SPANISH NATIONAL CANCER RESEARCH CENTRE, CNIO

SCIENTIFIC REPORT 2013

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Oncológicas

SCIENTIFIC REPORT 2013

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“2013 has been the most productive year in more than 10 years of CNIO´s history, both in terms of scientific discoveries and innovation achievements. This success is to be credited to CNIO scientists.”

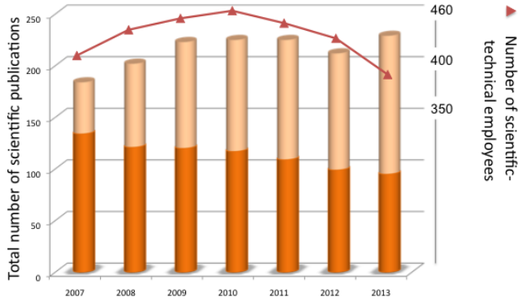
MARIA A. BLASCO
Director

FOREWORD

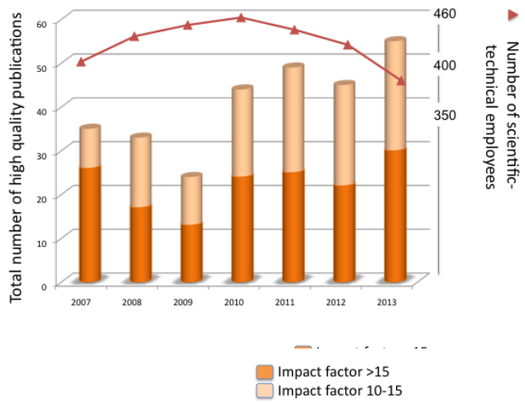
Maria A. Blasco Director

First of all, I would like to take this opportunity to thank all of those who have once again collaborated in the elaboration of this year's Annual Report. My special thanks goes out to the Scientific Management team that was heavily involved in its production, and in particular to Ana Merino and Sonia Cerdá, who once more demonstrated their efficiency and professionalism. I also wish to thank our external collaborators, Amparo Garrido (photography) and Underbau (design team).

I am very glad to start by saying that 2013 has been the most productive year in more than 10 years of CNIO's history, both in terms of scientific discoveries and innovation achievements. This success is to be credited to CNIO scientists; this year their scientific performance has excelled to an even greater extent.



In 2013, the CNIO published a total of 229 papers, 55 of which were published in journals with impact factors in the range of 10 to 15 and >15. This represents a 22% increase in high impact factor publications compared to 2012. This excellent performance consolidates our impressive output of scientific publications in top journals since 2010.



We are particularly proud of the groundbreaking discovery made by CNIO researchers from the Tumour Suppression Group, led by Manuel Serrano, who achieved *in vivo* generation of pluripotent stem cells; this important step forward in the field of regenerative medicine was published in *Nature* and was considered as one of the "notable advances" of 2013 by *Nature Medicine*. Serrano's Group deserves double congratulations, seeing as they also participated in the discovery of a role for cellular senescence in embryonic development, where, together with apoptosis, it plays an essential role in final organ design and functionality. This is a key discovery for the cellular senescence field; the study was published in *Cell*.

Erwin Wagner's Group alone published several papers in a variety of top journals (*Cell Metabolism*, *Immunity*, *Genes & Dev*, among others), strengthening our understanding on the role of inflammation in several types of cancer. CNIO scientists also made significant contributions in high-throughput cancer genome analysis through landmark papers published by the CNIO Groups led by Francisco Real on bladder cancer and Javier Benitez on breast and ovarian cancer; both were published in *Nature Genetics*.

2013 has also been outstanding in terms of CNIO translational research activities. In addition to consolidating the in-house Clinical Research Units of the Clinical Cancer Research Programme, directed by Manuel Hidalgo, the CNIO has signed agreements to create new Associated Clinical Units in two Hospitals of the Regional Government of Madrid, including the Paediatric Hospital *Hospital Niño Jesús* and the *Fundación Jiménez-Díaz*. We are particularly proud of launching an early phase Paediatric Clinical Trial Unit at the *Hospital Niño Jesús*. Paediatric Clinical Trials Units are scarce in Spain and the one led by CNIO oncologists will be the first early phase trial in Madrid. Moreover, CNIO oncologists are coordinating two large, country-wide, Clinical Trials Networks in Breast and Prostate Cancer.

In addition to the Clinical Research Programme, the CNIO performs important clinical activity through the Familiar Cancer Unit located at the *Hospital Universitario de Fuenlabrada*, as well as through the Molecular Diagnostics Unit and the Molecular Cytogenetics Group at CNIO; we provided genetic counselling to more than 160 patients and performed almost 1000 genetic determinations.

During 2013, the Experimental Therapeutics Programme (ETP) worked closely together with our researchers in order to validate new therapeutic targets. The Programme's current projects have attracted the interest of several companies who are interested in developing them into clinical candidates. 2013 proved to be an exceptional year thanks to our closing two very important licensing deals; namely, the licensing of the PIM inhibitor series to the Irish drug development company, *Inflection Biosciences* in March 2013, and the licensing of CNIO ATR inhibitors to Merck Serono in December 2013. For the first time, two very exciting projects headed by the Experimental Therapeutics Programme, in collaboration with CNIO scientists, have been partnered with industry for further development and commercialisation. We are hopeful that our joining forces with the pharmaceutical industry will allow us to take full advantage of our ability to find the next generation of breakthrough therapies to fight cancer. The final proof-of-concept for this Programme will emerge once one of our molecules enters a clinical trial. We believe that we are on the right track!

Research Collaborations with industrial partners have also been reinforced in 2013. Significantly, CNIO extended a long-standing partnership with the pharmaceutical company Eli Lilly in order to establish a new section in the field of cancer epigenetics. Furthermore, during 2013 two new research projects were granted funding under the Extended Innovation Network of Roche, which CNIO joined in 2012. This makes a total of three highly innovative and translational projects that are being funded at CNIO under the umbrella agreement with Roche. Our objective is to work together with industry to translate research results obtained at the CNIO into innovative ideas and products to improve diagnosis, prevention and treatment in the field of cancer.

It is my pleasure to announce that in 2013, Alfonso Valencia, who is the Director of the Structural Biology and Biocomputing Programme at the CNIO, was also appointed Vice-Director of Basic Research, and since then he has been helping the CNIO Direction in key managerial issues. I want to take this opportunity to wholeheartedly thank the former Vice-Director of Basic Research, Erwin Wagner, for his dedication to the CNIO and his enthusiastic efforts to make the CNIO a truly international Centre. Erwin will continue with this important task as Director of the BBVA Foundation-CNIO Cancer Cell Biology Programme.

In February 2013, the new CNIO Scientific Advisory Board undertook a site visit to CNIO in order to conduct a global evaluation of its activities, as well as carry out the 5 Year Evaluation of the BBVA Foundation-CNIO Cancer Cell Biology and Clinical Research Programmes. During this evaluation, the Groups led by Erwin Wagner, Christopher Heeschen and Manuel Hidalgo, were very positively evaluated. Two Junior Group Leaders of the Cancer Cell Biology Programme, Mirna Pérez-Moreno and Nabil Djouder, were also positively evaluated and their Junior Position at CNIO was extended for an additional three years. Congratulations Mirna and Nabil!

During 2013, two Junior Groups left the CNIO to take tenured positions at different institutions. On one hand, Marta Sánchez-Carbayo, who was with us for more than six years as Head of the Tumour Markers Group at the Molecular Pathology Programme, accepted a full professor position at the prestigious CIC-BIOGUNE; a multidisciplinary research centre in the Basque Country, Spain. We wish Marta lots of success in the beautiful city of Bilbao. Francesco Gervasio, a brilliant researcher in the field of computer biology, started at the CNIO as a Junior Group Leader three years ago, and was offered a full professorship at The University College of London. In spite of the fact that Marta and Francesco are no longer at CNIO, we maintain numerous collaborations with them.

In 2013, we celebrated the CNIO's 10th inauguration anniversary during the third *Nature*-CNIO Cancer Symposium. On this occasion the subject was Tumour Heterogeneity and Plasticity. Thanks again to all the *Nature* editors who were involved in the event! A special thank you goes out to Barbara Marte, Nicola McCarthy and Alexia-Ileana Zaromytidou, as well as to our

in-house organisers, Erwin Wagner and Mirna Pérez-Moreno, and to our external organiser, Scott Lowe, for the exciting conference that they managed to put together.

I would like to take this opportunity to thank those who sponsored our students, postdoctoral programmes, and the stays of several researchers. I hereby thank *Banco Santander* Foundation for funding postdoctoral stays at CNIO; *Obra Social "la Caixa"* Foundation for fostering international PhD fellowships; Seve Ballesteros Foundation that supports the Seve-Ballesteros Foundation-CNIO Brain Tumour Group; and the Jesus Serra Foundation for supporting the Visiting Scientists Programme. During 2013, Robert Benezra from the Memorial Sloan-Kettering Cancer Center in New York, was beneficiary of the Jesús Serra Foundation's Visiting Researcher Programme. I am also grateful to the Foundation Banc Sabadell for sponsoring conferences given by out-of–the box speakers who provided novel perspectives that contribute to the CNIO's trans-disciplinary environment. During 2013, we had the privilege of listening to: Pedro Alonso, one of the world's foremost global authorities on malaria research; Carl Djerassi, chemist and novelist but better known for his contribution to the development of the contraceptive pill; J.L. Arsuaga, a leader in human palaeontology and member of the research team of Atapuerca Pleistocene deposits, and finally Jose María Ordovás, globally known specialist in nutrition, nutrigenetics and nutrigenomics. In addition, I extend my thanks to *Fundación BBVA* that generously supports the BBVA Foundation-CNIO Cancer Cell Biology Programme, as well as to the *CRIS* Foundation against Cancer, which supports the *CRIS* Foundation-CNIO Prostate Cancer and Genitourinary Tumours Clinical Research Unit at CNIO. Further words of thanks are due to AVON, which funds the Breast Cancer Clinical Research Unit, and to the Botín Foundation for supporting the Telomeres and Telomerase Group, the Tumour Suppression Group and from 2014 onwards also the Genomic Instability Group; all of them located under the Molecular Oncology Programme.

I would also like to make a special mention of the success achieved by the Communications Department in 2013. After two years of hard work since its creation, the department has succeeded in increasing CNIO's press visibility by 75% compared with 2012. One of the top stories this year was undoubtedly the study on *in vivo* generation of pluripotent stem cells published in the journal *Nature*. All of the main Spanish media outlets reported the discovery, including print media, TV and radio. The discovery was also picked up by foreign media outlets around the world, including the BBC, The Wall Street Journal, The Financial Times, *Le Monde* and The Telegraph, amongst many others. Another example that I would like to highlight is the CNIO and Merck license agreement, which was deemed a success and reflected as such by national and international media, including the prominent Wall Street Journal. These listed examples are just a small selection amongst several, which stand testament to our motivation and commitment to society in terms of sharing the latest breakthroughs, as well as CNIO's capacity to translate knowledge into applications for the benefit of cancer patients.

As well as increasing our presence in traditional media, CNIO has also increased visibility on social media networks, such as Twitter and YouTube. During 2013, CNIO's press releases in the global news service EurekAlert! received over 70,000 views.

Finally, I want to whole-heartedly thank all CNIO employees for their professionalism during 2013. In a year of restrictions, it became more evident than ever before that we all consider ourselves to be an intrinsic part of the CNIO, and that we all continue to work closely together to ensure that the CNIO is one of the best scientific institutions in Europe. ■

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

BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

Erwin F. Wagner Programme Director

Erwin F. Wagner
Genes, Development and Disease 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

Francesco L. Gervasio (until June)
Computational Biophysics Junior 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 Core Unit

David G. Pisano
Bioinformatics Core Unit

Alfonso Valencia
National Bioinformatics Institute Core Unit

MANUEL HIDALGO VICE-DIRECTOR OF TRANSLATIONAL RESEARCH

MOLECULAR PATHOLOGY PROGRAMME

María S. Soengas Programme Director

María S. Soengas
Melanoma Group

Francisco X. Real
Epithelial Carcinogenesis Group

Christopher Heeschen
Stem Cells and Cancer Group

Marta Sánchez-Carbayo (until May)
Tumour Markers Junior Group

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez Programme Director

Javier Benítez
Human Genetics Group

Juan C. Cigudosa
Molecular Cytogenetics Group

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Miguel Urioste
Familial Cancer Clinical Unit

Anna González-Neira
Human Genotyping-*CEGEN* Core 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
CRIS Foundation-CNIO Prostate Cancer and Genitourinary Tumours Junior Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

Fátima Al-Shahrour
Translational Bioinformatics Unit

BIOBANK

Manuel M. Morente Director

MARISOL QUINTERO (until October) DIRECTOR 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

Marta Cañamero (until June)
Histopathology 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

Isabel Blanco (Charles River Laboratories)
Animal Facility

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling Therapies Section

Maria José Barrero (since July)
CNIO-Lilly Epigenetics Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Marisol Quintero Head of Office (until October)

Anabel Sanz Head of office (since October)

Vice-Direction of Basic Research

“My main mission, as Vice-Director for Basic Research, is to work together with CNIO´s Basic Research Groups to enhance scientific excellence, foster collaboration and optimise the use of our resources.”

ALFONSO VALENCIA
Vice-Director of Basic Research

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It may sound obvious, but it is always good to remember that the so-called "translational" or "applied" research necessarily requires "basic" science to be translated or applied. This is precisely the mission of the Molecular Oncology Programme: to generate knowledge that can be translated into better care for cancer patients.

This year, the Molecular Oncology Programme has continued to be on the frontline of oncology research. We were particularly proud this year because the Genomic Instability Group, in collaboration with the Experimental Therapeutics Programme, have generated extremely promising chemotherapeutic compounds that inhibit the DNA damage signalling kinase ATR, having signed an ambitious licensing agreement with the German pharmaceutical giant Merck Serono. Thanks to this agreement, these compounds will be tested in clinical assays, most likely with the participation of the CNIO Clinical Research Programme. This is a prime example of the great synergies that the CNIO can create, bringing together basic scientists, medical chemists and clinical oncologists. This is an ongoing story of great success and a motivation for all of us to continue along this road of progress.

I want to mention two other examples of research projects led by scientists of the Molecular Oncology Programme that further illustrate our ability to catalyse creative collaborations. The first one involves the collaboration of the CNIO Telomeres and Telomerase Group with the Spanish Project of the International Cancer Genome Consortium (ICGC), which resulted in the discovery of a novel type of oncogenic mutation that affects the stability of chromosomes in chronic lymphocytic leukaemia (CLL). The second example is a multi-partnered collaboration between the CNIO Tumour Suppression Group, almost all the Core Units at the CNIO, and a research group from the CNIC. This remarkable collaborative effort demonstrated the induction of pluripotency *in vivo*; the significance of this work was recognised by *Nature Medicine as* the most important discovery of the year 2013 in the field of stem cells. ■

"The Molecular Oncology Programme is CNIO´s flagship Programme. Part of our success lies in our collective goal to make our research more innovative and efficient. Many of the discoveries made in our Programme are already in translational phases and are, thus, contributing towards the improvement of cancer treatment."

TUMOUR SUPPRESSION GROUP

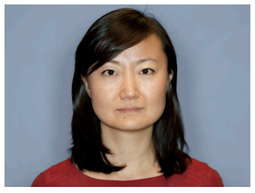
Manuel Serrano
Group Leader

Staff Scientists
Luis Enrique Donate (until February), Han Li, Susana Llanos, Antonio Maraver, Cristina Pantoja

Post-Doctoral Fellows
María Abad, Timothy Cash, Pablo J. Fernández-Marcos, Cian J. Lynch, Daniel Muñoz, Sandrina Nóbrega (until July), Daniela Piazzolla

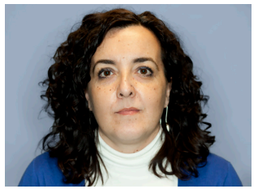
Graduate Students
Katharina Hess (until May), Elena López-Guadamillas, Lucía Morgado, Lluc Mosteiro, Adelaida Palla


Manuel Serrano ESP

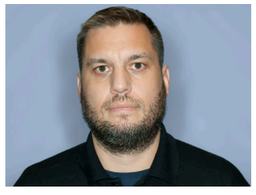

Han Li CHN


Susana Llanos ESP


Antonio Maraver ESP


Cristina Pantoja ESP


María Abad ESP


Timothy Cash USA


Pablo J. Fernández-Marcos ESP


Cian J. Lynch IRL


Daniel Muñoz ESP


Daniela Piazzolla ITA


Elena López-Guadamillas ESP


Lluc Mosteiro ESP


Adelaida Palla VEN

OVERVIEW

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes. However, despite their efficient protection against cancer, these genes can become defective over time. The affected cells thus become partially unprotected from cancer and, in combination with additional mutations in other genes, can give rise to the development of cancer.

Understanding how tumour suppressor genes work may help us to design drugs that block cancer. Our Group also manipulates the mouse genome to create novel alterations that increase or decrease tumour suppression potency.

The goals of our Group are:

- → To understand the mechanisms of tumour suppression and 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.

"During 2013, we have shown in mice that *in vivo* conditions are permissive to cellular de-differentiation and reprogramming. Moreover, *in vivo* reprogramming achieves a state of pluripotency that is more primitive than the one achieved *in vitro*. These findings were published in *Nature* and were considered Discovery of Year by the journal *Nature Medicine*."

RESEARCH HIGHLIGHTS

SIRT1 promotes thyroid cancer

While the existing evidence in mice indicated that SIRT1 has potent tumour suppressor activity in a variety of cancer models, we wanted to study the impact of SIRT1 on cancer associated to PTEN loss; for this purpose, we crossed our 2 lines of *Sirt1* transgenic mice with *Pten*-deficient mice. Surprisingly, rather than protecting, *Sirt1* overexpression decreased the survival of the *Pten*-deficient mice (FIGURE 1). Contrary to previous accumulated evidence in other cancer types where *Sirt1* acts as a tumour suppressor, *Sirt1* is oncogenic in the thyroid and prostate (FIGURE 1). This effect was particularly prominent in the case of thyroid cancers, which were all metastatic. The analyses of mRNA expression in pre-tumoural murine thyroids revealed that SIRT1 stabilises c-MYC protein and increases c-MYC transcriptional programmes. Interestingly, in the case of human thyroid cancer, SIRT1 is frequently overexpressed and its levels correlate positively with those of c-MYC. Our results implicate SIRT1 as a new candidate target for the treatment of thyroid carcinomas.

Cell reprogramming *in vivo*

Reprogramming into pluripotency is an intense field of investigation that is already providing many insights into cell plasticity. Cell reprogramming had so far been achieved

Figure 1 *Sirt1* overexpression in mice promotes thyroid tumorigenesis and correlates with increased c-MYC activity. **(A)** Survival of cohorts of male mice of the indicated genotypes. **(B, C)** Incidence of thyroid and prostate carcinomas, respectively, in 5 to 7-month-old mice of the indicated genotypes.

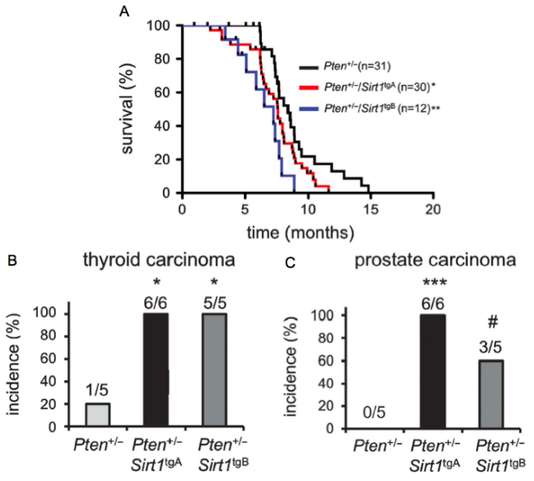
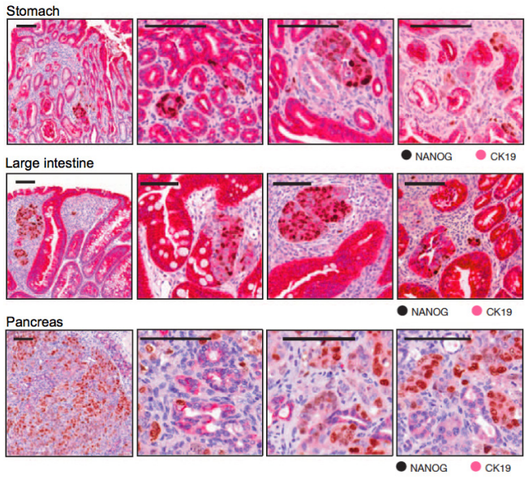


Figure 2 Many cell types are dedifferentiated and reprogrammed *in vivo*. Double immunohistochemistry of cytokeratin 19 (CK19, magenta, indicative of differentiation) and of NANOG (dark brown, indicative of reprogramming) in the stomach (top), the large intestine (middle), and the pancreas (bottom) of reprogrammable mice. All scale bars correspond to 100 μm.



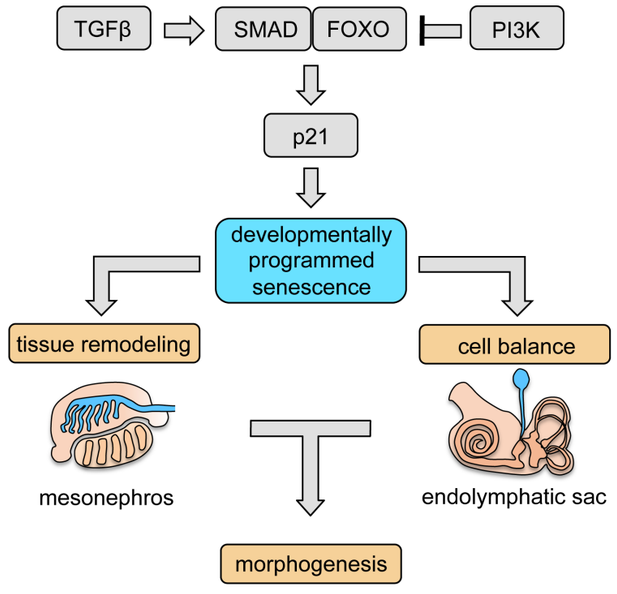


Figure 3 Summary of the molecular mechanisms and function of developmentally programmed senescence.

under carefully controlled *in vitro* culture conditions, whereas the *in vivo* tissue microenvironment would in principle steer towards cellular differentiation and is opposed to reprogramming. Bearing the former in mind, we have nevertheless attempted to achieve reprogramming *in vivo*.

