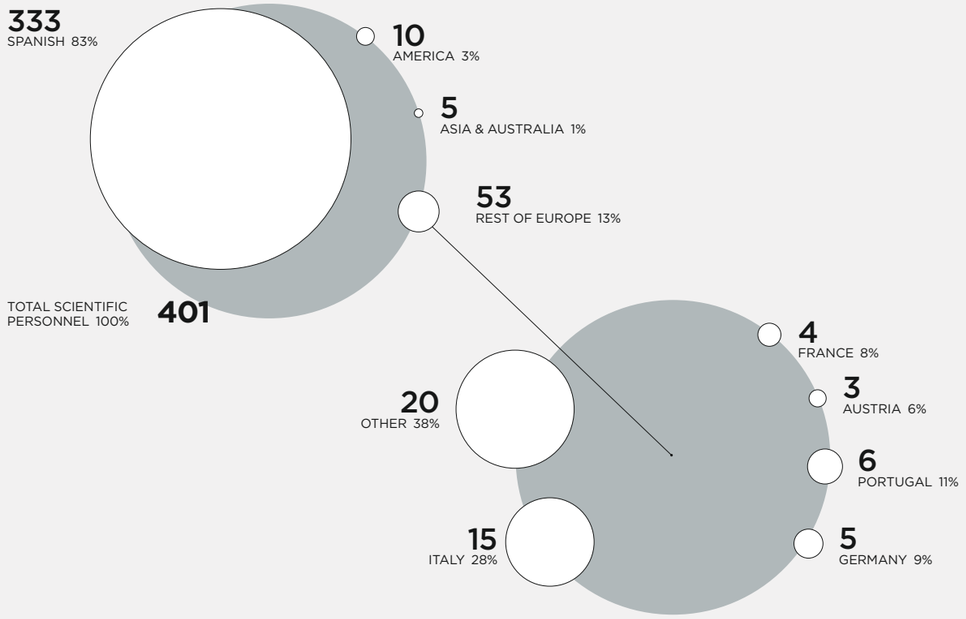


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 32%	14
GRADUATE STUDENTS 27%	25
STAFF SCIENTISTS 22%	15
PRINCIPAL INVESTIGATORS 10%	5
TECHNICIANS 1%	9

Total foreign scientific personnel 68
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

CNIO FRIENDS

One of the most exciting adventures we faced in 2015 was the implementation of the 'CNIO Friends' initiative, a philanthropic platform launched in late 2014 aimed at involving all social stakeholders in cancer research.

Under the slogan 'More research, less cancer', the CNIO is seeking a way to better fight the disease: through research and with everyone's commitment, we shall combat cancer. The initiative not only responds to the need to appeal for economic support, but also aims at sharing, with the general public, the knowledge generated at a top-level centre like the CNIO; knowledge that can be used in medicine in the future.

This year, more than 560 friends, benefactors and sponsors have placed their trust in and directed their generosity towards the Centre's research projects. The figures are extremely good and we hope to be able to continue progressing over the course of the next few years just as we have done to date. All our supporters can follow the Centre's latest discoveries and developments through our 'CNIO Friends' newsletter, which was launched at the beginning of the year every other month, as well as enjoy other benefits. These benefits come in the shape of guided visits to the centre or the inscription of their names on the seats of the Auditorium, one of the most emblematic and special places in the Centre where researchers discuss and share new ideas and discoveries that revolutionise biomedicine day after day; this gesture enables our friends to symbolically partake in this important activity.

To promote our message, the Centre announced the launch of the 'CNIO Friends' social networks in February, which is aimed at creating a community and to connect with those who are fully acquainted with cancer research. Many messages of encouragement, support and congratulations are received each day through these channels; especially via our Facebook page, which, in only 10 months, has exceeded 26,000 followers and reached more than 44,600,000 people.

In June, after 6 months of the initiative's launch, and to celebrate the occasion, the Centre paid tribute to its donors with a video that enabled them to explain the reasons that moved them to contribute so generously to the Centre's research projects. The video, produced by the visual artist Amparo Garrido, has been watched almost 23,500 times on YouTube channels. In October, CNIO reached an agreement with RENFE (the Spanish rail transport operator), which resulted in the video production travelling the length and breadth of Spain in December by being broadcasted in its high speed - long distance trains.

"A dose of solidarity against cancer"

Thanks to all the contributions received last year, the CNIO will launch 'CNIO Friends' contracts in 2016; a programme aimed at postdoctoral researchers developing new projects against cancer. Almost half of all tumours can be cured today, but there is still a long way to go. 'CNIO Friends' will serve to unite everyone's efforts in this important task.

“We must support research studies and the work of researchers. In Spain, these Centres of Excellence are essential; they should not only exist, but also have steady funding to carry out their work.”