This year, we have demonstrated that transitory induction of the factors *Oct4*, *Sox2*, *Klf4* and *c-Myc* in mice results in rapid dedifferentiation in multiple tissues, including the stomach, intestine, pancreas and kidney. Dedifferentiation occurred to a variable extent, including the loss of keratin expression and the acquisition of NANOG expression, a pluripotency marker indicative of reprogramming (FIGURE 2). At later periods of time, mice developed multiple teratomas. The reprogrammable mice also present circulating induced pluripotent stem cells (iPS cells) in the blood and, at the transcriptome level, these *in vivo* generated iPS cells are closer to embryonic stem cells (ES cells) than standard *in vitro* generated iPS cells. Interestingly, *in vivo* iPS cells efficiently contribute to the trophectoderm lineage, suggesting that they achieve a more plastic or primitive state than ES cells. We concluded that reprogramming *in vivo* is feasible and confers totipotency features absent in standard iPS or ES cells.

Developmentally-programmed cellular senescence

Cellular senescence is emerging as an important aspect of tissue remodelling. Until now, senescence had been associated to accidental, non-programmed, tissue damage; as it occurs in pathological states including cancer. This year, we have discovered that senescence also occurs during mammalian embryonic development in a programmed manner and actively participates in multiple tissue remodelling processes. We focused on the mesonephros and on the endolymphatic sac of the inner ear. Senescence at both structures depends strictly on p21, and it is independent of DNA damage, p53 or other cell cycle inhibitors. We have also demonstrated that the TGFβ/SMAD and PI3K/FOXO pathways regulate developmental senescence (FIGURE 3). Importantly, developmental senescence is followed by subsequent macrophage infiltration and clearance of the senescent cells, thus completing the cycle of tissue remodelling. In the absence of p21, loss of senescence is partially compensated by apoptosis, but still results in detectable developmental abnormalities. Human embryos also present senescence markers at the mesonephros and endolymphatic sac. We propose that senescence emerged during the course of evolution as an embryonic tissue remodelling process, and that it was subsequently adapted for tissue repair upon damage. ■

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AWARDS AND RECOGNITION

- *Jiménez Díaz* Distinction Award, XLV *Jiménez Díaz* Memorial Lecture, Spain.

EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Staff Scientists
Matthias Drosten, Carmen Guerra, Monica A. Musteanu, David Santamaría

OVERVIEW

K-*Ras* oncogenes have been implicated in about one fifth of all human cancers including those with the worst prognosis, such as lung adenocarcinoma and pancreatic ductal adenocarcinoma. We have developed genetically modified mouse models (GEMMs) that closely recapitulate the natural history of these human cancers. We have used these strains to validate targets of potential therapeutic value, with the ultimate goal of translating these findings into the clinic. We have crossed these GEM strains with mice that carried conditional knock-out mutations in loci encoding potential therapeutic targets. These targets can be ablated once the tumour has been generated to determine whether they are essential, or at least relevant, for tumour development. This genetic-based strategy has significant advantages over classical pharmacological studies since it does not rely on the quality of the drug/inhibitor and the observed effects are always mechanism-based, not off-target effects. Moreover, if the target is eliminated systemically, our studies offer relevant information regarding potential toxic effects that may occur when the target is pharmacologically inhibited in normal tissues. More recently, we are replacing the conditional knock-out strains with conditional knock-in mice so that we will express kinase dead isoforms in tumour tissue instead of eliminating the target. We hope that this experimental approach will mimic more accurately those pharmacological responses that will be observed in the clinic.

RESEARCH HIGHLIGHTS

Role of Ras Signalling in Skin Development

Proliferation in the epidermis is a tightly controlled process. During skin development, epidermis formation and hair follicle morphogenesis crucially depend on the regulated balance between proliferation and differentiation. We have deleted all three *Ras* loci (*H-Ras*, *N-Ras* and *K-Ras*) from keratinocytes *in vitro* as well as specifically from the epidermis in mice using a K5Cre strain. Upon Ras elimination, keratinocytes ceased proliferation and entered into senescence without any signs of apoptosis induction. Constitutive activation of the MAPK pathway was able to partially rescue the proliferative defects. In mice, Ras signalling was essential for proper development of the epidermis and hair follicles. Deletion of the three *Ras* loci during epidermis formation in mouse embryos caused a dramatic decrease in proliferation, substantially thinner epidermis and delayed appearance of differentiation markers. We did not detect apoptotic or senescent cells suggesting that loss of Ras protein expression only leads to severe hypoproliferation. These observations provide genetic evidence for an essential role of Ras proteins in the control of keratinocyte and epidermal proliferation.

Identification of cancer initiating cells in *K-Ras* driven lung adenocarcinoma

Ubiquitous expression of a *K-Ras*^{G12V} oncogene in adult mice only induced overt tumours in lungs. To identify these transformation-permissive cells, we induced *K-Ras*^{G12V} expression in a very limited number of adult lung cells. Four weeks later, 30% of these cells had proliferated to form small clusters. However, only surfactant protein C stained (SPC+) alveolar type II (ATII) cells were able to form hyperplastic lesions, some of which progressed to adenomas and adenocarcinomas. In contrast, induction of *K-Ras*^{G12V} expression in lung cells by intratracheal infection generated hyperplasias in all regions except the proximal airways. Bronchiolar and bronchioalveolar duct junction hyperplasias were primarily made of CC10+ Clara cells. Some of them progressed to benign adenomas. However, only alveolar hyperplasias made up of ATII cells, progressed to yield malignant adenocarcinomas. Adenoviral infection induced inflammatory infiltrates primarily made of T and B cells. This inflammatory response was essential for the development of *K-Ras*^{G12V}-driven bronchiolar hyperplasias and adenomas, but not for the generation of SPC+ ATII lesions. Finally, activation of *K-Ras*^{G12V} during embryonic development under the control

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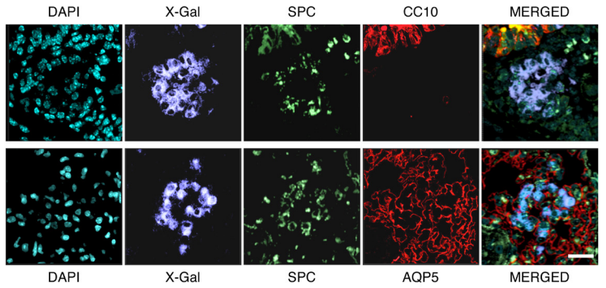

Figure Proliferating *K-Ras*^{G12V} oncogene expressing cells are SPC+ alveolar type II cells. Representative sections of X-Gal stained lungs were incubated with antibodies against (**A**) SPC and CC10 and (**B**) SPC and AQP5. Merged images represent the overlay of X-Gal staining and IF images. Nuclei were counterstained with DAPI. Scale bars represent 20 μm.

of a *Sca1* promoter exclusively yielded CC10+ lesions, including adenomas. These results illustrate that different types of lung cells, at various developmental stages, can generate tumour lesions in response to *K-Ras*. However, in adult mice, only SPC+ ATII cells were able to yield malignant adenocarcinomas.

K-Ras^{V14I}-induced Noonan syndrome predisposes to tumour development in mice

Noonan syndrome (NS) is an autosomal dominant genetic disorder characterised by short stature, craniofacial dysmorphism and congenital heart defects. A significant fraction of NS patients also develop myeloproliferative disorders (MPD). Mutations responsible for NS occur in at least eight different loci including *K-RAS*. We have generated a mouse model for NS induced by the most frequent *K-RAS* mutation in NS patients. Mutant mice displayed multiple NS-associated developmental defects such as growth delay, craniofacial dysmorphia, cardiac defects and haematological abnormalities including MPD. Homozygous animals had perinatal lethality, increased predisposition to tumour development other than MPD and cooperated with tumour suppressors. Exposure of pregnant mothers to a MEK inhibitor rescued perinatal lethality and prevented craniofacial dysmorphia and cardiac defects, but not haematological disorders including MPD. However, these defects were not corrected when mice were treated after weaning. These mice offer an experimental tool to study the molecular alterations underlying the clinical manifestations of NS and to test new therapies aimed at preventing or ameliorating those deficits associated with this syndrome. ■

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AWARDS AND RECOGNITION

- Foreign Member, National Academy of Sciences of the US, Section 41: Medical Genetics, Hematology and Oncology.

TELOMERES AND TELOMERASE GROUP

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Nora Soberón MEX

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 but is not present in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes that are essential for chromosome protection and genomic stability. One of the many factors that lead to ageing is the progressive shortening of telomeres associated with organism ageing. When telomeres are altered (in their length or their integrity) adult stem cells have a maimed regenerative capacity.

Telomere length defects are associated to cancer and ageing processes, and have a profound effect on stem cell behaviour. We aim to determine the role of genetic and epigenetic telomere regulators in cancer and ageing by generating new mouse models and studying the role of these factors in stem cell biology.

Our research aims at:

→ Understanding the biology of the telomeres and telomerase by generating mouse models to probe the role of telomeres and telomerase in cancer and ageing.


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Águeda M. Tejera ARG


Elisa Varela ESP


Christian Bär DEU


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Mercedes Gallardo ESP


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→ Deciphering the interplay between telomeres and DNA repair pathways.
→ Characterising the role of telomeric heterochromatin.
→ Developing strategies for telomerase activation.
→ Elucidating the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to induced Pluripotent Stem (iPS) cells.

“Our exploration of a new mechanism, by which mutations in the telomeric protein POT1 contribute to the development of human chronic lymphocytic leukaemia, may facilitate novel approaches for therapeutic intervention and clinical management of this disease.”

RESEARCH HIGHLIGHTS

Human chronic lymphocytic leukaemia and telomere dysfunction due to mutations in telomeric protein POT1

Chronic lymphocytic leukaemia (CLL), the most frequent leukaemia in adults in Western countries, affects more than one thousand new patients in Spain each year. Analysis of sequencing data from CLL patients uncovered that the gene *POT1* is one of the most frequently mutated genes in this illness. *POT1* encodes the telomeric protein POT1; a protein that, by acting as a staple, fixes in place the protective hood that safeguards the telomeres.

Somatic mutation of *POT1* affects key protein residues that are required for binding to telomeric DNA, thus preventing this gene from fulfilling its function. We showed that *POT1*-mutated CLL cells have numerous telomeric and chromosomal abnormalities (FIGURE 1) that suggest that *POT1* mutations favour the acquisition of the malignant features of CLL cells. The study of the biochemical pathways that lead from these chromosomal abnormalities to the uncontrolled growth of B lymphocytes could provide clues for a better understanding of CLL in particular and of human cancer in general.

Telomeric protein TRF1 is a stem cell marker and is essential for nuclear reprogramming

Deficiency of the telomeric protein TRF1 leads to early embryonic lethality – suggesting an essential role for TRF1 in early mouse development – and to severe organ atrophy and dysfunction when deleted in adult tissues; this suggests that TRF1 may also be important for adult stem cell compartments and maintenance of organ homeostasis. To study the role of TRF1 in stem biology and tissue homeostasis, we have generated a reporter mouse carrying eGFP-TRF1. In this context, generation of a reporter mouse for TRF1 is a valuable tool to understand the regulation of TRF1 expression in normal and pluripotent/adult stem cells, and also to address if cells with high TRF1 levels are associated with higher stemness/pluripotency.

We found that eGFP-TRF1 expression in mice is maximal in known adult stem cell compartments (FIGURE 2), and showed that TRF1 ensures their functionality. This discovery is useful for identifying and eventually isolating the stem cell population in tissues; something that is important for the development of regenerative medicine. We have determined that eGFP-TRF1 is highly expressed in induced pluripotent stem (iPS) cells and that TRF1 expression is uncoupled from the telomere elongation associated with reprogramming. We observed that selection of eGFP-TRF1-high iPS cell populations correlates with higher pluripotency as indicated by their ability to form teratomas and chimaeras. We also showed that TRF1 is necessary for both induction and maintenance of pluripotency and that TRF1 is a direct transcriptional target of Oct3/4.

Telomeric protein RAP1 protects from obesity through its extratelomeric role regulating gene expression

To investigate the non-telomeric roles of RAP1 *in vivo*, we have now generated a RAP1 whole-body *knock-out* mouse. These mice presented an early onset of obesity, which is more severe in females than in males and is aggravated under a high-fat diet. *Rap1*-deficient mice showed accumulation of fat in abdominal depots, developed hepatic steatosis, and had high fasting plasma levels of insulin, glucose, cholesterol, and alanine transaminase. Gene expression analyses of the liver and visceral white fat from *Rap1*-deficient mice before the onset of obesity indicated deregulation of key metabolic transcriptional programmes including fatty acid metabolism, PPARα signalling and glucose metabolism. We identified *Pparα* and *Pgc1α*, as well as their target genes, as the key metabolic pathways affected by *Rap1* deletion in the liver. We further showed that RAP1 binds to *Pparα* and *Pgc1α* loci and modulates their transcription. These findings have shown an unprecedented role for a telomere-binding protein in the regulation of metabolism. ■

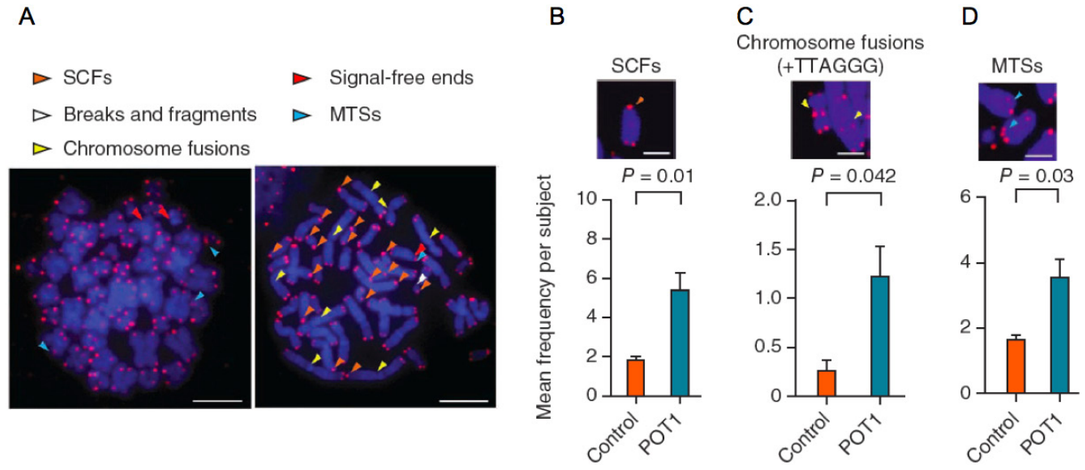

Figure 1 Chromosomal aberrations in cells from individuals with CLL expressing mutated *POT1*. (**A**) Telomeric FISH on metaphase spreads from *POT1*-mutated and control CLL samples. (**B**–**D**) Per-individual chromosomal aberrations for sister chromatid–type end-to-end fusions (SCFs) (**B**), chromosome-type end-to-end fusions (**C**) and multitelomeric signals (MTSs) (**D**).

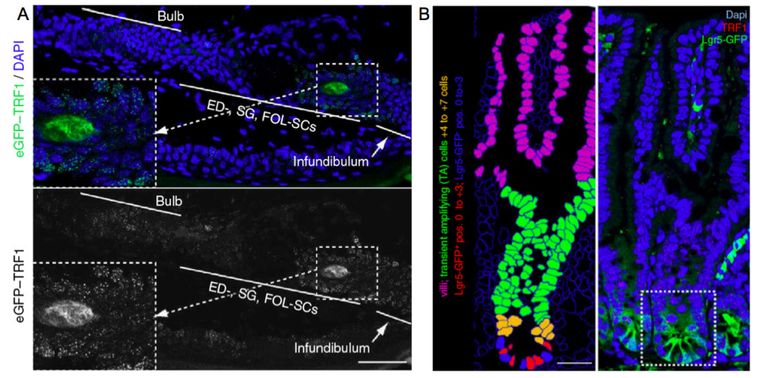

Figure 2 TRF1 levels are highest in adult stem cell compartments of the skin and the small intestine. (**A**) eGFP–TRF1 fluorescence in different compartments of tail-skin follicles of eGFP–TRF1 mice decreases from the putative stem cell niches towards the more differentiated compartments. (**B**) Small intestine cell type definition.

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AWARDS AND RECOGNITION

- Honorary Ambassador of the Spain Brand 2013 in the Science and Innovation category, Leading Brands of Spain Forum.
- Honorary Award from the *Cátedra Real Madrid* for an outstanding scientific career, Spain.
- Corresponding Fellow of the Spanish Royal Academy of Pharmacy.
- Member of the Selection Committee, 2013 Pezcoller Foundation-AACR International Award for Cancer Research.
- Member of the Jury, 2013 Inbev Baillet Latour Health Prize, Leuven, Belgium.

CELL DIVISION AND CANCER GROUP

Marcos Malumbres
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OVERVIEW

The main focus of our group is to understand the mechanisms by which mitosis is regulated. We are interested, not only in deciphering the control of chromosome segregation during mitosis, but also in finding new ways to block tumour progression through the inactivation of mitotic regulators. Using mouse models as a major research tool, we investigate mitotic kinases and phosphatases, as well as regulatory complexes involved in ubiquitin-dependent degradation of proteins during mitosis. Other research areas of our group include the study of microRNAs involved in the development and progression of hematopoietic tumours, as well as the control of asymmetric cell division in progenitor/stem cells and their relevance to development, tissue homeostasis and cancer.

"Our group has reported the *in vivo* relevance of three mitotic regulators; namely, Greatwall, Aurora-A and Cdh1. These proteins are putative cancer targets and our results will be critical to better understand the function of these proteins and the effects of their inhibition in cancer."

The Group's main objectives are the following:

→ To understand the basic control mechanisms of the mammalian cell cycle.
→ To characterise the physiological and therapeutic consequences of cell cycle deregulation *in vivo*.
→ To characterise the function of microRNAs in cell biology and tumour development, as well as their potential use in cancer therapy.
→ To understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties.

RESEARCH HIGHLIGHTS

Greatwall: a new target for cancer therapy?

Greatwall, also known as Mastl, is a recently identified kinase that regulates cell division. Until now, most studies on this protein were carried out in invertebrates. Our group, in collaboration with researchers from the National Centre for Scientific Research (CNRS) in Montpellier, France, has now generated the first genetic mouse model of this protein. Using this conditional knockout model, we have shown that Greatwall is essential for mouse embryonic development and cell cycle progression. This is due to mitotic collapse after nuclear envelope breakdown (NEB). We demonstrated that Greatwall is exported from the nucleus to the cytoplasm in a Chromosome Region Maintenance 1 (CRM1)-dependent manner before NEB. We postulate that, once at the cytoplasm, Greatwall inhibits the phosphatase 2 (PP2A)-B55 complexes to maintain the mitotic state (FIGURE 1).

Our findings may have therapeutic implications since Greatwall acts by blocking the function of the PP2A phosphatase; a tumour suppressor frequently altered in human cancer. This implies that the inhibition of Greatwall could, at the same time, slow down cell division and reactivate tumour suppressor PP2A; a protein capable of inhibiting many of the oncogenic molecular pathways involved in cancer development. We are actively working on this possibility.

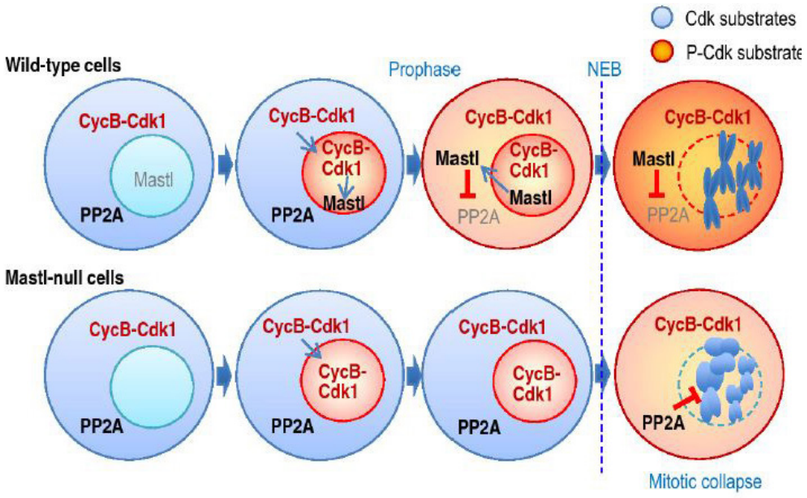

Figure 1 How to prepare a cell for nuclear envelope breakdown. Activation and nuclear import of CycB/Cdk1 triggers Greatwall export to the cytoplasm, where it inhibits PP2A/B55. A defective inhibition of PP2A in early mitosis would cause a defective phosphorylation of Cdk substrates upon NEB leading to the mitotic collapse observed in Greatwall null cells (modified from Alvarez-Fernández et al., 2013).

Aurora kinases, biomarkers and cancer treatment

Aurora-A, a major cell cycle regulator, is highly expressed in human tumours; it correlates with poor prognosis in some tumour types. Although the most important roles of this molecule have been studied in the past in other model organisms and in mouse embryos, the requirements of this kinase in adult tissues or in young individuals remain unknown. Our group, in collaboration with T. Van Dyke and D. Cowley at The University of North Carolina, has demonstrated that inhibition of this kinase results in a premature ageing phenotype when applied to young individuals. This phenotype mainly results from an increase in the levels of senescent cells. These phenomena were accompanied with a significant increase in the percentage of cells that accumulate high levels of DNA content, indicating a defect in how cells segregate their DNA upon inhibition of this kinase. Our study also has important therapeutic consequences since the genetic elimination of Aurora-A efficiently inhibits the proliferation of tumours in mice (FIGURE 2). The fact that inhibition of Aurora-A also generated a significant amount of DNA damage is of special relevance in cancer therapy, since it implies that the inhibition of Aurora-A could sensitise tumours to anticancer agents that work better against cancer cells with high levels of DNA damage.

We have found that Aurora-A-deficient tumours are characterised by an accumulation of polyploid cells. These cells display a low proliferative potential, resulting in a defect in the ability of the tumour to progress. From these results, we propose that scoring the number of polyploid cells in patients treated with these compounds should be highly informative for evaluating the efficiency of these drugs in clinical studies.

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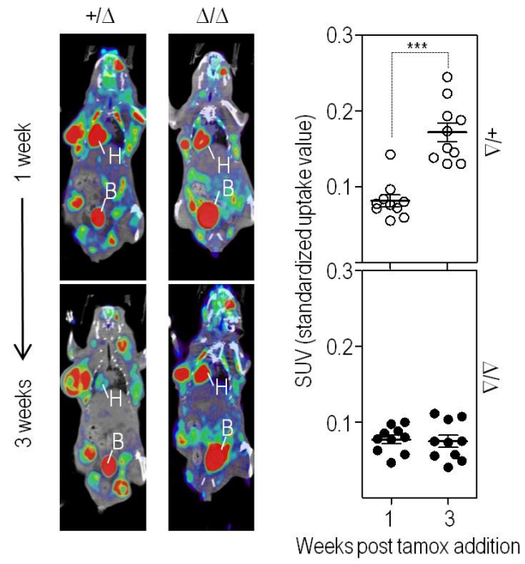

Figure 2 Depletion of Aurora-A inhibits the growth of oncogene-induced mammary tumours, whereas, control tumours significantly increase their metabolic activity measured by PET. Aurora-A null tumours did not increase their metabolic activity with time (Pérez de Castro et al., 2013).

The APC/C cofactor Cdh1, replicative stress and neural progenitors

The requirements for the APC/C during mitotic exit have recently been proposed as new therapeutic strategies against cancer. One possible complication of these strategies resides in the possible undesired effect of inhibiting APC/C-Cdh1, since lack of Cdh1 activity may result in the accumulation of proliferative molecules such as cyclins, or oncogenes – such as Pttg1/Securin or Aurora kinases – that could drive increased cellular proliferation. We have shown that genetic ablation of *Cdh1* in the developing nervous system results in hypoplastic brain and hydrocephalus. These defects correlate with enhanced levels of Cdh1 substrates and increased entry into S-phase in neural progenitors. However, cell division is prevented in the absence of Cdh1 due to hyperactivation of cyclin-dependent kinases, increased phosphorylation of H2AX, induction of p53, G2 arrest, and apoptotic death of these progenitor cells. This particular requirement for Cdh1 during neurogenesis is related to the ability of Cdh1 to prevent replicative stress in progenitors of the developing brain. Contrary to initial expectations, our genetic data suggest that ablation of *Cdh1* results in replicative stress *in vivo* and a general antiproliferative response that is not p53-dependent. Thus, putative APC/C inhibitors are unlikely to generate proliferative responses, even in the case of unspecific inhibition of Cdh1 and with independence of the p53 status of tumour cells. ■

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OVERVIEW

DNA damage is the source of pro-cancerous mutations but 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 arises, unavoidably, 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 has significantly limited their study, particularly at the organismal level. In order to overcome these limitations, a major part of our work these past years has 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 RS on cancer and ageing, and have resulted in putative drugs that can be used to test our conceptual approaches to cancer therapy. Overall, our main goal is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge as a way to fight cancer.

"During 2013, we have invested in the implementation of technology for performing forward genetic screenings in haploid mammalian cells, and discovered that the mechanisms that deal with the dissolution of inter-molecular DNA links are important for the suppression of cancer and ageing in mammals."

RESEARCH HIGHLIGHTS

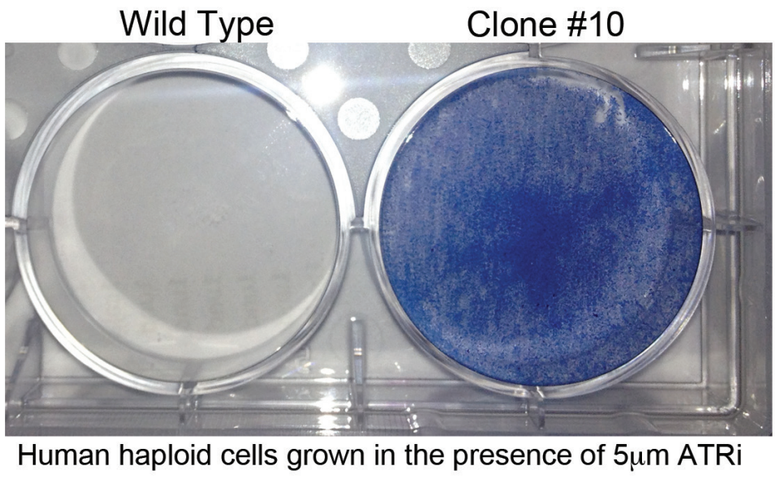

Figure 1 Isolation of human cells resistant to ATR inhibition. The picture represents the growth of a mutant clone of human haploid cells at doses of ATR inhibitor that are toxic for "wild type" cells. This clone was isolated from a library of 125,000 clones mutagenised with a piggyBac transposon.

Searching for players of the DNA damage response through forward genetics in haploid mammalian cells

One of the key advantages of using yeast as a model system is the capacity to grow it as a haploid organism, which has greatly facilitated genetic screenings based on gene-deletions. In mammals, RNA interference (RNAi) emerged as a powerful alternative, but unfortunately suffers from significant off-target effects and/or incomplete knockdowns that frequently limit its potential. The availability of human haploid cell lines (KBM7 and HAP1) isolated from a leukemic cell line, as well as the capacity to generate primary mouse haploid embryonic stem cells (mES^{h}), are rapidly shaking the field. During the last year we have invested in implementing the technology in our laboratory in order to perform forward genetic screenings in human KBM7/HAP1 cells and mES^{h}, using piggyBac transposons as mutagens. We have already performed some initial screenings that were directed at finding mutations that generate resistance against commonly used genotoxic drugs. One of these screenings was directed at exploring whether resistances can arise against ATR inhibitors; compounds that we have generated in collaboration with the Experimental Therapeutics Programme, and which we believe might be particularly useful for the treatment of tumours with high levels of replication stress. We have currently identified several mutant clones that can grow normally, even in the presence of high doses of ATR inhibitors (FIGURE 1), and are working on the characterisation of the genes responsible for this resistance. Our next steps in this area include setting up a pipeline for the identification of mutants through Next Generation Sequencing, as well as developing mES^{h} lines at the CNIO with the help of CNIO's Transgenic Mice Unit. The *in house* generation of haploid lines will enable us to perform synthetic viability screens oriented to unmask genes that, when mutated, enable the growth of cells lacking tumour suppressors that are otherwise essential in normal cells (i.e. *BRCA1*).

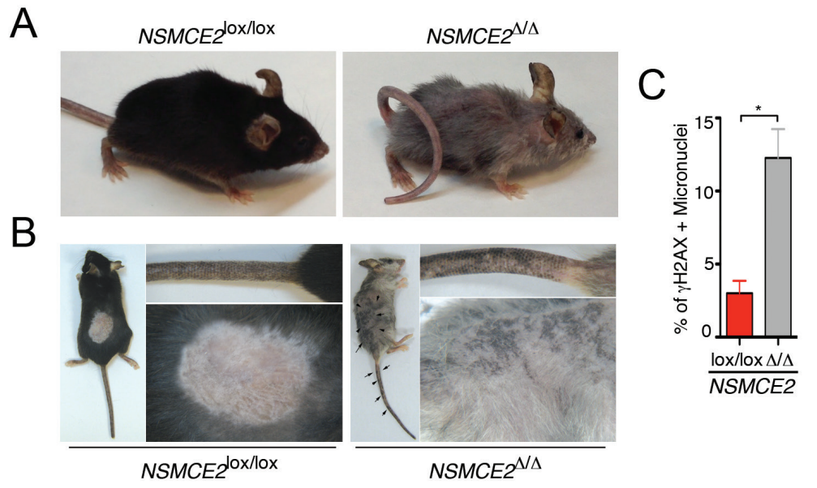

Figure 2 *NSMCE2* deletion accelerates ageing in mice. (**A**) The picture exemplifies the outcome of *NSMCE2* deletion in adult mice. Mice develop a progeroid syndrome with several features that are reminiscent of Bloom's Syndrome, such as (**B**) pigmentation problems or (**C**) the accumulation of micronuclei.

A SUMO ligase involved in the dissolution of joint DNA molecules suppresses cancer and ageing in mammals

Most of our work focuses on understanding how cells are protected from the accumulation of replication stress through a phosphorylation-based signalling cascade coordinated by ATR and Chk1 kinases. However, there is emerging evidence that additional signalling pathways based on other post-translational modifications, such as SUMOylation or Ubiquitinylation, are also key for these responses. In this regard, we have focused our research on NSMCE2; a SUMO ligase that is part of the so-called SMC5/6 complex. This complex is similar to other SMC complexes such as condensins and cohesins, but its real function remains unclear. Studies in yeast have suggested that the complex plays a role in dissolving DNA linkages that arise between sister chromatids. Through the generation of 3 independent mouse models of NSMCE2 (genetrap, conditional knockout and a mutant strain lacking SUMO ligase activity) we have discovered that this complex is essential for the suppression of mitotic recombination, cancer and ageing in mice (FIGURE 2). These (and other) phenotypes resemble those found on a human hereditary disease known as Bloom's Syndrome. It is noteworthy that mice lacking the SUMO ligase activity of NSMCE2 do not show an obvious phenotype, so that its role in the SMC5/6 complex seems to be independent of this activity. We are currently exploring the potential relationship between the SMC5/6 complex and BLM; the protein that is defective in Bloom´s Syndrome patients. In addition, we are looking for genes that are particularly toxic to cells harbouring mutations in this complex; the aim being to design future chemotherapeutic strategies that would be particularly useful for tumours that present an accumulation of inter-molecular DNA links. ■

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AWARDS AND RECOGNITION

- *Doctores Diz-Pintado* National Award for Cancer Research, Spain.

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OVERVIEW

Proper development of a multicellular organism entails 2 major processes. One is proliferation; i.e. the cell duplicates its genetic material and divides into 2 identical daughter cells. The other is differentiation; i.e. the specialisation of naive precursors into specific cell types. This is accomplished through the activation of tissue-specific transcriptional programmes that establish cell identity. Higher order genome structure is a major determinant of such regulation of gene expression. Our research focuses on a protein complex named cohesin that occupies a central position in both of these processes. On the one hand, cohesin mediates sister chromatid cohesion and thereby ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. On the other hand, cohesin contributes to the spatial organisation of the genome by promoting or stabilising the formation of chromatin loops. Mutations in cohesin and its regulatory factors have been identified in a group of human syndromes, collectively known as cohesinopathies, and also in several tumour types. Our goal is to understand how cohesin works, how it is regulated, and how its dysfunction contributes to cancer and other human diseases. In addition to the study of cohesin, we are interested in the epigenetic inheritance of centromeres mediated by the histone H3 variant CENP-A; another essential aspect of chromosome segregation.

"We have found that the cohesin-associated factors Pds5A and Pds5B have non-redundant roles in embryonic development and cell proliferation. In particular, Pds5B is essential for centromeric cohesion and in its absence the cells often mis-segregate their chromosomes and become aneuploid."

RESEARCH HIGHLIGHTS

The specific functions of Pds5 proteins in vertebrate cells

In vertebrate somatic cells, cohesin consists of 4 subunits, Smc1, Smc3, Rad21, and either SA1 or SA2. Three additional factors, Pds5, Wapl, and Sororin bind cohesin and modulate its dynamic association with chromatin. There are 2 Pds5 proteins in vertebrates, Pds5A and Pds5B, but their functional specificity has remained elusive. We have generated conditional knockout alleles for the genes encoding Pds5A and Pds5B. Both genes are individually required for embryonic development although lethality occurs in late post-implantation stages. This has allowed us to obtain mouse embryonic fibroblasts in which we could study the functions of the 2 proteins. Our results show that Pds5 proteins have positive and negative effects on the stability of cohesin's association with chromatin. In concert with Wapl, Pds5 proteins promote cohesin release from chromatin both during interphase and mitosis. In interphase, this dynamic association could be important to facilitate chromatin processes like transcription and replication. In mitosis, dissociation of most cohesin during prophase allows sister chromatid resolution and thereby ensures efficient chromosome segregation. Pds5 proteins have additional functions. They are required for Smc3 acetylation by the cohesin acetyl transferases (CoATs) Esco1/2 during S phase and for subsequent binding of Sororin; these are the two key steps for cohesion establishment. While both Pds5A and Pds5B contribute to telomere and arm cohesion, Pds5B is specifically required for centromeric cohesion (FIGURE 1). In the Pds5B null cells, both acetylation of cohesin by Esco2 and binding of Sororin at pericentromeric heterochromatin are significantly decreased. Moreover, reduced accumulation of Aurora B at the inner centromere region in mitotic chromosomes lacking Pds5B impairs its error correction function, promoting chromosome mis-segregation and aneuploidy. Decreased proliferation of Pds5B null cells could be explained by mitotic cell death and aneuploidy. Cells lacking Pds5A have a stronger proliferation defect and Pds5A null embryos present an earlier lethality, but in this case cells display correct ploidy and no mitotic defects. We speculate that the Pds5A null phenotypes may be related to altered transcription. Future experiments will have to address the genome-wide distribution of Pds5A and Pds5B, as well as the effects of their ablation in gene expression during development.

Analysis of cohesin functions in a mouse model for Cornelia de Lange Syndrome

Cornelia de Lange Syndrome (CdLS) is a genetic disorder that affects around 1 in 30,000 newborns and is linked to mutations in cohesin and its regulators. To date, it is unclear which function of cohesin is more relevant to the pathology of the syndrome. A mouse heterozygous for the gene encoding the cohesin loader Nipbl – generated by the group of A. Calof and A. Lander (University of California at Irvine, USA) – recapitulates many features of CdLS. We have carefully examined Nipbl deficient cells and found that they have robust cohesion all along the chromosome. DNA replication, DNA repair and chromosome segregation are carried out efficiently in these cells. While bulk cohesin loading is unperturbed, binding to certain promoters such as the Protocadherin genes in the brain is notably affected and alters gene expression (FIGURE 2). These results provide further support for the idea that developmental defects in CdLS are caused by deregulated transcription and not by malfunction of cohesion-related processes. ■

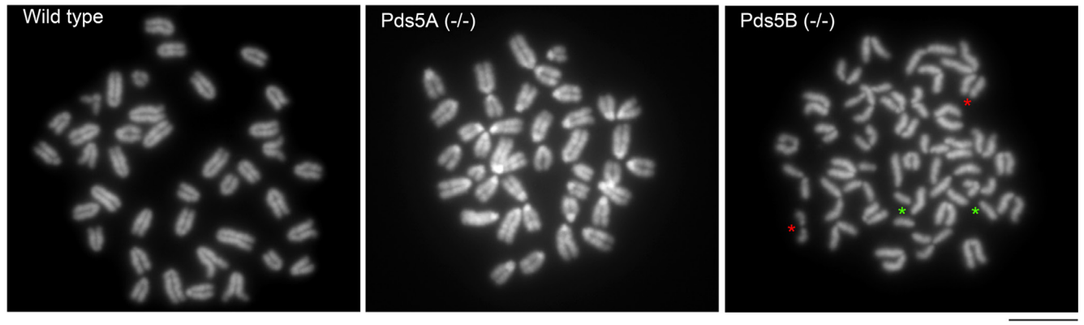

Figure 1 (up) Pds5B is specifically required for the establishment and maintenance of centromeric cohesion. Chromosome spreads were prepared from hepatocytes isolated from E14.5 embryos of the indicated genotypes. In the case of Pds5B null cells (right panel) several chromosomes display separated centromeres (red asterisks) or complete loss of cohesion between sister chromatids (green asterisks). Scale bar, 10 μm.

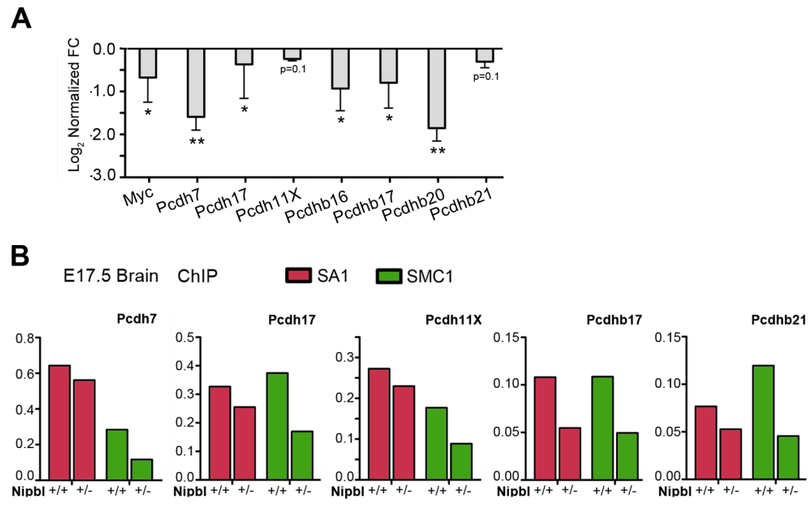

Figure 2 (right) Nipbl deficiency alters cohesin binding at certain promoters and affects gene expression. (**A**) Down regulation of Protocadherin genes in the brains of E17.5 *Nipbl +/-* embryos could be observed after measuring mRNA levels of the indicated genes by qPCR. (**B**) Chromatin immunoprecipitation followed by qPCR (ChIP-qPCR) of SA1 and SMC1 cohesin subunits was performed to assess binding of cohesin to their promoters.

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OVERVIEW

As part of the physiological renovation of our tissues, cells regularly undergo rounds of division that are preceded by the duplication of their DNA. The process of DNA replication entails risks to the genome, ranging from sporadic mutations to the generation of chromosomal breaks and translocations that may activate oncogenes or inactivate tumour suppressor genes. At the molecular level, the vulnerability associated to DNA replication is caused by the temporary exposure of stretches of ssDNA; a phenomenon called “replication stress”. Cellular exposure to UV irradiation increases replication stress by introducing chemical modifications in the DNA that standard DNA polymerases cannot recognise. In this situation, the protein complexes in charge of synthesising new DNA, known as replisomes, are initially stalled and may eventually collapse and generate double-strand DNA breaks. Cells are equipped with defence mechanisms, including a checkpoint pathway that detects ssDNA and attracts several factors that stabilise stalled replisomes. In our laboratory we study two complementary aspects of DNA replication: (1) the characterisation of proteins and mechanisms that counteract replication stress; these include the activation of “dormant” origins upon the collapse of nearby replication forks and the existence of enzymes that facilitate fork progression; and (2), the effects of deregulated DNA replication *in vivo* in mammalian organisms, particularly on ageing and cancer predisposition.

“We have identified a new enzyme, which facilitates the replication and repair of DNA molecules damaged by UV irradiation, and that is required to maintain genomic stability. We have also discovered that deregulated DNA replication in transgenic mice impairs embryonic haematopoiesis and favours the development of haematological cancers.”

RESEARCH HIGHLIGHTS

PrimPol facilitates DNA replication on damaged DNA

We have identified and characterised a new enzyme that facilitates DNA replication fork progression under stress conditions that are induced by either dNTP attrition or UV irradiation. This enzyme, encoded by gene *CCDC111* located on chromosome 4q35.1, has been named PrimPol because it displays both DNA primase and polymerase activities. *In vitro*, PrimPol is capable of bypassing the two most common types of UV light-induced lesions, CPD and (6-4)pp thymine adducts. Using a combination of time-lapse microscopy in living cells and single-molecule analysis of DNA replication, we have discovered that PrimPol is rapidly recruited to DNA damage sites and uses its primase activity to mediate uninterrupted fork progression (FIGURE 1). PrimPol's biochemical activity promotes the reinitiation of DNA synthesis downstream of the lesion. The replisome then effectively skips the damaged DNA, leaving an unreplicated gap to be repaired after replication by specialised DNA polymerases or through homologous recombination. Interestingly, PrimPol can also be found inside mitochondria, where it plays a role in the maintenance of mitochondrial DNA. As an enzyme involved in DNA damage tolerance, PrimPol has potential application as a target for cancer therapy.

Overexpression of *CDC6* is oncogenic in the mouse

CDC6 and *CDT1* genes encode two proteins responsible for the first stages of replisome assembly at replication origins. In a process referred to as "origin licensing", the combined action of Cdc6 and Cdt1 attracts and engages the hexameric MCM helicase with the DNA. MCM, in turn, becomes part of the main DNA helicase acting at the replication forks. The protein level and activity of both Cdc6 and Cdt1 are strongly regulated; both proteins are inactivated during S phase to prevent unscheduled events of origin licensing that could result in partial DNA re-replication. These control mechanisms, however, could be partially lost in tumour cells. *CDC6* and *CDT1* are indeed highly expressed in several cancer types, such as non-small cell lung carcinoma (NSCLC). Recent work in model systems has shown that even modest amounts of re-replication can lead to gene amplification. To study this possibility, we have generated several transgenic and *knock-in* mouse strains that allow the inducible expression of *CDC6* and *CDT1*, either individually or in combination. We have already determined that the continued overexpression of *CDC6* in adult mice leads to early-onset lymphomas and a shorter life span. Consistent with its proto-oncogenic function, *CDC6* overexpression driven from the keratin 5 promoter sensitises mice to papilloma formation in the skin (FIGURE 2). We are currently characterising the *in vivo* effects of simultaneous *CDC6* and *CDT1* overexpression.