JAVIER GÁLLEGO

I’m investing in something that is being constructed and will endure over time. I have confidence in the team of researchers, whom I trust fully. They are a team of highly qualified professionals, and on a humane level it is number one.

CRUZ DÍAZ

“I felt I also had to do my bit to help. It gives you a sense of self-esteem and personal satisfaction to say: I am contributing a little to our society. A way of giving back to society in return for all it has provided me in other ways.”

NEMESIO CARRO

“I donate with my family. We consider research is a fundamental pillar for the advancement of society as a whole. You feel a certain satisfaction in saying: “They are doing something, partly ‘in memory of’, but that will also benefit future generations to come.”

MARINA LIMIÑANA

SUPER FRIEND MARCOS

“Every Euro invested on this is a Euro invested in happiness. The other day I read that the CNIO is one of Europe’s leading Cancer Research Centres. And it is in Madrid, so you don’t have to go too far to collaborate here.”

MARCOS ARGUMOSA

In April, the sports world allied its efforts with the CNIO when the athlete Marcos Argumosa, our Super Friend Marcos, began a difficult but not impossible challenge: to run 10 consecutive marathons from Santander (Cantabria) to Madrid — 420 km in 10 days — to raise funds in support of ‘CNIO Friends’. All the companies and individuals interested in supporting Marcos could do so by purchasing kilometres through our online platform.

During the challenge, Marcos was not alone and received the support of city councils and provincial governments. On the 9th marathon, the finish line was set up at the CNIO headquarters with the support of the *Banco Sabadell* Foundation. That same day, CNIO scientists, including the Director, also grabbed their shoes and joined him for the last kilometres of the challenge.

As his endeavour received attention in the media, both in local radio stations and the news bulletins of Spain’s main TV channels, the engagement of new donors was boosted during those days, and in the weeks and months to come. We thank Marcos and our friends for his generosity and inspiration to help us spread the word of the initiative.

‘CONSTANTES Y VITALES’ CAMPAIGN

Héctor Peinado receives €100,000 from ‘Constantes y Vitales’ to support metastasis research at the CNIO. Pictured at the check presentation, from left, are: Josep Alfonso, Director of the ‘Fundación AXA’; Héctor Peinado, Group Leader of the CNIO Microenvironment and Metastasis Group; Maria Blasco, CNIO Director; and Mario López, *Director de Antena de laSexta*.

Another one of our achievements in 2015 was the agreement established with the TV & Media Group ‘Atresmedia’, one of the leading media groups in Spain. Through this agreement, ‘Constantes y Vitales’ – the social responsibility initiative of the TV channel *laSexta* and the AXA Foundation – launched the engaging action *#CadaPasoEsVital* (#EachStepMatters) with the aim of raising €100,000 in support of metastasis research at the CNIO led by Héctor Peinado, Group Leader of the Microenvironment and Metastasis Group. This campaign received enormous support from the community: more than 6,000 people donated 650,000 exercise kilometres through this platform, raising €100,000 destined to fund metastasis research led by Héctor Peinado at the CNIO.

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 €400,000 this year); in doing so they have contributed to society for generations to come.