Hypomorphic expression of MCM3 causes incomplete erythropoiesis

Following a complementary approach to the "gain of function" models described above, we have continued to study MCM3$^{GFPLuc\text{-}loxP}$; a mouse strain carrying a genetically modified *MCM3* allele with hypomorphic expression that also allows its ablation with Cre recombinase. Heterozygous *MCM3*$^{+/GFPLuc\text{-}LoxP}$ and *MCM3*$^{+/-}$ mice are viable, but their lifespan is reduced due to early-onset lymphomas and mesenchymal tumours. In addition, homozygous *MCM3*$^{GFPLuc\text{-}LoxP/GFPLuc\text{-}LoxP}$ embryos die *in utero* around embryonic day (E) 14.5-16.5; the gestational stage in which liver haematopoietic stem cells (HSCs) proliferate rapidly in order to build the erythropoietic system. Given the very short S-phase of foetal liver HSCs (1.5-2 h), our hypothesis is that MCM3-defective HSCs cannot activate sufficient origins of replication to sustain efficient erythropoiesis. In 2013, we initiated a collaborative study with E. Passegué (University of California, San Francisco, USA) in order to evaluate the DNA replication dynamics of HSCs in the MCM3$^{GFPLuc\text{-}LoxP}$ strain and to test their functionality in engraftment assays. ■

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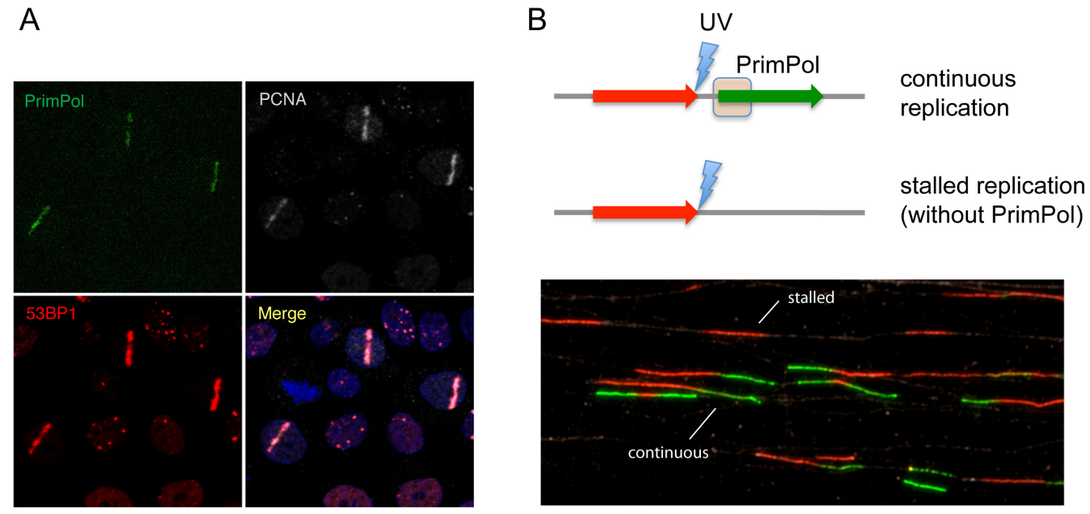


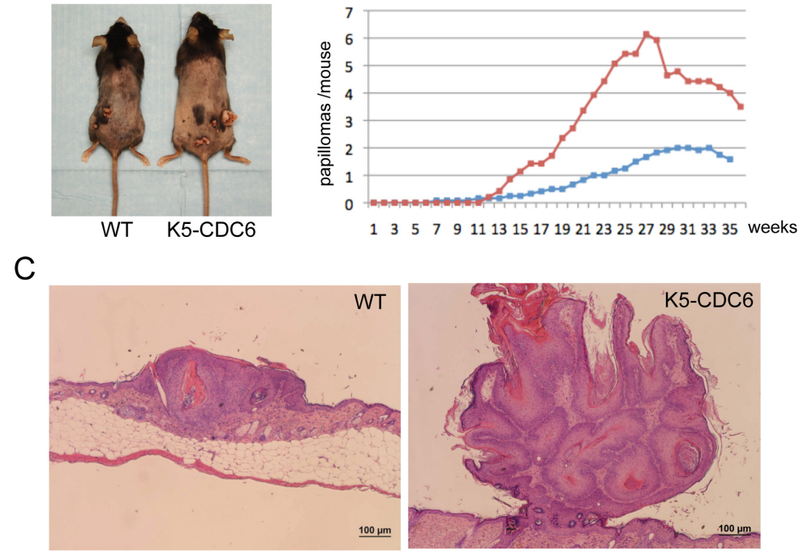
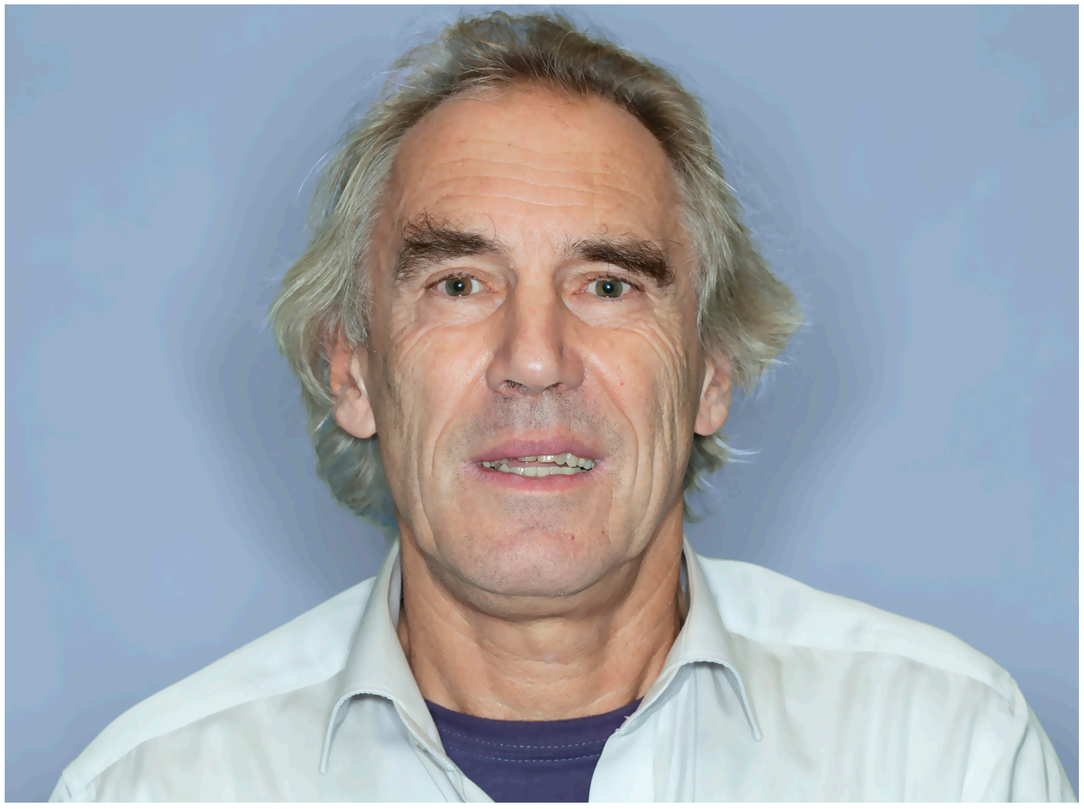


Figure 1 (up) PrimPol facilitates replication through damaged DNA. (**A**) PrimPol, PCNA and 53BP1 localise to nuclear sites irradiated with an UV-A laser beam. (**B**) Analysis of stalled forks (red tracks) or forks with continuous synthesis (red-green tracks) in individual DNA molecules.

Figure 2 (left) Oncogenic effects of CDC6 overexpression in mice. (**A**) Representative examples of WT and K5-CDC6 mice treated with DMBA/TPA to induce papillomas. (**B**) Number of papillomas detected in WT (blue) or K5-CDC6 mice (red) along the duration of the experiment. (**C**) Histology section of papillomas from WT or K5-CDC6 mice.

BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director



The overall strategic goals of the BBVA Foundation - CNIO Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development, progression and metastasis, as well as to discover molecular mechanisms that could provide a basis for novel therapies. Our Programme investigates how a tumour can grow as an 'extrinsic organ'. Our research covers various aspects of tumour cell biology, 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, angiogenesis, hypoxia, 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 1 Senior and 3 Junior Groups.

My own Research Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes. Our studies focus on liver fibrosis and fatty liver disease, inflammation and cancer, bone homeostasis and osteosarcomas, and also aim to molecularly define the causes of skin cancer and inflammatory skin diseases, such as psoriasis. Mirna Pérez-Moreno's Group concentrates on the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer development; whereas Nabil Djouder's Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development.

The third Junior Group was incorporated into the Programme in late 2012 and is headed by Massimo Squatrito, who moved from the Memorial Sloan Kettering Institute in New York. This Group is in part funded by the Seve Ballesteros Foundation and studies how brain tumours, mainly glioblastomas and medulloblastomas, develop and how they respond to therapy. ■

"We aim to make CNIO a more international institution; we have 17 different nationalities in our Programme and have hosted one foreign Visiting Professor with support from the Jesús Serra Foundation. With the generous support from the BBVA Foundation, we recruited –besides my Group– three foreign Group Leaders, one of whom receives funding from the Seve Ballesteros Foundation, to perform first-class cancer cell biology research and 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, Juan Guinea-Viniegra, María Jiménez, Helia B. Schönthaler, Özge Uluçkan


Erwin F. Wagner AUT


Latifa Bakiri FRA


Juan Guinea-Viniegra ESP


María Jiménez ESP


Helia B. Schönthaler DEU


Özge Uluçkan CYP


Rainer W. Hamacher DEU


Michele Petruzzelli ITA


Álvaro Ucero ESP


Hui Wu CHN


Stefanie Wurm AUT


Vanessa Bermeo ESP


Ana Guío ESP

Post-Doctoral Fellows
Rainer W. Hamacher, Michele Petruzzelli, Jochen Schulze (until July), Martin K. Thomsen (until October), Álvaro Ucero (since October), Hui Wu (until November)

Graduate Students
Eva Briso de Montiano (until June), Sebastian Hasenfuss (until June), Stefanie Wurm

Technicians
Vanessa Bermeo (since July), Marta García (until June), Ana Guío

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, and furthermore employing patient-derived samples. Specifically the functions of the AP-1 (Fos/Jun) transcription factor complex in regulating cell proliferation, differentiation and transformation are being investigated. The ultimate goal is to define the molecular pathways leading to disease development and to identify novel therapeutic targets. We focus on:

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

"We aim to make CNIO a more international institution; we represent 17 different nationalities and have hosted a Visiting Professor with support from the Jesús Serra Foundation. With the invaluable support from the BBVA Foundation, 4 foreign Group Leaders, 1 of whom receives funding from the Seve Ballesteros Foundation, are focusing on unravelling 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 or dimers in the liver, skin and bone (FIGURE 1). This technology has been transferred to CNIO's Transgenic Mice Core Unit. Mouse and human tissue samples are used for large scale studies, such as RNA expression profiling, large scale sequencing (RNA-Seq, ChIP-Seq) and mass spec analyses.

Bone development and sarcomas

We are studying the function of Fos proteins and TGFBI (βIG-H3), a Fos target gene, using loss- and gain-of-function mouse models. Preliminary results show that TGFBI has a role in bone homeostasis and osteosarcoma development. In addition, Fra-2 transcriptionally controls osteocalcin, collagen and adiponectin, thereby affecting osteoblast function and systemic metabolism.

Liver disease – inflammation, metabolism, fibrosis and cancer

In hepatitis, c-Jun is a mediator of cell survival specifically in hepatocytes, while the absence of JunB in immune cells is beneficial. Mechanistically, JunB promotes cell death during

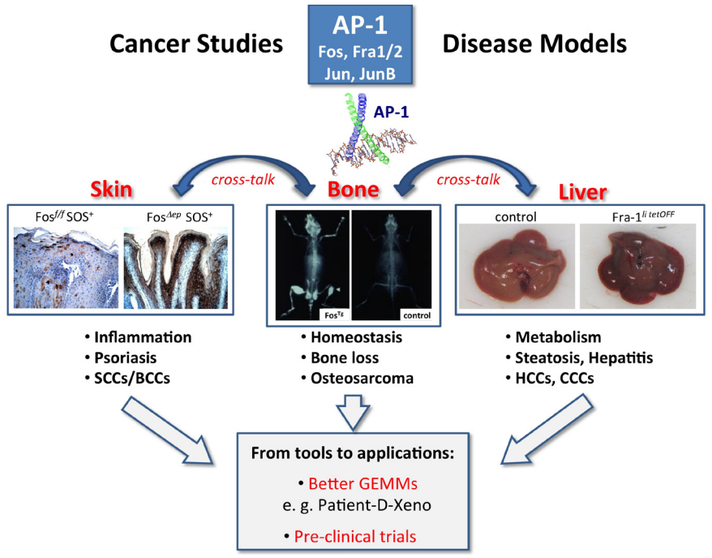


Figure 1 Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers or dimers in the liver, skin and bone. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease and to identify novel targets. Preclinical studies are performed in our AP-1-dependent mouse models with compounds that target the identified molecules to determine the potential of translating our findings to treating human disease.

acute hepatitis by regulating interferon-γ production in NK and NKT cells, thus functionally antagonising the hepatoprotective function of c-Jun.

Fra-1 and Fra-2 proteins seem dispensable for liver fibrosis, while they are important novel modulators of hepatic lipid metabolism. AP-1 modulates hepatic lipid storage and steatosis formation by controlling PPARγ transcription. Strikingly, AP-1 dimers can either induce or repress PPARγ expression. Therefore, fatty liver disease and obesity likely depend on the composition of AP-1 dimers.

Ectopic expression of c-Fos and its dimers leads to spontaneous liver inflammation, fibrosis, hepatocyte/bile duct hyperproliferation, and cancer. Conversely, deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis. Interestingly, additional deletion of c-Fos in immune cells abrogates this protective effect.

Role of white adipose tissue in cancer-associated cachexia

Various cancer mouse models were employed to discover a consistent metabolic and phenotypic switch from white to brown fat (browning) in cachectic mice. The role of browning as a contributor to the wasting process was further characterised providing a promising new target to prevent/delay cachexia in cancer patients.

A function for AP-1 in the lung

The contribution of Fra proteins to lung fibrosis and cancer is currently being studied using GEMMs, as well as lung cancer samples from patients; this study is conducted in collaboration with the Medical University Erlangen (Germany), Mariano

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- Bakiri L, Wagner EF (2013). Mouse models for liver cancer. *Mol Oncol* 7, 206-223.
- Bozec A, Bakiri L, Jimenez M, Rosen ED, Catalá-Lehnen P, Schinke T, Schett G, Amling M, Wagner EF (2013). Osteoblast-specific expression of Fra-2/AP-1 controls adiponectin and osteocalcin expression and affects metabolism. *J Cell Sci* 26, 5432-5440.
- Evellin S, Galvagni F, Zippo A, Neri F, Orlandini M, Incarnato D, Dettori D, Neubauer S, Kessler H, Wagner EF, Oliviero S (2013). FOSL1 controls the assembly of endothelial cells into capillary tubes by direct repression of av and ß3 integrin transcription. *Mol Cell Biol* 33, 1198-1209.

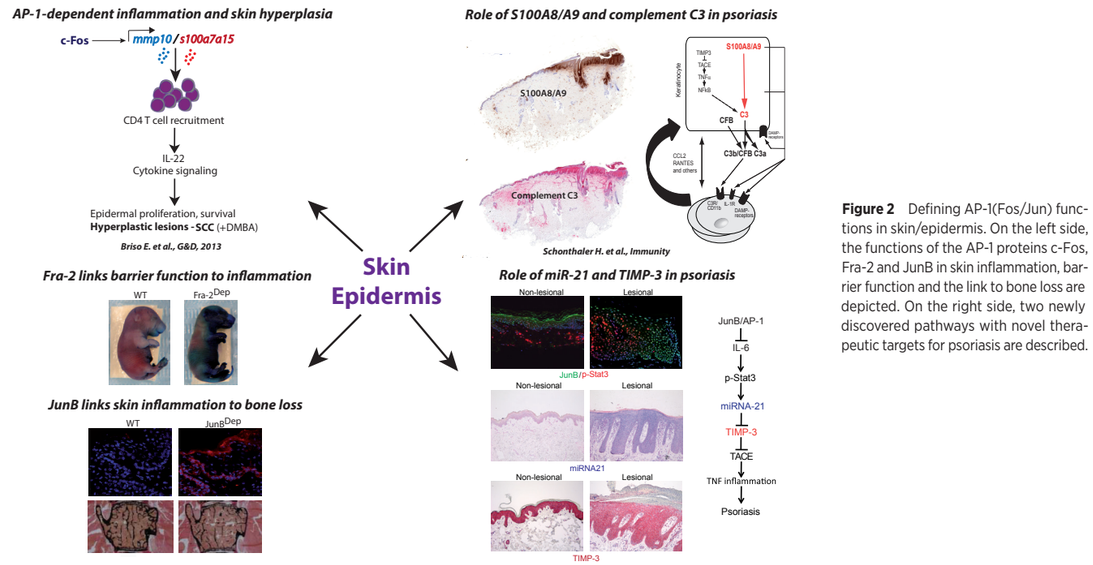

Figure 2 Defining AP-1(Fos/Jun) functions in skin/epidermis. On the left side, the functions of the AP-1 proteins c-Fos, Fra-2 and JunB in skin inflammation, barrier function and the link to bone loss are depicted. On the right side, two newly discovered pathways with novel therapeutic targets for psoriasis are described.

Barbacid´s Experimental Oncology Group at the CNIO, and Daiichi Sankyo Company.

Skin cancer, inflammation and human disease

Increased c-Fos expression is detected in Squamous Cell Carcinomas (SCCs). We have modelled SCC development in a mouse model with inducible c-Fos expression and identified an essential role of c-Fos in modulating immune cell recruitment to the skin, which contributes to skin cancer development.

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. Therefore, targeting these cytokines can prevent bone loss in these diseases.

Using GEMMs, we demonstrate that Fra-2 transcriptionally promotes expression of epidermal differentiation genes. Loss of epidermal Fra-2 results in skin barrier defects and cell autonomous secretion of TSLP by keratinocytes.

Several new approaches including genetic and biochemical analyses by proteomics of mouse and human skin samples were performed and unravelled novel pathways and molecules for targeted therapies (FIGURE 2). Furthermore, the potential role of specific miRNAs involved in the pathogenesis of psoriasis is being studied. Human skin samples are provided by our collaborator, Esteban Daudén, from *Hospital Universitario de La Princesa* (Madrid, Spain). ■

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- Schumacher M, Schuster C, Rogon ZM, Bauer T, Caushaj N, Baars S, Szabowski S, Bauer C, Schorpp-Kistner M, Hess J, Holland-Cunz S, Wagner EF, Eils R, Angel P, Hartenstein B. Efficient keratinocyte differentiation strictly depends on JNK-induced soluble factors in fibroblasts. *J Invest Dermatol* (in press). PMID: 24335928.

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

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Carolina Epifano

Graduate Students
Ljiljana Dukanovic,
Marta N. Shahbazi (until November)

Technician
Francesca Antonucci

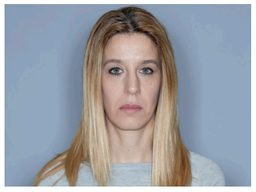

Mirna Pérez-Moreno MEX


Donatello Castellana ITA


Carolina Epifano ESP


Ljiljana Dukanovic BIH


Marta N. Shahbazi ESP


Francesca Antonucci ITA

OVERVIEW

Our research aims at advancing insight into the events that regulate the physiology of epithelial tissues and how, when perturbed, these result in disease, including cancer. The primary epithelial tissue we study is the skin. In adult skin, epithelial progenitor cells have been identified as the cell of origin of skin carcinomas; the most common cancers in the world. These cells reside in the basal proliferative layer of the epidermis, whereas in the hair follicle they localise in a restricted area known as the bulge. We focus on dissecting how the interactions between epithelial progenitor cells, including interactions with their surrounding microenvironment, maintain tissue architecture and modulate cell adhesion, proliferation, migration, and gene expression.

In order to investigate how alterations in the interactions between epithelial progenitor cells and their neighbouring cells, and/or surrounding tissue microenvironment, correlate with hair and epidermal diseases, we employ mouse genetics, *in vitro* culture systems, and human skin samples analyses.

"During 2013, we continued our efforts to uncover novel events controlling the behaviour of skin progenitor cells, and to increase our understanding of how their malfunction may lead to cancer; the ultimate goal is to enable their use in regenerative and anti-cancer therapeutic approaches."

RESEARCH HIGHLIGHTS

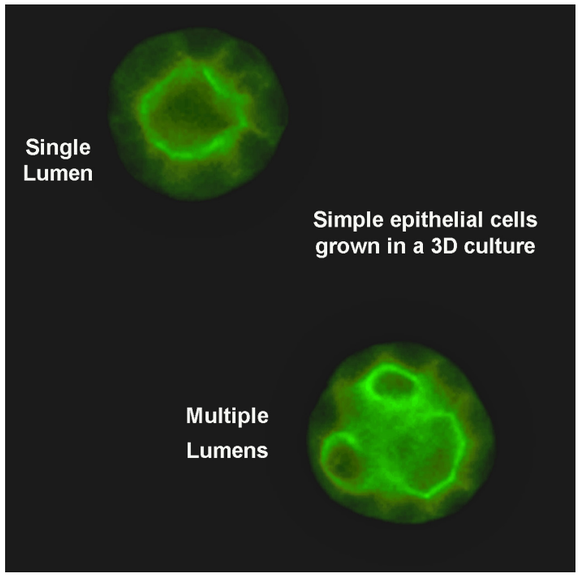


Figure Microscope image of simple epithelial cells grown in a three-dimensional culture. Under these conditions cells form cysts, characterised by a hollow internal lumen surrounded by polarised cells. Alterations in cell polarity proteins lead to disruption of the cyst architecture and the formation of multiple lumens. The actin cytoskeleton is labelled in green.

PUBLICATION

Shahbazi MN, Megías D, Epifano C, Akhmanova A, Gundersen GG, Fuchs E, Perez-Moreno M (2013). CLASP2 interacts with p120-catenin and governs microtubule dynamics at Adherens Junctions. *J Cell Biol* 203, 1043-1061.

Using skin as the primary tissue of study, and mice as a genetic model system, we continued investigating how epithelial skin progenitor cells preserve their dynamic interactions and communicate with their microenvironment in order to maintain tissue homeostasis and promote tissue repair upon injury.

The results obtained from our research are helping us to understand how deregulations in these events lead to alterations in skin regeneration and promote tumourigenesis.

During 2013, we achieved the following key accomplishments:

→ We have identified novel players that control the polarised architecture of both simple epithelial cells (FIGURE) and epidermal cells, and that also play a potential role in regulating the orientation of the mitotic divisions of progenitor basal cells of the epidermis.

→ We have found that epidermal progenitor cells are held together through the stabilisation of adhesion complexes, termed adherens junctions, via the microtubule cytoskeleton. Specifically, we have found that the adherens junction protein p120-catenin binds to the microtubule-associated protein CLASP2 and contributes to the correct epidermal architecture and skin homeostasis.

→ We have uncovered a mechanism by which epidermal progenitor cells sense injury and promote the repair of epithelial layers. This involves the adherens junction protein p120-catenin, whose roles extend beyond intercellular adhesion, to the regulation of inflammatory responses and epithelial remodelling upon tissue injury, as well as being potentially implicated in chronic inflammation and cancer.

→ We have identified a novel interaction between skin progenitor cells and macrophages, which modulates stem cell properties and their regenerative potential. This is an important step to decipher how alterations in this crosstalk are implicated in cancer. These findings could facilitate the design of targeted therapeutic approaches for skin regeneration. ■

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Nabil Djouder
Junior Group Leader

Post-Doctoral Fellows
Hugo Bernard, Stefan Burén, Mohamad-Ali Fawal, Ana Gomes

Graduate Students
Marta Brandt, Almudena Chaves, Krishna Seshu Tummala


Nabil Djouder FRA


Hugo Bernard FRA


Stefan Burén SWE


Mohamad-Ali Fawal LBN


Ana Gomes PRT


Marta Brandt POL


Almudena Chaves ESP


Krishna Seshu Tummala IND

OVERVIEW

Ever since Western society has shifted to a higher caloric diet with nutrients overload and a more sedentary lifestyle, the incidence of metabolic syndrome and cancer has increased to epidemic proportions. Using *in vivo* mouse models combined with biochemical techniques, we are interested in dissecting the growth factor and nutrient signalling cascades that impact the patho-physiolological states of metabolic disorders and cancer. Successful outcomes in new mechanistic insights of circuits associated to growth factors and nutrients may have significant predictive clinical potential and should facilitate the development of innovative mechanism-based therapies to treat metabolic diseases and cancer.

"The identification and validation of gatekeeper pathways in early disease stages offers new therapeutic strategies to prevent and cure metabolic dysfunctions and cancer."

PUBLICATION

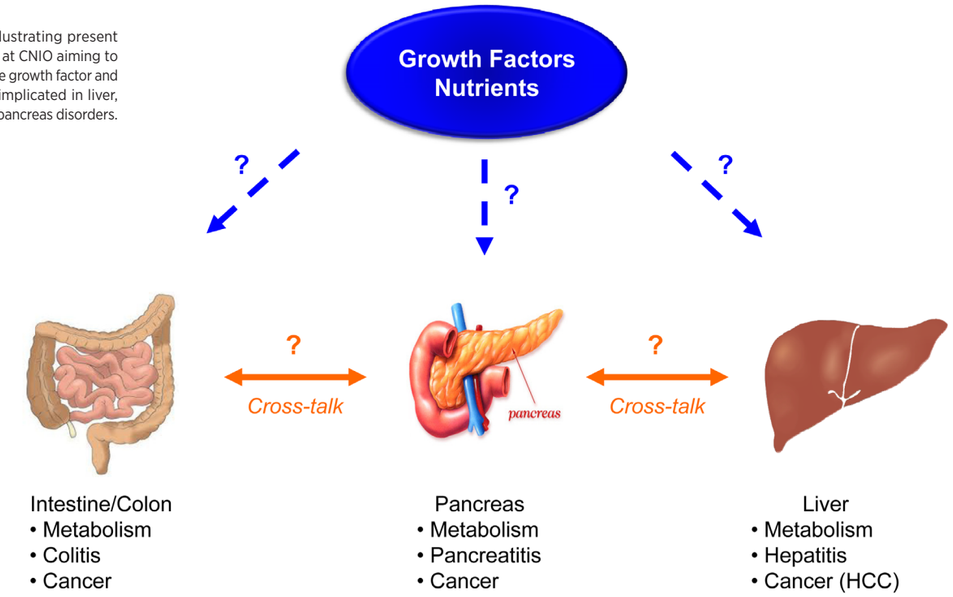
- Mita P, Savas JN, Ha S, Djouder N, Yates JR 3rd, Logan SK (2013). Analysis of URI nuclear interaction with RPB5 and components of the R2TP/prefoldin-like complex. *PLoS One* 8, e63879.

Awards and recognition

- *Ad hoc* Reviewer of *Gastroenterology, Molecular Oncology* and *Cell Death and Differentiation.*

RESEARCH HIGHLIGHTS

Figure Scheme illustrating present and future research at CNIO aiming to better understand the growth factor and nutrient circuitries implicated in liver, intestine/colon and pancreas disorders.



We are studying diseases and associated molecular mechanisms linked to the mammalian/mechanistic target of rapamycin (mTOR) dysfunctions, critical in sensing growth factors and nutrients. We are particularly interested in the liver, intestine and pancreas, as these three organs are physiologically interconnected and influenced by their exocrine and/or endocrine functions. Nutrients overload, through elevated levels of insulin and related insulin-like growth factors (IGF), dysregulate the organ through cellular overactivation of mTOR. In this context, the function and balance of whole-body energy metabolism may be affected, leading to severe metabolic disorders that can ultimately progress to cancer.

This year, we achieved the following lines of research: either achieved a milestone or followed lines of research.

Generation of *in vivo* mouse models

In our laboratory, we recently discovered new components of the mTOR signalling pathways. Genetically engineered mouse models (GEMMs) with gain- and loss- of function of these mTOR regulators were generated in our lab in order to study *in vivo* the impact of nutrients and growth factors in cancer development and metabolic disorders associated to liver, colon and pancreas.

Identifying new components of growth factor and nutrient circuits

Using microscopy and live-cell imaging, we created a new tool based on fluorescence resonance energy transfer (FRET) that is currently being used to screen and find new components of the growth factor and nutrient signalling cascade. ■

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientists
Alberto J. Schuhmacher (since March),
Bárbara Oldrini (since February)

Graduate Student
Carolina Almeida

Massimo Squatrito ITA

Alberto J. Schuhmacher ESP

Bárbara Oldrini ITA

Carolina Almeida PRT

OVERVIEW

Malignant brain tumours represent about 3% of all cancers, and annually around 100,000 new cases are diagnosed worldwide. In Spain, there are about 4,000 new cases each year. Gliomas are a large group of brain tumours of which Glioblastoma Multiforme (GBM) is the most frequent and aggressive primary central nervous system (CNS) tumour in adults. Regardless of the recent advances in treatment modalities, GBM patients usually respond poorly to all therapeutic approaches and prognosis remains dismal (approximately 15 months).

Our laboratory uses a combination of genomic analyses, mouse models and primary tumour cell cultures, in order to identify molecular mechanisms that could provide a basis for the development of novel therapeutic modalities for GBM patients.

"The main focus of our group is to uncover the genetic defects present in GBM patients that might be responsible for the aggressiveness of this tumour type; in particular, we are interested in the identification of the genetic alterations that lead to the modulation of the activity of the DNA damage response (DDR)."

RESEARCH HIGHLIGHTS

Role of AP-1 transcription factors in gliomagenesis

AP-1 (activating protein-1) transcription factors have been involved in numerous biological processes, including cell proliferation, differentiation, apoptosis, inflammation, and oncogenic transformation. Abnormal expression and/or activation of AP-1 proteins contribute to the development of various diseases, including cancer. AP-1 is constitutively activated in various human cancers, including glial tumours, for which there is some evidence of its direct association with chemotherapeutic resistance and a decreased response to apoptotic signals. Through the analysis of various glioma datasets, we have observed that AP-1 components are frequently overexpressed in GBM patients. In collaboration with the Genes, Development and Disease Group, we are using a series of loss-of-function mouse models to dissect the role of the various AP-1 members in glioma tumour formation and treatment response.

DNA Damage Response (DDR) signalling in glioma tumour formation and therapeutic resistance

Preservation of genomic integrity is an essential process for cell homeostasis. Defects in the DNA Damage Response (DDR), a network of protein complexes capable of detecting DNA lesions and signalling to downstream effector pathways (cell cycle checkpoints, DNA repair, apoptosis, etc.), are linked to numerous pathological states including brain cancers. We have previously shown that genes encoding key molecules of the DDR (such as ATM, Chk2 and 53BP1) are subjected to frequent copy number alterations in GBM patients and are required for glioma tumour suppression in mice. In close collaboration with the CNIO Genomic Instability Group, we are currently studying other important components of the DDR, such as the ATR and Chk1 kinases, by means of different loss-of-function and gain-of-function mouse models. We are also conducting preclinical analysis of specific DDR inhibitors developed here at CNIO. ■

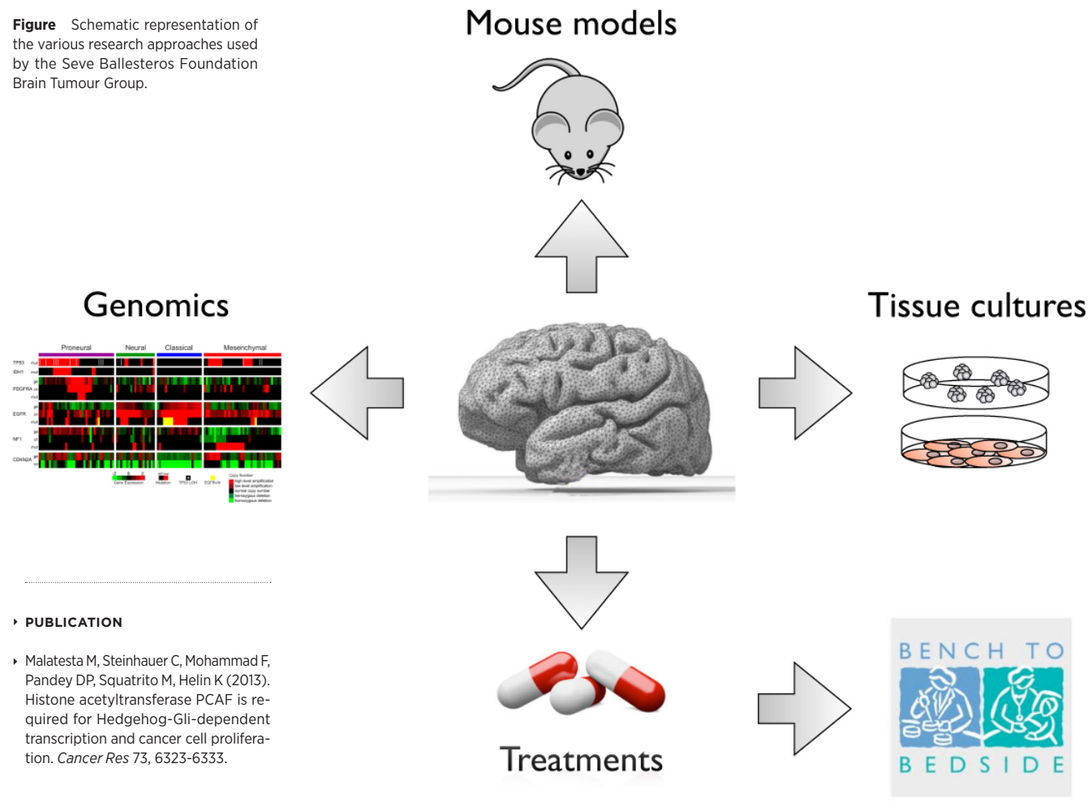


Figure Schematic representation of the various research approaches used by the Seve Ballesteros Foundation Brain Tumour Group.

PUBLICATION

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STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

ALFONSO VALENCIA Programme Director

The main objective of the Structural Biology and Biocomputing Programme is to expand the mechanistic understanding of key cancer related molecular systems. The strength of the Programme resides in its capacity to combine computational, biochemical, biophysical and structural approaches. Our Programme is deeply involved in collaborations with basic and translational Research Programmes at the CNIO, as well as in a number of international consortia.

Our 3 main research goals are to:

- Reconstruct the structural details of protein complexes that are active in cell cycle control, DNA repair, genomic stability and growth factor signalling.
- Predict the consequences of cancer related alterations; we are focusing on alterations of compensatory nature (co-evolutionary related mutations) as well as those affecting alterative splicing patterns.
- Model the dynamics of tumour progression through the integration of epi- and genomic information.

“The work of our Programme — on the building of mechanistic models that capture the details of the underlying molecular interactions — provides the essential link between basic biological knowledge and actionable biomedical applications.”

Research highlights of the year include: the participation of the Bioinformatics Unit in top CNIO publications – including the paper by Manuel Serrano´s Tumour Suppression Group on *in vivo* reprogramming; the development of a conceptual model of the role of genome structure in the evolution of tissue-specific expression; deciphering the genes and pathways implicated in the inverse comorbidity of cancer, and neurological diseases; the inclusion of conformational flexibility in the allosteric action model of non-receptor tyrosine kinases c-Src and c-Abl; the development of computational methods based on co-evolution for the prediction of epistatic interactions; employing a multidisciplinary approach to demonstrate the activation of the membrane clustering of Focal Adhesion Kinase (FAK) by phosphatidylinositol 4,5-biphosphate (PIP_2); deciphering the three-dimensional structure of CAD, an anti-tumour target controlling the biosynthesis of pyrimidines; the combined structural and biochemical analysis of the binding of AvrBs3 TALE (Transcription Activator-Like Effector) to its DNA target; and, using a combination of X-ray and electron microscopy studies, the structural analysis of the eukaryotic CMG system, including the MCM2-7 complex and its activity in the unwinding of DNA during S phase. ■

STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Alfonso Valencia
Group Leader

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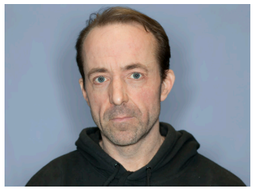
Alfonso Valencia ESP


Federico Abascal ESP


Milana Morgenstern ISR


Tirso Pons CUB


Daniel Rico ESP


Michael Tress GBR


Vera Pancaldi ITA


Miguel Vázquez ESP


César Boullosa ESP


Simone Ecker AUT


Paolo Maietta ITA


Juan Rodríguez ESP


David Juan ESP


Martin Krallinger AUT


Florian Leitner AUT


Miriam Rubio ESP

Post-Doctoral Fellows
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OVERVIEW

The main interest of our group is the study of the molecular bases of cancer progression. We bring an evolutionary perspective to how global genome organisation influences tumour progression, i.e. how the interplay between genomics and epigenomics determines the cause and course of the progression of tumours.

Our research is largely carried out in the context of large-scale projects, where we develop and apply computational methods to reveal general properties of the genome-cancer relationships. In parallel, these methods and ideas are applied to specific molecular systems from which we can gain detailed molecular information.

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 statistical 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.

"In 2013, we carried out a comprehensive analysis of genomic (gene duplications and copy number variations) and epigenomic (replication timing, GC content) information, and proposed a model that explains the influence of genomic structure on the evolution of genomes. This model has important implications for tissue differentiation and tumour progression."

RESEARCH HIGHLIGHTS

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

CLL-ICGC

In the context of the Chronic Lymphocytic Leukaemia project, which is part of the International Cancer Genome Consortium (ICGC), we have continued developing methods for the analysis of complex cancer genome data. In particular, we have implemented novel approaches to improve the prediction of the consequences of point mutations in oncogenesis and tumour progression.

During this year, we have made significant progress in the analysis of mutations that can affect drug binding sites. This information was incorporated into our APPRIS system; the largest current collection of drug binding sites in proteins that

have been extracted systematically from protein structure databases (APPRIS). The predictions of binding sites, based on this information, were the best performing system in the latest CASP conference (Critical Assessment of techniques for protein Structure Prediction). This approach is complemented with parallel research efforts, which are dedicated to the extraction of information from the literature and to the organisation of a large compendium of texts annotated with chemical compounds and drugs (part of the BioCreative text mining challenge).

This year, we have also completed the development of the KINMUT system. This system incorporates information that makes it specific for predicting the consequences of mutations in protein kinases, and can also outperform generalist systems. Given the importance of this protein family in cancer research, the accurate prediction of the consequences of mutations in these kinases may be of particular relevance.

BluePrint-IHEC

The goal of Blueprint is to generate large-scale datasets derived from our epigenetics research efforts, as part of the International Human Epigenome Consortium (IHEC). During the starting phase of the project, we obtained from monocytes and neutrophils a complete collection of results (i.e. DNA methylation, Chip-Seq of Transcription factors, DNAase accessibility and others). Based on this complex array of data, we have produced the first ever evidence of the intrinsic difference between these two cell types.

By adopting a generic approach, we have analysed how genome organisation and replication timing influence the process of gene duplication, and how the accumulation process of duplicated genes follows a historical time course, i.e. newer genes, in evolutionary time, accumulate faster in late replication/heterochromatic regions than older genes. This model has important implications for the evolution of tissue-specific genes and, given the importance of duplications in cancer, it might be applicable for addressing the relationship between genome organisation and duplications in cancer.

We have applied the same ideas to a specific case, the evolution of the ASF1 isoforms. In this case, the detailed reconstruction of the evolutionary history of these proteins showed the intimate relation between the structure of the genome (i.e. GC content) and the acquisition of new functions.

GENCODE-ENCODE

In the context of this NIH-funded project, our Group has continued to develop our system for the annotation of splice isoforms (APPRIS), as part of the project´s efforts to map the functional elements in the human genome. In the case of cancer, the annotation of splice isoforms is essential for the correct analysis of cancer-associated mutations.

Previous studies have demonstrated the actual existence of many mRNAs, leading to the common believe that all human genes express multiple mRNA variants. We have challenged this view by analysing the existence of proteins supposedly encoded by those mRNAs. The result of analysing all the available proteomics data is that, for most genes, only one protein product exists. Even if, in normal conditions each gene produces only one main isoform and a single protein, it is by now clear that in pathological conditions the regulation of the splicing process can change this balance. This has been demonstrated in chronic lymphocytic leukaemia, where mutations in splice factors lead to alterations in the production of splice isoforms that are directly associated to the progression of the leukaemia. ■

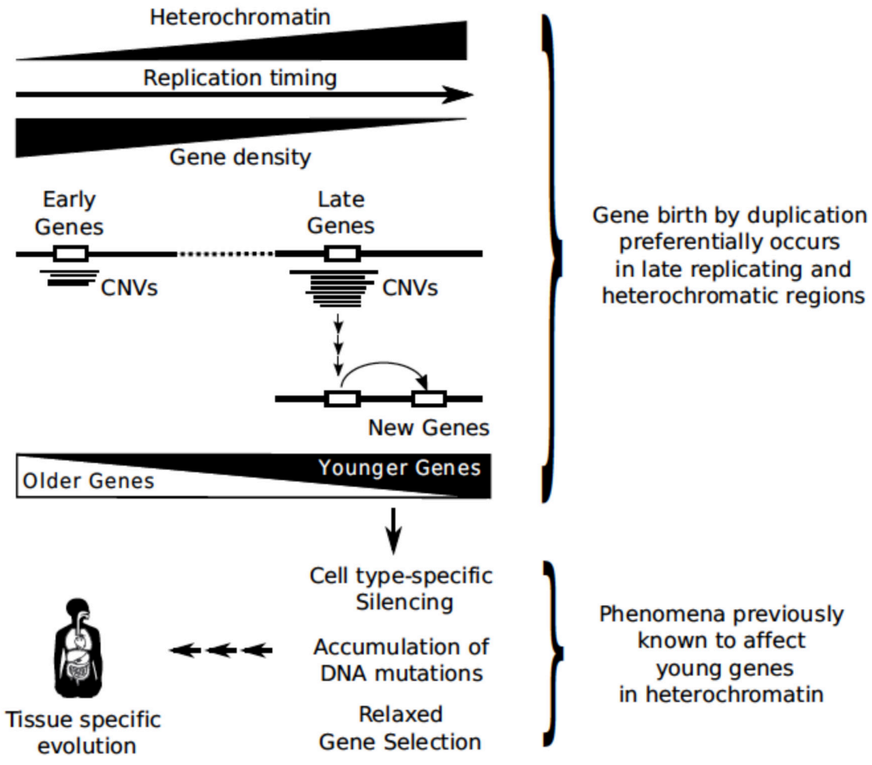

Figure Model proposing the influence of the genomic structure in the evolution of genome organisation. The model proposes that replicative stress will preferentially lead to the accumulation of copies of genes in heterochromatin-rich regions, and in an evolutionary time scale to the accumulation of genes in late-replicating regions. The location of newly duplicated genes in heterochromatin could be a factor favouring the expression of tissue-specific genes, thus contributing to tissue differentiation. This hypothetical scenario is supported by the accumulated information on gene expression, chromatin organisation, replication time maps and the evolutionary reconstruction of duplication events at the species and population levels; furthermore, it might have important implications once that it is translated to the parallel scenario of tumour progression.

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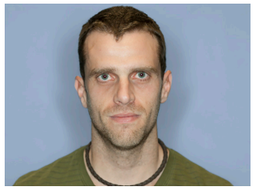
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OVERVIEW

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. The focus of our Group is 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 in an unparalleled view into 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.

"The human genome is a sophisticated and complex coding system that is capable of producing thousands of different proteins in a tightly controlled way, regulated in time and location. Proteins interact with other macromolecules, forming assemblies that perform particular cellular tasks. The structural determination of these complexes will help us to decipher the mechanisms that regulate these processes."

RESEARCH HIGHLIGHTS

S-phase and replication

DNA replication is indispensable for the reliable inheritance of the genome at each cell division. To which extent this process uses similar mechanisms in bacteria and complex organisms is still under debate. Higher eukaryotic organisms require supplementary factors to cope with larger genomes, diverse cell fates, and to increase DNA replication fidelity; thus adding extra complexity to the process. To replicate, a DNA double helix must open up to allow the DNA synthesis machinery to copy each DNA strand. In mammals, thousands of origins of replication are activated at each cell cycle. However, not all origins are activated at the same time; their activation follows the specific timing of DNA replication during the cell cycle. To initiate replication, a number of protein complexes assemble at a given replication origin in a tightly regulated and temporally controlled manner. Among these complexes, we studied a module of proteins that contains the hexameric minichromosome maintenance (MCM) 2-7 complex. This complex is responsible for the unwinding of DNA after origin firing during S phase in association with two additional partners: the initiation factor Cdc45 and a 4-subunit complex called GINS. Together, they form the CMG complex that has ATP dependent helicase activity. Our Group attempts to decipher the molecular mechanisms of this essential cellular machinery for eukaryotic DNA replication. With this aim in mind, we have been able to obtain the structural information of an MCM homologue that contains a domain bearing primase and polymerase activities (FIGURE 1). This study has helped us to propose a working mechanism for the helicase that may have important implications for our understanding of the eukaryotic complex. Thus, besides from the eukaryotic CMG, we are also attempting to gain mechanistic information about the MCM complex using X-ray crystallography and electron microscopy studies in order to decipher its structure.