BENEFACTOR FRIENDS/SPONSOR FRIENDS

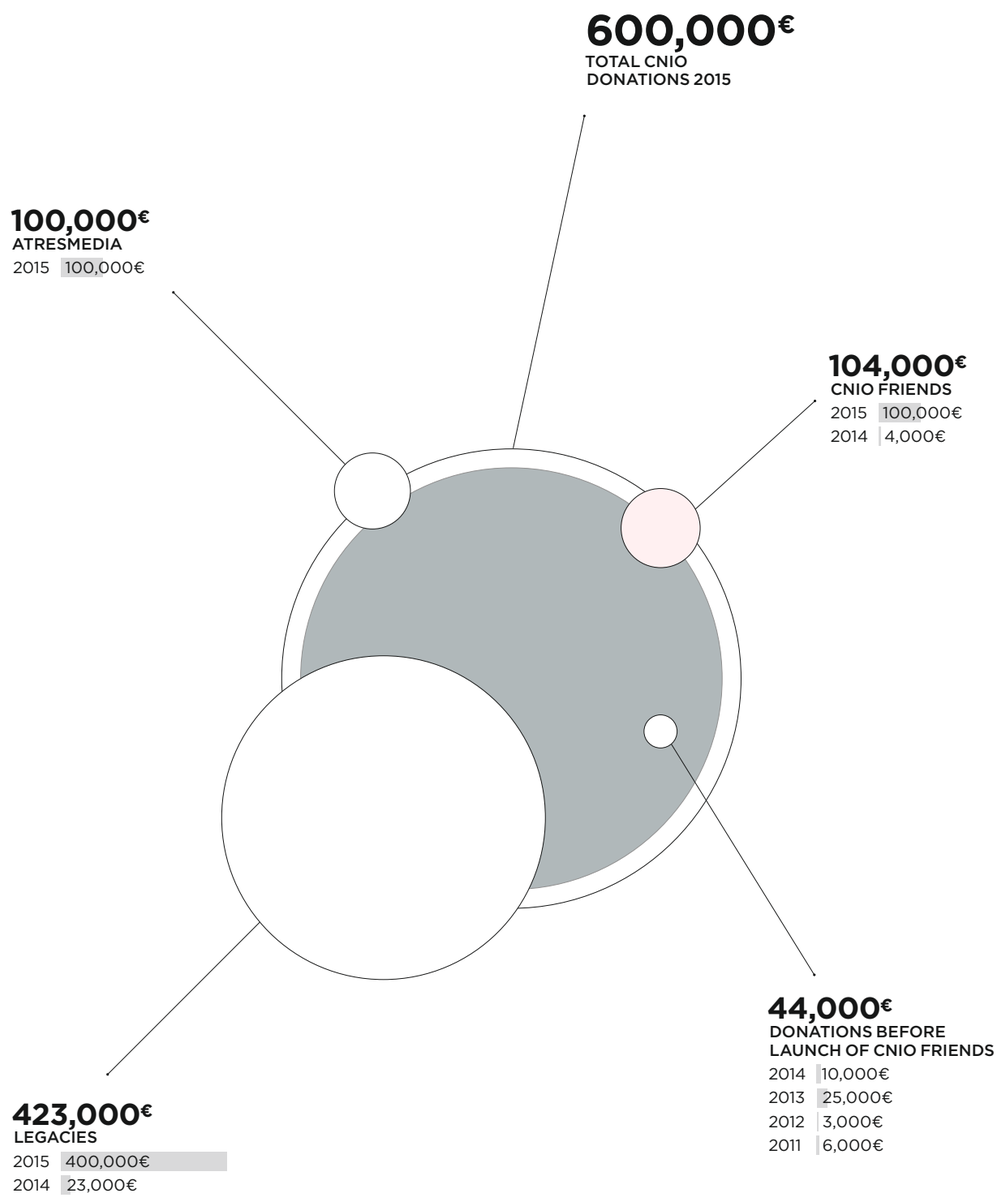
→ Benefactor Friends

- **Alberto Otero Hermida**
  Bilbao, Vizcaya
- **Alfonso Agüera Nieto**
  Santa Ana-Cartagena, Murcia
- **Amando Palomino López**
  Cáceres, Cáceres
- **Andrés Sánchez Arranz**
  Madrid, Madrid
- **Andrés Viedma Medina**
  Jaén, Jaén
- **Concepción Garófano Obregón**
  San Fernando, Cádiz
- **Diana Limiñana Gregori**
  Mutxamel, Alicante
- **Francisco Javier Gállego Franco**
  Barbastro, Huesca
- **Gema Rubio González**
  Madrid, Madrid
- **José Carlos Fernández Martínez**
  Madrid, Madrid
- **José Limiñana Gregori**
  Mutxamel, Alicante
- **José Limiñana Valero**
  Alicante, Alicante
- **Jesús Miguel Iglesias Retuerto**
  Valladolid, Valladolid
- **Lesley Jackman**
  Club femenino social, Castalla, Alicante
- **Luisa Vázquez Bejarano**
  Leganés, Madrid
- **Marcelino Cordero Hernández**
  Madrid, Madrid
- **María Alonso Vaquero**
  Madrid, Madrid
- **María Begoña Toca**
  Irún, Guipúzcoa
- **María del Carmen Pérez Laborda**
  Murcia, Murcia
- **María Dolores Díaz Almagro**
  Sevilla, Sevilla
- **María Rodríguez López**
  Celada de los Calderones, Cantabria
- **Marina Limiñana Gregori**
  Alicante, Alicante
- **Miguel Ángel Fernandez de Betoño Villanueva**
  Madrid, Madrid
- **Nemesio Carro Carro**
  León, León
- **Patricia Limiñana Gregori**
  Alcoi, Alicante
- **Santiago Rodríguez Uriel**
  Bruselas, Bélgica

→ Sponsor Friends

- **Ana Cristina Jardón Ruiz**
  Madrid, Madrid
- **Maria Rosa Fernández García**
  Selaya, Cantabria

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 authority, the Coca-Cola Foundation, and the *Unicaja* Foundation, among many others. Most recently, her latest exhibition at the *Romantic Museum* in Madrid, "*Tiergarten*" – a romantic German garden – a project that shows the relationship between contemporary art and romanticism, has received numerous praises and recognition.

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* 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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Edition **Sonia Cerdá and An Devriese**
Supervision **Isabel Barthelemy**
Text, data and figures **CNIO Faculty and Management**
Publication database **Victoria López**
Photography **Amparo Garrido**
Design **underbau**

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ANNUAL REPORT 2015

Centro Nacional de Investigaciones Oncológicas

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