Mitotic Complexes

Cellular growth and division are regulated by an integrated protein network that ensures the genomic integrity of all eukaryotic cells during mitosis. These processes involve a completely different set of genes that serve diverse functions ranging from cell motility to cell growth, genome replication, genome maintenance, etc. However, all these genes are interconnected through cellular crossroads and share common cellular homeostatic mechanisms. We have recently solved the structure of a kinase that is involved in chromatin remodelling and DNA repair. The crystals of this protein diffracted to 2.8 Å resolution. The biochemical characterisation of this enzyme has allowed us to design new inhibitory compounds. This

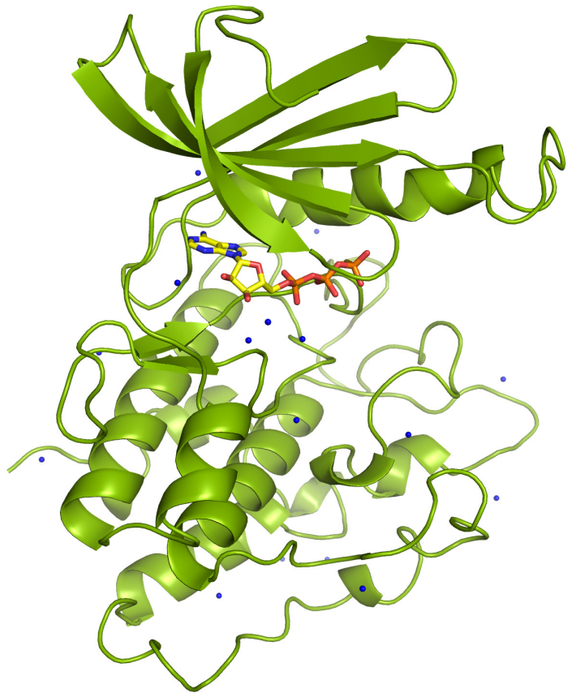


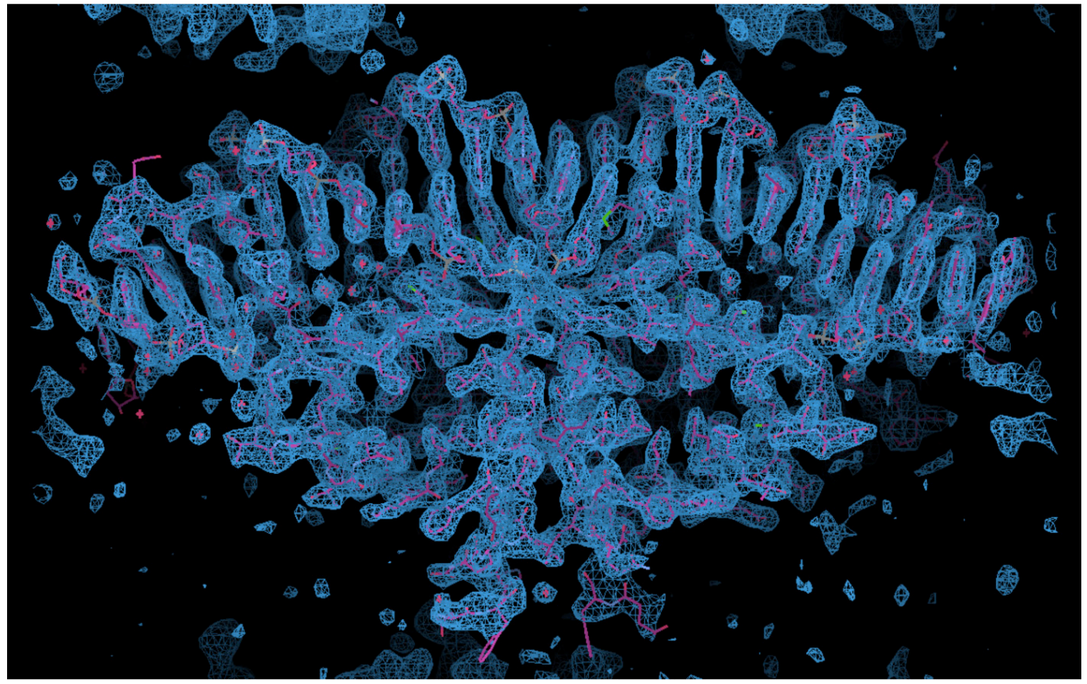
Figure 1 Crystal structure of a new kinase involved in DNA repair solved at 2.8 Å resolution. The picture shows an ATP analogue bound in the nucleotide pocket.

kinase forms dimers in solution. The oligomers may be part of a strategy to enhance its activity for key events through autophosphorylation.

Structural design of protein-DNA interactions for gene targeting

We have extended our work on homing endonucleases to a new protein-DNA binding scaffold: the TALE (Transcription Activator-Like Effector) proteins that contain a DNA binding domain constituted by tandem repeats of a 33-35 amino acid sequence. The amino acid sequence of each repeat is well conserved, with the exception of a combination of 2 adjacent amino acids (positions 12 and 13 in the repeat) that specifically recognise a single nucleotide, establishing a direct code between this pair of amino acids and each nucleotide. The assembly of several repeats by redesigned TALE that recognise new DNA targets has confirmed the modularity of these DNA binding domains. Their heterodimeric binding to adjacent

Figure 2 Crystal structure of a protein-DNA complex revealing a new protein DNA binding domain. The 2fo-fc electron density map shows the protein wrapping the DNA molecule.



DNA target sites in specific chromosomal loci, together with the fusion of these scaffolds with the catalytic domain of FokI, can generate a double-strand break (DSB) that is mainly repaired through homologous recombination. These scaffolds can present new perspectives for a wide range of applications, such as the correction of mutations linked with monogenic inherited diseases. Our Group has solved the crystallographic structures of different variants, revealing the molecular basis of new target DNA recognition domains (FIGURE 2). In addition, we have shown that the repair of the damaged gene can be done at its locus in human cells, opening avenues to possible therapeutic applications. ■

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COMPUTATIONAL BIOPHYSICS JUNIOR GROUP

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OVERVIEW

The Group is a key player in the field of kinase dynamics as well as in the development of computational methods to calculate the free energy landscapes that are associated with conformational change, ligand binding and allosterism. The use of advanced nuclear magnetic resonance (NMR) techniques, together with converged free energy calculations, sheds light on the functional consequences of oncogenic mutations in terms of changing the conformational landscape and favouring active *versus* inactive states. This in-depth knowledge is useful to rationally design inhibitors that selectively target mutant kinases, and to understand the mode of action of allosteric kinase inhibitors.

"We contributed to the understanding of the effect of oncogenic mutations on the activation mechanism of EGFR, and played a key role in elucidating the mode of action of the first allosteric inhibitors of the FGF receptor tyrosine kinase."

RESEARCH HIGHLIGHTS

A promising area in cancer research in recent years has been the search for drugs that target the growth of blood vessels within tumours. Cancerous tumours have a huge appetite for blood, and their continued growth depends on blood vessels. But success at targeting these vessels has been mixed, due to the emergence of resistance (e.g. anti-VEGF therapies in clinical trials). The receptors on tumour cells mutate quickly and drugs that can bind them now may not do so in the future.

The new drug SSR developed by Sanofi-Aventis bypasses these pitfalls by targeting receptors in a new way; instead of targeting the main ('orthosteric') receptors, they target a slower-mutating site on the receptor. This does not physically block the binding of the ligand to the receptor, but it inhibits the receptor-dependent signalling pathway and consequent cell response. Key to this research was our work simulating the response of the receptor to the drug in a supercomputer. In response to SSR, the extracellular D3 domain of FGFR undergoes a significant conformational change. The new conformer could not be seen by either crystallography or by NMR due to its extreme flexibility. Only using accurate free energy calculation and extensive all-atom MD simulations, it was possible to obtain a model for the new conformer and understand the mode of action of SSR. Based on the computational model, more potent and selective derivatives of SSR were produced. The new drugs have been tested in mice with promising results. However, as with all novel drugs, they will have to undergo comprehensive tests in humans to determine their safety and effectiveness before approval. ■

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CELL SIGNALLING AND ADHESION JUNIOR GROUP

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OVERVIEW

Our Group is interested in mechanisms regulating growth and adhesion signals that control key cellular programmes such as proliferation, growth, adhesion and survival. We use X-ray crystallography and biochemical techniques to study how some of the important signalling switches are regulated and how oncogenic events keep the signals on. We study these mechanisms at atomic resolution, which enables us to use structural information for the rational discovery of potential anti-cancer therapeutics.

Several growth and adhesion signalling molecules are regulated by specific phosphoinositides in the plasma membrane. We focus on three related systems: (i) how does 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 the serine/threonine kinase Akt/protein kinase B (Akt/PKB); and iii), how are the SH2-domain-containing inositol 5-phosphatases (SHIP) regulated to reduce PIP_3 levels in the plasma membrane.

"We have gained important insight into the structure and mechanisms regulating the cancer targets FAK, Akt/PKB and SHIP. For FAK we have discovered the first fragment compounds interacting with allosteric sites, and we aim to develop these compounds into highly selective FAK inhibitors."

RESEARCH HIGHLIGHTS

Focal Adhesion Kinase

Focal Adhesion Kinase (FAK) integrates signals from integrin and growth factor receptors to control cell migration and survival. In cancer, FAK is a major driver of disease progression, invasion and metastasis. We previously identified autoinhibitory mechanisms of FAK, showing how the regulatory 4.1-ezrin-radixin-moesin (FERM) domain docks onto the FAK kinase to induce a closed conformation. Recently, we have gained important insight into the activation mechanism of FAK. Employing an interdisciplinary approach, we show that the phospholipid PIP_2 induces FAK clustering on the cell membrane. In these clusters, FAK adopts an open conformation allowing efficient autophosphorylation. FAK autophosphorylation recruits the proto-oncogene tyrosine-protein kinase Src, which in turn phosphorylates the FAK kinase to induce the release of the FERM domain and subsequent full activation of FAK.

We utilise such detailed structural and mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ a combination of experimental and computational approaches to identify fragment compounds interacting with allosteric pockets, with the aim of extending and/or combining fragments to obtain inhibitory lead compounds. We have already discovered several novel compounds interacting with FAK.

SH2-domain-containing inositol 5-phosphatases

The SH2-domain-containing inositol 5-phosphatase (SHIP) removes the 5-phosphate from PIP_3 and therefore, like PTEN, negatively regulates PIP_3 levels. SHIP is an important regulator of Akt/PKB signalling, however, despite its importance in physiology and disease, little is known about the mechanisms regulating its activity or membrane targeting. To this end, we recently solved a crystal structure of the catalytic and C2 domains of SHIP (FIGURE), and we are now performing extensive biochemical studies to define the role of the C2 domain. We have found that the catalytic activity is greatly enhanced by the C2 domain, but to different extents depending on whether PIP_3 or its head group $Ins(1,3,4,5)P_4$ is used as substrate. We are currently performing molecular dynamics simulations to understand the allosteric effect of the C2 domain at atomic detail. ■

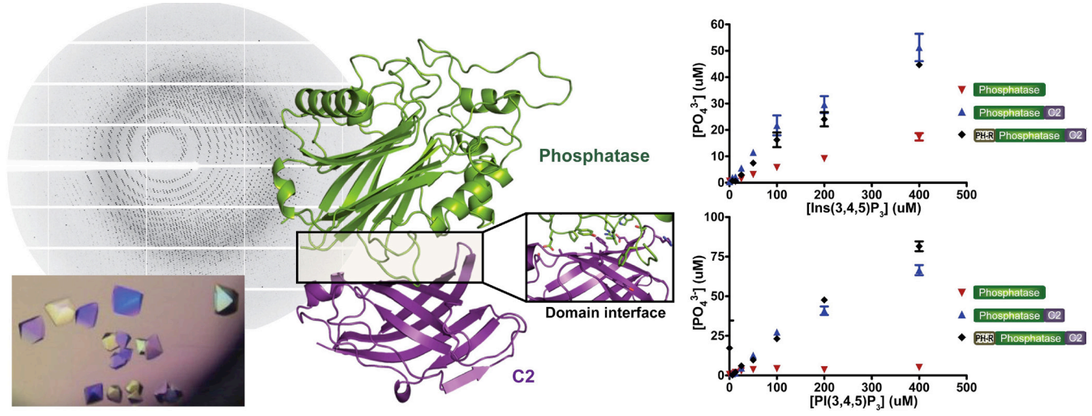

Figure Structure and activity of SHIP. The structure of the phosphatase and C2 domains of SHIP (centre) was solved from X-ray diffraction data (left) obtained from SHIP crystals (bottom left). The presence of the C2 domain in SHIP constructs is required for full catalytic activity (right).

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Dao P, Jarray R, Le Coq J, Lietha D, Loukaci A, Lepelletier Y, Hadj-Slimane R, Garbay C, Raynaud F, Chen H (2013). Synthesis of novel diarylamino-1,3,5-triazine derivatives as FAK inhibitors with anti-angiogenic activity. *Bioorg Med Chem Lett* 23, 4552-4556.

STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

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Araceli Grande ESP

OVERVIEW

Safeguarding genome integrity is essential in order to maintain correct cell functioning and to prevent cancer. At the same time, genome integrity can be an Achilles' heel of tumour cells; exacerbating the problems derived from a fast replication rate owing to nucleotide pool depletion, replicative stress or aneuploidy, may provide novel opportunities to fight tumours. Our group is interested in understanding central cellular processes that affect the integrity of the genome, such as the production of pyrimidine nucleotides, site-specific DNA recombination or the maintenance of chromatin architecture. These processes depend on the assembly of large and dynamic macromolecular complexes. We combine protein engineering, X-ray crystallography and single-particle electron microscopy, together with biochemical and functional studies, to decipher the architecture of these protein-protein and protein-DNA complexes, and to understand their catalysis and regulatory mechanisms at the atomic level.

"We have discovered a novel mechanism of targeting DNA for recombination mediated by MuB protein. We found that MuB is a new AAA+ ATPase that forms helical filaments on the DNA. We have also obtained the first atomic information about CAD by solving the crystal structures of the dihydroorotase and aspartate transcarbamoylase domains of this human complex."

RESEARCH HIGHLIGHTS

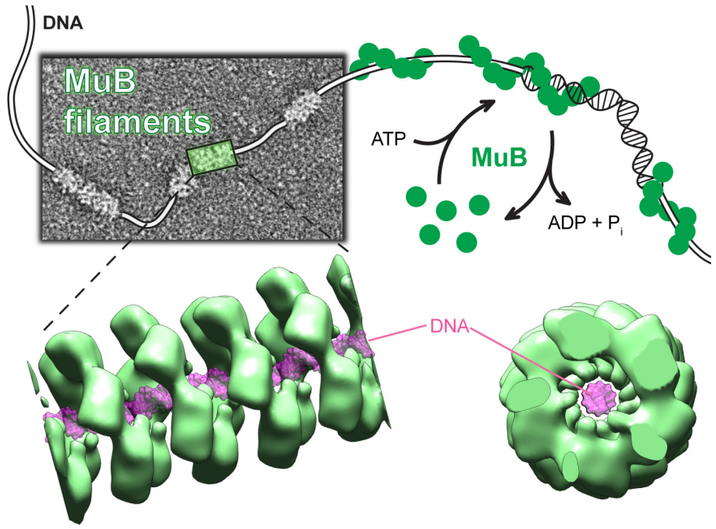

Figure MuB targets DNA for transposition. Negative staining electron microscopy image and cartoon representation of MuB helical filaments on the DNA. The 3D representation is a reconstruction of the MuB filament (carried out by Prof. N. Mizuno from the Max Planck Institute of Biochemistry in Martinsried, Germany) with a simulated DNA molecule in the axial channel.

Deciphering CAD, an anti-tumour target that controls the biosynthesis of pyrimidines

Pyrimidine nucleotides are essential building blocks for nucleic acid synthesis and DNA repair. In animals, the *de novo* biosynthesis of pyrimidines is initiated and controlled by CAD, a ~243 kDa multifunctional polypeptide that harbours the first three enzymatic activities of the pathway: glutamine-dependent carbamoyl phosphate synthetase (GLN-CPS), aspartate transcarbamoylase (ATC) and dihydroorotase (DHO). CAD is under strict allosteric control and its activity is modulated by phosphorylation through the ERK, PKA and mTORC1 signalling cascades. Up-regulation of CAD is essential for the proliferation of normal and tumour cells. Thus, targeting this central pathway opens new avenues for the development of novel therapeutic strategies. There is however no structural information about CAD, other than it forms a ~1.5 MegaDa particle that, for unclear reasons, shuttles between nucleus and the cytoplasm during the cell cycle. To understand the architecture and functioning of human CAD, we have determined the crystal structures of the DHO and ATC domains. We have also made progress in our aim to decipher the structures of the GLN and CPS domains, as well as of the entire CAD complex.

Basic mechanisms of DNA recombination

We have discovered a new mechanism that involves DNA binding and selection for translocation. We combined bioinformatics, mutagenic, biochemical and electron microscopy techniques to understand the mechanism by which MuB binds and targets DNA for recombination. We discovered that MuB is a new member of the AAA+ ATPase family and identified key residues for ATP hydrolysis, DNA binding, and protein polymerisation. We also found that MuB forms helical filaments on the DNA, with a unique symmetry that suggests a double role for this protein in DNA signalling. MuB protects the DNA within the filament from recombination, but at the same time, it promotes DNA untwisting at the end of the filament; this favours DNA bending and recognition by the transposase. ■

PUBLICATIONS

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Article in press

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SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE CORE UNIT

Ramón Campos-Olivas
Core Unit Head

Technician
Blanca López-Méndez

Ramón Campos-Olivas ESP

Blanca López-Méndez ESP

OVERVIEW

The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and other biophysical instrumentation available at the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of spectroscopic and other 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 intracellular extracts, as well as of intact cells and tissues from both animal models of cancer and human samples.

"In 2013, we quantified by Surface Plasmon Resonance (SPR) the affinity and kinetics of different protein interactions, providing essential information to unravel the molecular function of cancer-related proteins, and establishing structure-to-function relationships for the rational elaboration of ligands into high-potency and protein-specific modulators."

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RESEARCH HIGHLIGHTS

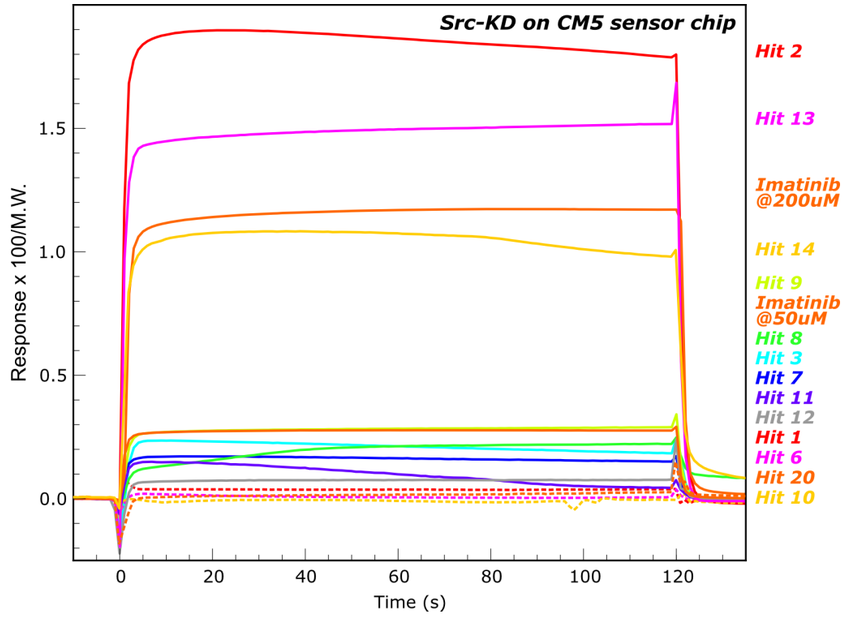


Figure SPR sensorgrams performed to evaluate the binding to SrcKD of a subset of positive hits from the primary ^{19}F NMR screening. Reference SPR data is also shown for Imatinib, a known binder, at 50 μM and 200 μM. SrcKD was immobilized onto a CM5 sensor chip and fragments flown independently at 100-200 μM. Hits 2, 13, and 14 show larger responses, indicating higher binding affinity; these hits were prioritised for follow up.

Our Core Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions. This includes spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, a multi-angle static light scattering apparatus, analytical ultracentrifugation, and a recently-installed surface plasmon resonance machine (Biacore X100). Research groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2013.

In addition, the Unit hosts a 700 MHz NMR spectrometer that is well-equipped with probes (HR-MAS, dual fluorine/proton, and triple and quadruple resonance) and a sample changer for running up to 120 samples automatically. This provides the required throughput for screening of small molecule protein binders (in collaboration with the CNIO's Structural Biology and Biocomputing and the Experimental Therapeutics Programmes), as well as for metabonomics measurements that are performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the Experimental Therapeutics Programme), the Cell Division and Cancer Group and the Tumour Suppression Group (from the Molecular Oncology Programme), and the Genes Development and Disease Group (BBVA Foundation–CNIO Cancer Cell Biology Programme). Collectively with these groups, we have implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples. As part of *REDLAB* – a research laboratory network in the Autonomous Community of Madrid – the Unit also offers all the above mentioned research technologies to the wider research community.

To illustrate some research activities of the Core Unit in 2013, the FIGURE shows how SPR was used to examine the ability of several fragment hits, identified using ^{19}F NMR screening, to bind to the Src kinase catalytic domain (SrcKD). Src is a *bona fide* cancer target that is relevant to multiple aspects of tumour biology. ■

BIOINFORMATICS CORE UNIT

David G. Pisano
Core Unit Head

Technicians
Eduardo Andrés (until September), Ángel Carro, Gonzalo Gómez, Osvaldo Graña


David G. Pisano ESP


Ángel Carro ESP


Gonzalo Gómez ESP


Osvaldo Graña ESP

OVERVIEW

The Bioinformatics Unit helps CNIO scientists to analyse and interpret their data when it requires complex numerical or computational analyses. We design and maintain the Centre´s scientific computing facilities, and train students and scientists in bioinformatics tools and methods. In collaboration with Alfonso Valencia from the Structural Computational Biology Group (CNIO), Fátima Al-Shahrour from the Translational Bioinformatics Unit (CNIO), and the National Bioinformatics Institute (INB), we contribute to several cancer genomics initiatives ranging from the personalised to the population levels.

"In most biomedical research institutions, one of the limiting factors in understanding the wealth of information is the effective integration of biological, clinical and computational biology approaches. The mission of the Bioinformatics Unit is to provide resources to enable such integration in the CNIO."

RESEARCH HIGHLIGHTS

Bioinformatics analyses for cancer research

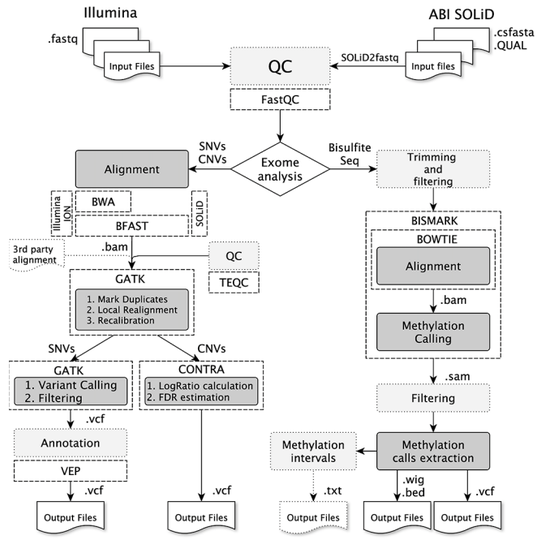
Our mission is to support CNIO Research Groups in the understanding of their experimental data with the development of computational systems and the application of numerical analysis methods.

We helped Alfonso Valencia (CNIO) to implement the web version of *ChiTaRS* – an online chimeric transcript database (Frenkel-Morgenstern et al., *Nucleic Acids Res*, 2013) – and M. Tarsounas (Cancer Research UK) to show that senescence induced by ARF was linked to p53 targets upon DNA damage (Rita Carlos et al., *Nat Communications*, 2013). Together with Manuel Serrano (CNIO), we described the transcriptional signatures of senescent cells during embryonic development (Muñoz-Espín et al., *Cell*, 2013). Our methods in miRNA expression analysis and target prediction also helped Javier Benitez (CNIO) to profile miRNA signatures in breast cancer (Tanic et al., *British J. of Cancer*, 2013 and *Breast Cancer Res*, 2012).

Next-generation sequencing – next generation bioinformatics

Next Generation Sequencing (NGS) techniques are revolutionising genomics and bioinformatics at many levels, including the

RESEARCH HIGHLIGHTS



way we interrogate complex experimental datasets. Many special applications demand specific and novel bioinformatics methodologies, which are implemented in complex computational processes. In 2013, we publicly released *RUbioSeq* (Rubio-Camarillo et al., *Bioinformatics*, 2013); the suite of software pipelines that we generated and developed internally over the past few years for the analysis of CNIO´s NGS experiments (FIGURE).

Using exome sequencing, we helped Juan C. Cigudosa (CNIO) to explore the mutational landscape of blastic plasmacytoid dendritic cell neoplasm (Menezes et al., *Leukemia*, 2013) and chronic neutrophilic leukaemia (Menezes et al., *Blood Cancer J*, 2013). We also assisted Francisco X. Real (CNIO) to delineate the presence and effect of ARID1A and STAG2 mutations in bladder cancer (Balbás-Martínez et al., *PLoS ONE* and *Nat Genet*, 2013). Using RNA-seq data we characterised, with M. Serrano (CNIO), the expression profiles of reprogrammed totipotent cells (Abad et al., *Nature*, 2013). ■

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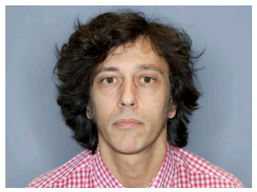
NATIONAL BIOINFORMATICS INSTITUTE CORE UNIT

Alfonso Valencia
Core Unit Head

Technicians
Andrés Cañada, Guillermo Comesaña, José M. Fernández, José M. Rodríguez, Victor de la Torre


Alfonso Valencia ESP


Andrés Cañada ESP


Guillermo Comesaña ESP


José M. Fernández ESP


José M. Rodríguez ESP


Victor de la Torre CUB

OVERVIEW

The Spanish National Bioinformatics Institute (*Instituto Nacional de Bioinformática*, INB) is a platform of the *Instituto de Salud Carlos III*. The INB integrates 10 distributed nodes that cover the main areas of Bioinformatics. The CNIO hosts the Central Coordination Node as well as the Node specialised in genome scale annotation.

The main objectives of the INB Core Unit are to:

- → Generate and supply bioinformatics solutions to genomics projects with particular emphasis on solutions related to human health.
- → Collaborate with national and international bioinformatics activities and consortia.
- → Support the development of bioinformatics and computational biology in Spain.
- → Provide training and support training activities in bioinformatics.
- → Integrate the Unit's activities in the context of the European Infrastructure for Bioinformatics (ELIXIR).

"During 2013, the INB has continued supporting the systems behind large-scale genome projects, including the participation of the European Bioinformatics Infrastructure ELIXIR in the EGA (European Genotype-Phenotype Archive) and the related (epi) genome/diseases data warehouse"

RESEARCH HIGHLIGHTS

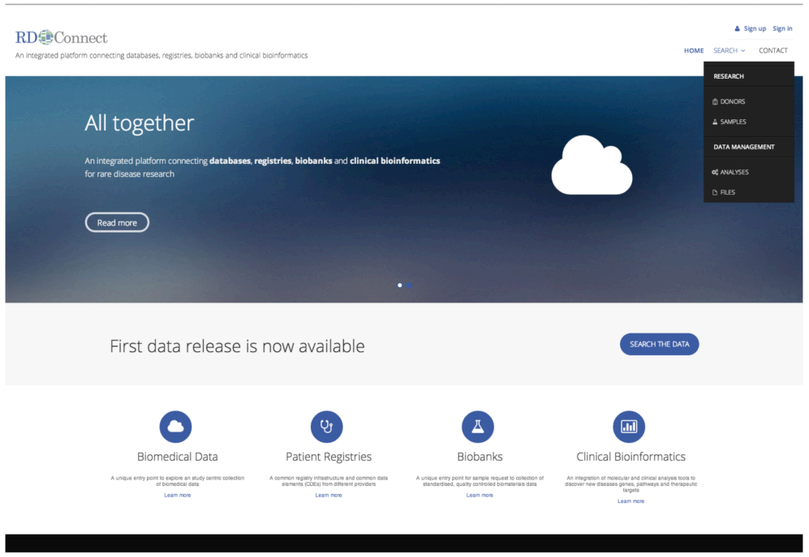


Figure The RD-connect data warehouse: an integrated platform connecting databases, registries, biobanks and clinical bioinformatics for rare diseases research.

During this year, our Core Unit has focused on the following bioinformatics analyses and support systems:

- → The Unit contributed to the development of APPRIS; an integrated system for the annotation of splice isoforms and the detection of principal isoforms. The Unit has further contributed to the development and implementation of the system, and maintains the associated database. APPRIS is being developed in the context of the INB's collaboration in the ENCODE/GENCODE project.
- → The Unit maintained basic components of the platform for the analysis of cancer genomes, and supported the necessary infrastructure for the analysis of Chronic Lymphocytic Leukaemia (CLL) genomes as part of its participation in the CLL-ICGC (International Cancer Genome Consortium) project.
- → Our Core Unit also designed and implemented the database for the BLUEPRINT project; BLUEPRINT is part of the International Human Epigenome Consortium (IHEC).
- → The Unit built the first prototype of the data warehouse infrastructure for the RD-connect project; RD-connect is part of the International Consortium on Rare Diseases (IRDiRC) (FIGURE).
- → Finally, the Unit contributed to the development of the LiMTox hepatotoxicity text mining system. The LiMTox system is the first text mining approach that tries to extract associations between compounds and a particular toxicological end point at various levels of granularity and evidence types, all inspired by the content of toxicology reports. This system has been developed in the context of eTOX IMI-funded project. ■

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Vice-Direction of Translational Research

MANUEL HIDALGO
Vice-Director of Translational Research

"Our Research Groups strive to make discoveries that are readily applicable to prevent cancer and to improve the life of patients suffering from these diseases."

The CNIO continues to strengthen and grow in the domain of translational research. The goal of these Research Programmes is to conduct research that benefits cancer patients. During 2013, we published our research findings and collaborated in projects of great impact on cancer medicine, such as the unveiling of the genetic makeup of bladder cancer, genetic risks of breast cancer, and the development of *Nab*-paclitaxel in pancreatic cancer. In addition, we have made significant progress towards the clinical development of BO-101; an anticancer agent discovered at the CNIO. This has been made possible through the collaboration with several hospitals and cancer cooperative groups, as well as by strengthening our strategic partnerships with pharmaceutical companies. ■

MOLECULAR PATHOLOGY PROGRAMME

MARÍA S. SOENGAS Programme Director

We share a common goal within the Molecular Pathology Programme: a rational approach to the prevention, diagnosis and treatment of aggressive cancers. A distinctive feature of our laboratories is their particular emphasis on clinical specimens, which provide a physiological platform for gene discovery and target validation. A variety of cellular systems and genetically engineered mice are employed to complement these studies.

This has been a highly dynamic year for the Molecular Pathology Programme. In June 2013, after having discovered multiple genes involved in the initiation and progression of bladder cancer, Junior Group Leader Marta Sánchez-Carbayo left the CNIO to further pursue her career at the Centre for Cooperative Research in Biosciences (CIC bioGUNE) in Vizcaya, Spain. We commend Marta for the biomarkers her team identified, and look forward to hearing more about her strategies to translate these findings into more accurate diagnostic tools. In turn, Christopher Heeschen joined the Molecular Pathology Programme as a Senior Group Leader and Head of the Stem Cells and Cancer Group. Exciting discoveries from the Heeschen laboratory include proof-of-concept support for mitochondrial metabolism as an "Achilles´ heel" for therapeutic intervention in pancreatic cancer stem cells. His team is also actively involved in large pan-European genetic, transcriptomic and functional analyses to differentiate "driver" – and potentially druggable – genes, from inconsequential "passenger" alterations. These areas of investigation further expand the fields of research covered by other Senior Laboratories of the Programme. These are the Epithelial Carcinogenesis Group and the Melanoma Group, headed by Francisco X. Real, and by myself, respectively. Francisco Real's Group has made great strides on pancreatic and bladder cancer. For example, they have uncovered new transcriptional networks involved in exocrine cell differentiation and have defined how these signalling cascades impact on the development of pancreatic cancer. In addition, they have completed the first exome analysis of non-muscle invasive bladder cancer. These results have important implications as they led to the discovery of novel tumour suppressive roles for STAG2, as well as to the identification of several genes involved in DNA repair and chromatin remodelling. My own laboratory is interested in malignant melanoma, a paradigm of histopathologically heterogeneous tumours. In a search for lineage-specific oncogenes, we have identified a unique wiring of the endo/lysosomal machinery that distinguishes melanoma from over 30 other tumour types. Moreover, we have found unexpected roles of RNA-binding factors in melanoma cells that define putative novel tumour markers and prognostic indicators in this disease. From a therapeutic perspective, we are also involved in large collaborative consortia for the validation of an anti-tumoural strategy based on dsRNA nanoparticles developed in our Group.

"Groups at the Molecular Pathology Programme strive to help the cancer community during all the stages of the disease; through the identification of risk factors, tumour biomarkers, prognostic indicators and novel targets for rational drug design. The road ahead is still challenging, but we are committed to riding it together towards the ultimate goal of translating our knowledge to the patient´s bedside."

The perspectives for the molecular pathology field are exciting. It is becoming apparent that tumour cells are not fixed entities. Understanding pathophysiological events that affect the crosstalk between tumour cells and their microenvironment will be key for the development of personalised and more efficient therapies. As an example of the clinical applicability of our results, the Programme´s translational activities have led to the creation of a spin-off company (*Bioncotech therapeutics*) and multiple ongoing collaborations with various biotechnology companies. ■

MELANOMA GROUP

María S. Soengas
Group Leader

Staff Scientists
Alicia Gónzalez, David Olmeda

Post-Doctoral Fellows
María García, Lisa Osterloh

Graduate Students
Direna Alonso (until June), Daniela Cerezo, Metehan Cifdaloz, Panagiotis Karras, Raúl Martínez (since April), Lina Sofia Odqvist (until May), Eva Pérez, Napala Ransom Pratini (until May)

Technicians
Tonantzin Calvo, Estela Cañón, Ángel Colmenar


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Eva Pérez ESP


Tonantzin Calvo ESP


Estela Cañón ESP


Ángel Colmenar ESP

OVERVIEW

Cutaneous melanoma is the deadliest form of skin cancer. Despite great progress in the identification of key (epi)genetic defects expressed in melanoma cells, the efficacy of current treatments is either transient or restricted to only a limited fraction of melanoma patients. The long-term goal of our Group is to identify molecular mechanisms that are involved in the initiation and progression of malignant melanoma, as a platform for a more rational design of improved therapies. Specifically, we are interested in stress response programmes (involving apoptosis, autophagy, senescence and endosome mobilisation), with particular interest in genes that are deregulated in a melanoma-specific manner. Multimeric complexes controlling RNA stability, transcription and translation, are also central themes in our research. Our experimental settings include normal and tumour cells, as well as comprehensive collections of tissue specimens spanning early, intermediate and late stages of melanoma development. In addition, we have generated a unique set of genetically modified mice for non-invasive imaging of metastatic processes. These studies are performed in the context of multidisciplinary consortia of specialists in biology, chemistry, pharmacy, nanotechnology, molecular imaging, dermatopathology and clinical oncology. We also work in partnership with biotechnology companies to translate our discovery efforts to the bedside. Examples of these collaborative efforts are dsRNA-based nanocomplexes, which are being developed for clinical testing by *Bioncotech Therapeutics*.

“Our group pursues a challenging and ambitious objective: to improve melanoma diagnosis and treatment response. To this end, we join forces with experts in a wide range of basic and clinical specialties. We have also created a spin-off biotechnology company to help translate our scientific discoveries to the bedside.”

RESEARCH HIGHLIGHTS

New melanoma drivers and tumour markers

The long-term goal of our laboratory is to identify and validate novel prognostic markers and drug targets in melanoma. In this context, we dedicate particular attention to the concept of “tumour cell addiction” (i.e. to genes and pathways that are selectively required for the survival of malignant melanoma cells, but are dispensable for their normal counterparts). Combining genetic and functional analyses in normal and malignant melanocytic cells, mouse models and tissue specimens, we have previously reported a variety of factors with melanoma cell-selective roles. These include the anti-apoptotic factors MCL1 and BCL-XL, as well as other genes such as *MYC*, *DEK* or *MDM2*, which we have found to be essential to support melanoma cell proliferation and to bypass suppressive effects of premature senescence (FIGURE 1). More recently, we joined forces with the groups of Juan Valcárcel, Fátima Gebauer (the Centre for Genomic Regulation, CRG) and Raúl Méndez (the Institute for Research in Biomedicine, IRB) in Barcelona, in order to interrogate molecular mechanisms underlying the pleiotropic changes in mRNA expression that are

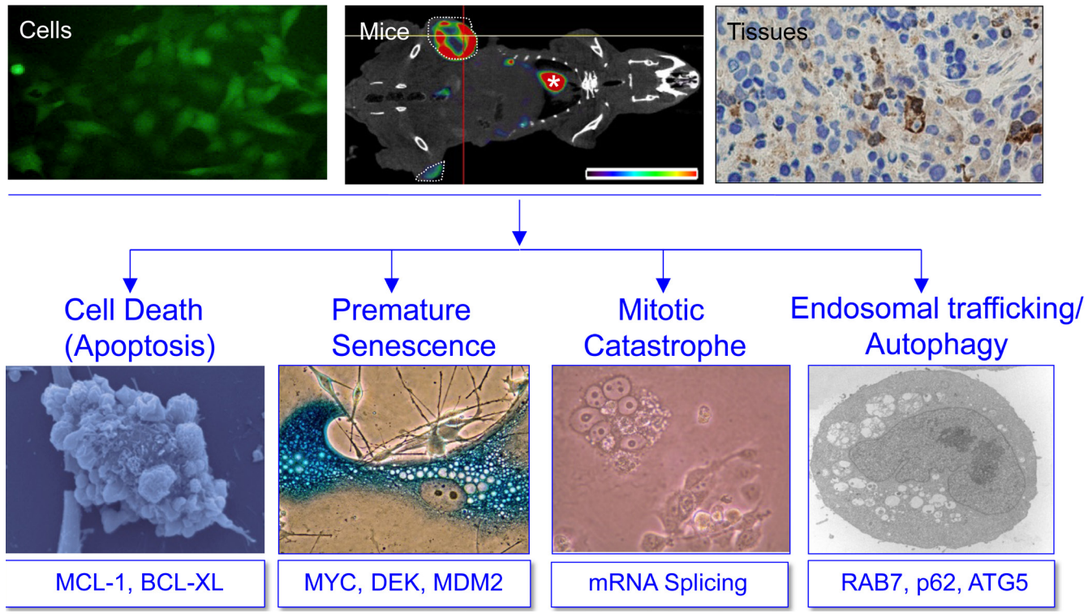

Figure 1 Experimental models and main signalling cascades explored by the CNIO Melanoma Group. Our studies involve genetic and functional analyses in normal and tumour cells, mechanistic studies in inducible animal models, as well as the comprehensive histological characterisation of clinical biopsies isolated from early, intermediate and late stages of melanoma progression. Indicated are examples of key genes involved in the control of apoptosis, premature senescence, cell cycle progression and vesicular trafficking, which we have found to be essential mediators of melanoma cell proliferation and/or maintenance *in vivo*.

associated with melanoma development. These analyses led to the identification of essential modulators of mRNA stability, alternative splicing, transcription and translation. Mechanistic analyses demonstrated key unanticipated tumour-selective roles of these RNA binding proteins in the control of mitotic progression. We are very excited about these results as they open new avenues for research on RNA regulators as diagnostic markers and therapeutic targets in melanoma.

In parallel to the characterisation of classical tumour "hallmarks" (namely, alterations in cellular processes such as apoptosis, senescence, cell proliferation and immortality that are commonly deregulated in all cancers), we pursued the less explored strategy of indentifying oncogenic drivers that are activated in a lineage-specific manner. To this end, we teamed up with researchers from the Bioinformatics Core Unit at the CNIO, the *Hospital 12 de Octubre* in Madrid, the Hospital of Zurich, and the Memorial Sloan-Kettering Cancer Center in New York. These studies identified a cluster of endolysosomal-associated genes that are uniquely upregulated in melanoma, and distinguish this cancer from 35 other tumour types. Embedded in this gene cluster, we found the small GTPase *RAB7* to be an early-induced melanoma driver gene whose expression can be tuned to favour tumour invasion, ultimately defining metastatic risk. These data illustrate how lysosomal-dependent degradation, otherwise a rather universal feature of eukaryotic cells, can be hijacked in a tumor type- and stage-dependent manner. Further analyses of lysosomal-dependent and -independent catabolic processes have revealed new pro-metastatic roles of p62/sequestosome protein and the autophagy factor ATG5**.**

Non-invasive imaging of neo-lymphangiogenesis to monitor metastatic dissemination and identify novel anticancer agents in melanoma

A main limitation for rational drug design in cancer, particularly in melanoma, is the lack of physiologically-relevant models

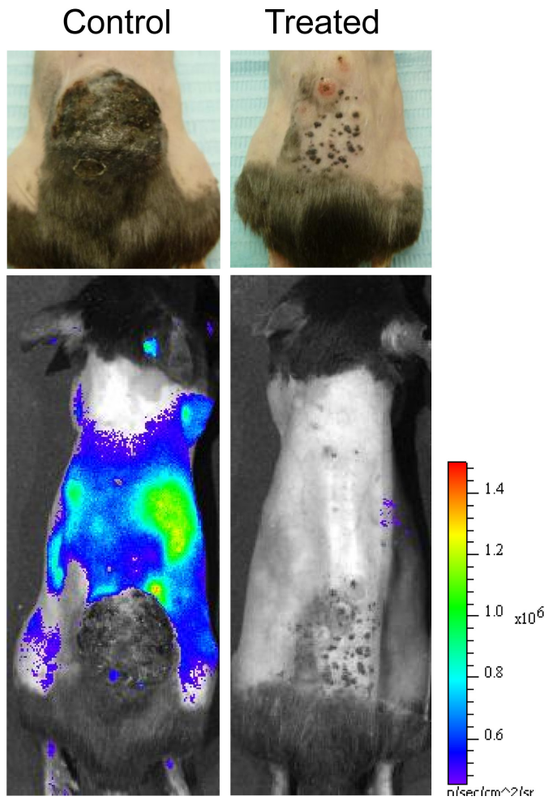

Figure 2 Use of melanoma models for the identification of anti-metastatic agents that block Vegfr (a main mediator of lymphangiogenesis). Upper panels show representative examples of cutaneous melanomas generated in the *Vegfr3*-Luc; Tyr.*::CreERT2; Braf*^{CA}*; Pten*$^{fl/fl}$ mice. The corresponding Vegfr3-driven luciferase signal induced by untreated melanomas is shown in the bottom panels.

and tracers to monitor metastatic cells *in vivo*. In particular, no genetically engineered strains were available to visualise the dissemination of melanoma cells to lymph nodes, a key process considered to precede the colonisation of distal organs (metastasis). In collaboration with the CNIO Transgenic Mice Core Unit of Sagrario Ortega, we have generated the first in-class melanoma "lymphoreporter" mice. These animals are based on a knock-in of a GFP-Luciferase fusion cassette at the 3' UTR region of *Flt4* (Vegfr3). Crosses into the Tyr::*CreERT2*; *Braf*^{CA} strain showed no significant activation of neo-lymphangiogenesis before or after the generation of Braf-driven benign nevi. Instead, in the context of melanoma development (generated in *Vegfr3*-Luc; Tyr::*CreERT2*; *Braf*^{CA}; *Pten*$^{fl/fl}$ mice), lymphangiogenesis could be clearly imaged in sentinel and distal lymph nodes, marking pre-metastatic niches. Moreover, we demonstrated that the *Vegfr3*-Luc lymphoreporter mice represent a cost-effective platform for pharmacological testing of anticancer agents (FIGURE 2). These results are currently guiding regulatory studies whose ultimate goal is to support phase I clinical trials. ■

PUBLICATION

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AWARDS AND RECOGNITION

- Elected Member, Society for Melanoma Research Steering Committee, USA.
- Member, European Cell Senescence Association.
- Team Science Award, Melanoma Research Alliance, USA.

EPITHELIAL CARCINOGENESIS GROUP

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Group Leader

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Graduate Students
Cristina Balbás, Isidoro Cobo, Francesc Madriles, Laia Richart

Technicians
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Francesc Madriles ESP


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Natalia del Pozo ESP

OVERVIEW

Our research interest focuses on understanding the molecular/cellular mechanisms involved in pancreatic and bladder cancer through a disease-oriented approach. Our strategy builds on a structure similar to a pyramid, having an equilateral triangle as a base. The 3 vertices correspond to the models used: patient samples, cell cultures, and genetically modified mice; each of these 3 models holds equal weight in our work. The third dimension comes from the projection of this knowledge to the "population" level whereby we bring the biology to large-scale studies in patients. We are interested in the genetic susceptibility to cancer and in developing better molecular tools to predict patient outcome or response to therapy. Our primary observations can be made at either of these levels and then be extended through additional work.

The focus on pancreatic ductal adenocarcinoma (PDAC) relates to the early events involved in tumour development, especially in relation to the control of cell differentiation as a critical tumour suppressor mechanism. Using genetic mouse models, PDAC can originate both in pancreatic progenitors and acinar cells. The elucidation of the contribution of these cell types is crucial to better design strategies for early tumour detection and prevention in subjects at risk of developing PDAC. Regarding urothelial cell carcinoma (UCC), the focus is on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms through which they participate in tumourigenesis, and applying this knowledge for improved prediction of outcome.

"Our group has shown how Nr5a2 is implicated as a tumour suppressor in pancreatic cancer using mouse models. We have also identified several new genes/pathways that are involved in UCC using exome sequencing and have shown that *TERT* promoter mutations are the most common genetic alterations in this tumour."

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

Cell differentiation as a tumour suppressor mechanism in the pancreas. Genomic analysis of PDAC shows that *KRAS, p16*, *TP53*, and *SMAD4* are the major genes involved in this tumour and that a wide variety of genetic alterations converge to perturb a few critical genetic pathways. We have acquired evidence that novel genes controlling acinar cell differentiation (i.e. *Gata6* and *Nr5a2*) also play an important role. *Gata6* inactivation in pancreatic progenitors accelerates *KRas*-driven PDAC progression in mice and GATA6 is commonly deleted in a subset of human PDAC, supporting its role as a tumour suppressor. These effects are mediated, at least in part, through a unique control mechanism of the epithelial-mesenchymal transition, as revealed by a genome-wide chromatin occupancy analysis.

We are also studying other genes that play important roles in development, differentiation, pancreatitis and PDAC, including *Nr5a2*, *Hnf1a*, *Foxa1/2*, and *Myc*. For example, *Nr5a2* haploinsufficiency cooperates with mutant *KRas* and pancreatitis in the development of murine PDAC, and Myc controls cell differentiation while promoting tumourigenesis (FIGURE 1). RNA-Seq and ChIP-Seq studies have identified intricate relationships between these genes through regulatory networks controlling tumour suppressors, epigenetic regulation, metabolic processes, and inflammatory cytokine cascades. Components of these networks can contribute to PDAC by modulating the risk of developing chronic pancreatitis and by controlling the expression of genes that can be targeted at the therapeutic level.

This work is strengthened through our close collaboration with other CNIO Groups who are also working on PDAC (Mariano Barbacid, Christopher Heeschen, Manuel Hidalgo, and Núria Malats).

Urothelial cell carcinoma (UCC) genetics and biology

We are making progress in our efforts to establish the genomic landscape of UCC: hotspot *TERT* promoter mutations occur in more than 70% of tumours and are the most common genetic alteration in UCC, regardless of stage and grade. *TERT* mutations can be identified in tumour cells in voided urine and are promising candidates for non-invasive tumour detection. A major goal of our future studies will be to determine when mutations occur and what their biological effects are.

We are also using massive parallel exome sequencing to identify new genes and pathways involved in UCC, focusing on non-muscle-invasive tumours. Our analysis of 17 exomes, followed by a prevalence screen in 60 additional tumours, has confirmed an important role of both known and novel chromatin remodellers: *ARID1A* inactivation occurs mainly in *FGFR3* wild-type, *Tp53* wild-type, aggressive tumours. We have also identified *STAG2* as a recurrently mutated gene (FIGURE 2), together with other cohesin members. *STAG2* inactivation is more frequent in non muscle-invasive tumours and is not associated with aneuploidy. Furthermore, STAG2 knockdown in UCC lines does not lead to consistent changes in chromosome number. These observations suggest that STAG2 participates in UCC through mechanisms different from those involved in chromosome segregation; we are currently investigating these mechanisms in our laboratory. Loss of STAG2 expression is associated with a better patient outcome. We have also found that genes involved in DNA repair – such as *ERCC2*, *ATM*, and *FANCA* – are frequently altered in UCC, thus providing opportunities for targeted therapies. We plan to extend these studies to a larger series of non muscle-invasive tumours, in order to provide a comprehensive landscape of the genetic alterations differentially associated with aggressive vs. non-aggressive UCC that could, in turn, help to predict outcome and to identify targeted therapies for precision medicine.

This work is being conducted in collaboration with the Groups of Núria Malats, Alfonso Valencia, and Ana Losada at CNIO, S. Chanock (Translational Genomics Laboratory, US-NCI), I. Gut (*CNAG*, Barcelona), several hospital-based groups, and a consortium of European collaborators. ■

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AWARDS AND RECOGNITION

- Editorial Board Member, The Pancreapedia: Exocrine Pancreas Knowledge Base (http://www.pancreapedia.org/).

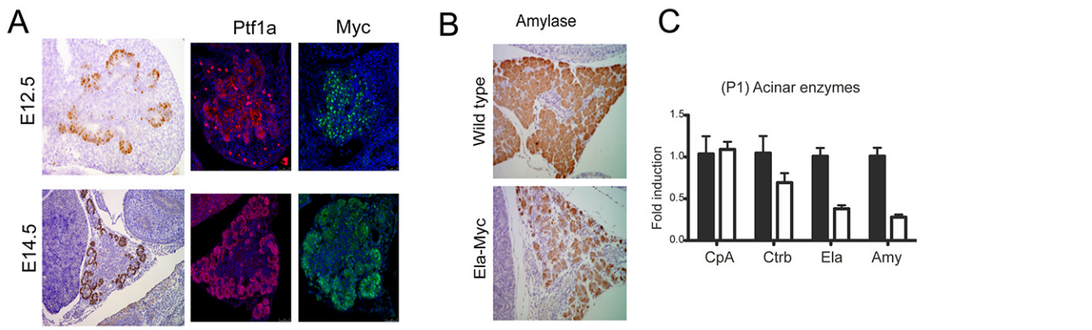

Figure 1 Myc is expressed in pancreatic multipotent progenitors; upon its overexpression, it blocks acinar differentiation while inducing pancreatic tumours. (**A**) Expression of Ptf1a and Myc at early stages of mouse pancreas development. At embryonic day 12.5 (E12.5), pancreatic epithelial cells are multipotent, while at E14.5 lineage restriction has started. *Left Panel* shows Ptf1a expression at E12.5 and E14.5. *Middle* and *Right Panels* show expression of Ptf1a and c-Myc. At E12.5, high levels of both proteins are expressed in pancreatic progenitors; at E14.5, Ptf1a becomes restricted to acinar progenitors and c-Myc is progressively down-regulated. Immunofluorescence with DAPI counterstaining. (**B**) Down-regulation of amylase in the pancreas of P1 Ela-myc mice. (**C**) Acinar enzyme transcripts are expressed at lower levels in the pancreas of P1 Ela-myc mice (by quantitative RT-PCR); further reduction is observed at 4 and 8 weeks, indicating that Myc overexpression suppresses cell differentiation in the exocrine pancreas. (CpA, carboxypeptidase A; Ctrb, chymotrypsin B; Ela, elastase; Amy, amylase).

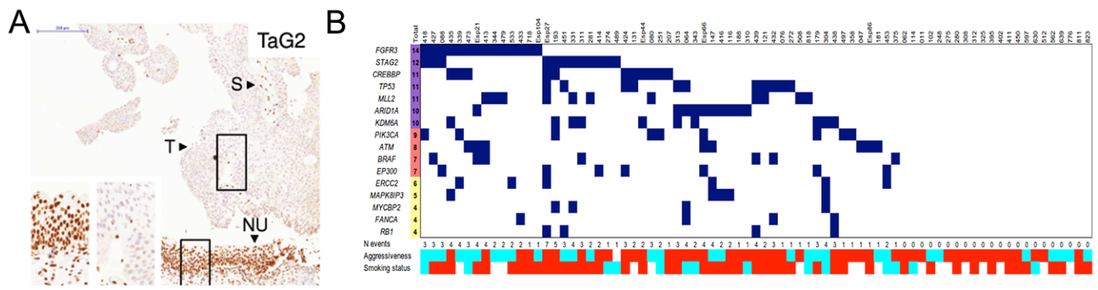

Figure 2 Redefining the landscape of genetic alterations involved in UCC. (**A**) STAG2 is recurrently inactivated in UCC and loss of expression occurs in 35% of non muscle-invasive tumours. (**B**) Distribution of mutations in genes that are recurrently inactivated in UCC and are expressed in >30% of tumours.

STEM CELLS AND CANCER GROUP

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Group Leader

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Sladjana Zagorac SRB


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Álvaro Castells ESP


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Anja Fries USA


Sonia Alcalá ESP


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Catarina L. Reis PRT

OVERVIEW

With the availability of more sophisticated model systems and technologies it has now become evident that cancer heterogeneity is even greater than anticipated from the multiple genetic alterations, but it is also driven by the phenotypic and functional heterogeneity and plasticity within each subclone of the tumour. Indeed, pancreatic cancer stem cells (CSCs) represent a subset of cancer cells, for which we, and others, have provided conclusive evidence that these cells represent the root of the disease by giving rise to all the differentiated progenies within each cancer subclone (FIGURE 1).

"Our research should ultimately allow us to develop novel multimodal therapies to eliminate both CSCs, as the root of pancreatic cancer, and their differentiated progenies. Targeted delivery of new therapies in combination with advanced imaging technologies will be achieved by nanoparticle technology and tested in well-designed clinical trials."

Even more important, from a clinical perspective, these cells are driving the metastatic behaviour of pancreatic cancer and are the putative source for disease relapse. Therefore, CSCs are responsible for the intraclonal heterogeneity of the tumour and represent a crucial component for the development of novel treatments. Noteworthy is the fact that CSCs do not represent *bona fide* stem cells based on most stringent criteria, nor do they necessarily arise from tissue stem cells. Instead, CSCs have acquired certain features of stem cells. While CSCs and their differentiated progenies demonstrate an identical genetic ground state with respect to genetic alterations, as demonstrated by single-cell implantation experiments, CSCs exhibit distinct gene expression profiles that share modules with pluripotent stem cells. Most of the genes involved in generating induced pluripotent stem cells (iPS cells) – such as *Nanog*, *Oct3/4*, *Klf4*, and *Sox2*– have been linked to cancer and are strikingly overexpressed in the pancreatic CSC compartment.

RESEARCH HIGHLIGHTS

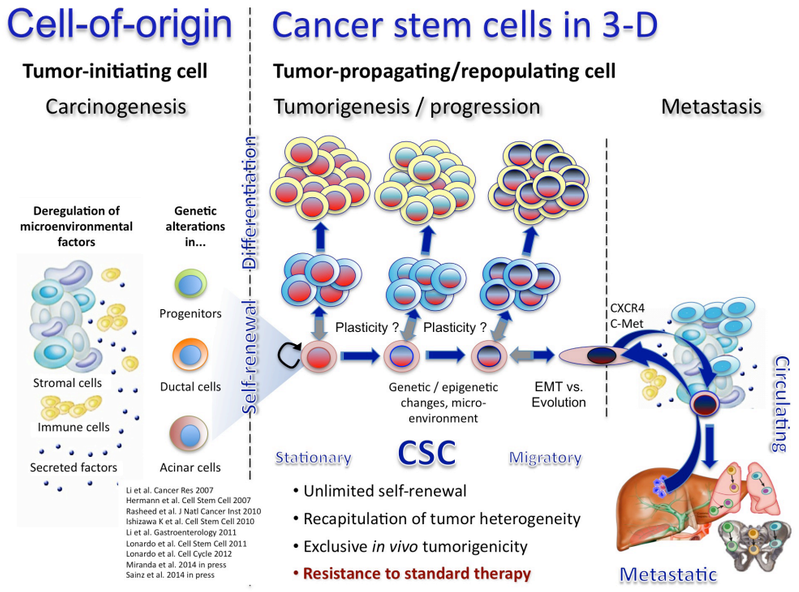

Figure 1 Cancer Stem Cell (CSC) concept. Although the origin of CSCs still remains elusive for most cancer entities and may actually vary between patients, distinct populations of CSCs have already been identified (once the tumours have been formed). CSCs are defined by their unlimited self-renewal, the capability to recapitulate the tumour heterogeneity (differentiation) and their exclusive *in vivo* tumourigenicity. CSCs are not a single population of uniform cells; rather, they undergo evolution through the acquisition of genetic and epigenetic alternations. Also, the tumour microenvironment can induce dramatic changes in their phenotypic characteristics. For example, a distinct subpopulation of migrating CSCs, identified by additional CXCR4 expression, can be detected in the invasive front in the pancreas as well as in the circulating blood. Detection of these circulating CSCs could serve as prognostic and predictive biomarkers and, even more importantly, their prospective isolation as a liquid biopsy should eventually provide non-invasive access to metastatic CSCs.

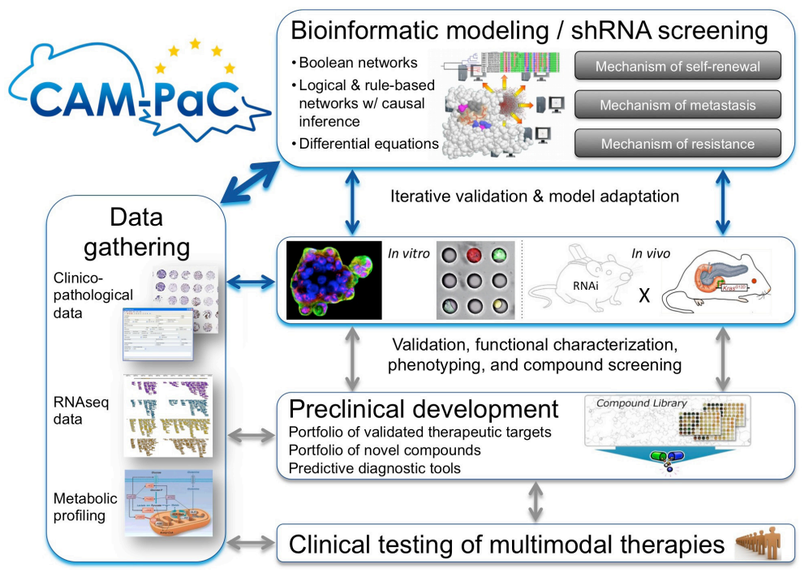

Figure 2 CAM-PaC strategy. Gathering of (pre-) clinical, histopathological, RNAseq, and metabolic data. Data integration using a combination of bioinformatics and tailored shRNA library screens for self-renewal, metastasis and resistance. Target screen using established *in vitro* models. Target validation using RNAi mice. Eventually, we want to identify novel therapeutic targets and predictive biomarkers, which would then be tested in innovative early-phase clinical trials.

Targeting the metabolic Achilles heel of human pancreatic CSCs

Epidemiologic studies have suggested that diabetes mellitus, particularly type II, is associated with enhanced risk for pancreatic cancer. Strikingly, in a retrospective analysis, oral administration of metformin in patients with diabetes mellitus type II was found to be associated with a reduced risk for developing pancreatic cancer as well as better outcome in patients with established pancreatic cancer. When evaluating metformin for the treatment of pancreatic cancer in large preclinical studies, we found that the heterogeneous populations of cancer cells harboured in primary human pancreatic cancer tissues differed strikingly in their response to metformin depending on their level of stemness. While the bulk of more differentiated cancer cells reacted to metformin treatment with cell cycle arrest, a subset of cells with distinct stemness features, namely CSCs, actually underwent rapid apoptotic death due to energy crisis.

Our data demonstrate that metformin virtually exhausted the CSC fraction, but are also consistent with the notion that non-CSCs do not replenish the pancreatic CSC pool after termination of metformin treatment. Further metabolic studies have suggested that pancreatic CSCs actually bear a highly mitochondrial-dependent metabolic profile, which is in striking contrast to normal stem cells, but also distinguishes them from the bulk cancer cells. Metformin is slowly accumulated 1000-fold within mitochondria and directly inhibits Complex 1 (NADH dehydrogenase), thus interfering with this proton gradient across the inner mitochondrial membrane. Subsequent alteration in the electron transport chain and oxidative phosphorylation appear to be particularly lethal for CSCs. Therefore, drugs such as metformin that target the oxidative mitochondrial metabolism represent powerful therapeutic tools for attacking the CSC pool.

Launching an interdisciplinary research programme to identify novel targets against CSCs

For many human malignancies, large-scale genomic, transcriptomic and, to a somewhat lesser degree, proteomic analyses have been instrumental in establishing a comprehensive catalogue of molecules that are altered in their structure and/or abundance in bulk tumours. Far less developed are concepts and methods for the integration of data from CSCs and their progenies, and to perform systematic interrogation of gene functions for CSC features in order to differentiate 'driver' alterations – which directly contribute to tumourigenicity and/or metastasis – from "passenger" alterations, which have minimal or no influence on CSC biology.

Therefore, the goal of our new pan-European project CAM-PaC is to functionally interrogate transcriptomic data from a large set of primary human pancreatic CSCs in order to systematically identify these driver genes/pathways (FIGURE 2). This will be achieved by tailored shRNA screening (~500 pre-specified genes). The obtained shortlist of genes/pathways (~20 per functional feature) will then be comprehensively characterised and, if indeed of crucial functional relevance for the CSC phenotype, further validated as novel CSC targets for therapeutic intervention. Eventually, a unique collection of pre-selected and pre-characterised CSC candidate genes will be selected for systematic functional characterisation using RNAi mouse models in combination with novel genetically engineered mouse models of pancreatic cancer. The latter will allow for the independent temporal control of up to four initiating genetic events (e.g. 1st hit: KrasG12D/+, 2nd hit: Tp53R172H/+, 3rd hit: DPC4 shRNA) in a postnatal setting and should more accurately reflect human disease. ■

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TUMOUR MARKERS JUNIOR GROUP

Marta Sánchez-Carbayo (until May)
Junior Group Leader

OVERVIEW

The main goal of the Tumour Markers Group is to contribute to the molecular characterisation of bladder cancer by integrating valuable information from high-throughput approaches, with 2 aims:

- To understand how identified molecular alterations contribute to tumourigenesis and cancer progression.
- To translate the resulting knowledge into improved clinical management of bladder cancer patients, providing multiplexed tools for the analysis of tissue biopsies (for disease stratification and outcome prediction) and body fluids (for early diagnosis and follow-up).

Our studies are designed to address 4 major clinical needs for biomarkers in bladder cancer: diagnosis, surveillance, progression into invasive and metastatic disease, and prediction of therapeutic response. We are currently using epigenetic and proteomic approaches to identify and validate individual and multiplexed biomarkers, as well as to dissect the molecular pathways through which genes of interest contribute to bladder cancer initiation and disease progression. ■

"Our Group has contributed to the understanding of key mechanisms involved in bladder cancer and has identified biomarkers that will improve non-invasive diagnostics of these tumours. Our analysis of gene methylation and protein expression patterns in bladder and colon cancer has yielded novel biomarkers that will facilitate patient stratification and guide therapeutic decisions."

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HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Programme Director

"Genetics (in our case human genetics) is the basis of evolution. Although we are going through a critical and difficult period, we learnt that evolution never goes backwards and so we can only go forwards!"

The Human Cancer Genetics Programme conducts research in a wide range of areas related to translation studies. The Programme is currently composed of 4 Research Groups, 1 Genotyping Unit and 1 Familial Cancer Unit. There is also a Familial Cancer Consultancy that allows the assessment of cancer families and the selection of appropriate candidates for genetic studies in order to perform a correct diagnosis and genetic counselling. The Consultancy is located in the *Hospital Universitario de Fuenlabrada* and works in strong collaboration with the Oncology Service. The key goals of the Programme are the genetic characterisation and diagnosis of families with cancer, the genetic and cytogenetic study of tumours, the search for diagnostic and prognostic markers, as well as the discovery of novel cancer-related genes. Another area of work that complements our research activities is the study of the genetic and environmental factors that confer cancer susceptibility and drug response (pharmacogenetics). This research line focuses on the analysis of a wide variety of tumours, taking advantage of the high-throughput genotyping technologies provided by the Genotyping Unit.

The Programme works in close collaboration with the clinical community in order to foster cooperation in genetic diagnosis and promotes training and education. During 2013, the Research Groups hosted 3-month training programmes for 7 resident physicians from different hospitals in Spain. We also offer short-stay opportunities (2-6 weeks) for professionals from different international research centres (a total of 4 visitors in 2013; 3 foreign nationals and 1 national). In terms of education, since the beginning of 2013, a total of 11 national and 10 international students from outside Spain have worked on their PhD research projects, 7 of whom have already successfully defended their thesis.

We participate in various international and national consortia; this permits us to apply for international project funding, hold international meetings and publish in the best scientific journals.

The major milestones of the Programme in 2013 include:

- The Familial Cancer Unit was re-established.
- The Programme organised the final meeting of the Collaborative Oncological Gene-Environment Study (COGS) European project held in *El Escorial* (Spain), April 2013, with the participation of the 3 consortia working on breast, prostate and ovarian cancer. ■

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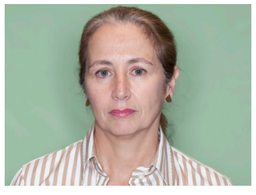
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OVERVIEW

For several years, the Human Genetics Group has been working on research aiming to better understand the genetic bases of familial cancer, especially breast and ovarian cancer. Our main objective is the translation of our discoveries into clinical practice in order to improve knowledge about these cancers.

The Group´s activities include the identification of new high-, moderate- and low susceptibility genes that explain either families with cancer or individuals with cancer susceptibility and, whenever possible, the description of functional mechanisms. We are also involved in the detection of modifier genes that modulate disease evolution and cancer risk. Finally, we are interested in the discovery of diagnostic and prognostic biomarkers by using new high-throughput technologies.

Our experimental approaches are based on the study of patients and healthy subjects, as well as on the analysis of genes and genomes across a broad range of constitutional and somatic samples. We employ classical genetics, functional studies and mouse models, and apply the most advanced technology for this endeavour.

The Group´s strategic goals are:

"Our group has contributed to the identification of new low susceptibility alleles in breast cancer (41 genes) and new high susceptibility genes in families with rare cancers. Furthermore, we have defined new diagnostic and prognostic markers in breast and ovarian cancer, based on microRNA (miRNA) and array-based comparative genomic hybridisation (aCGH) studies."

- → To better characterise the genetic profile of familial and sporadic breast and ovarian cancer by identifying new cancer susceptibility genes.
- → 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 approaches.
- → To translate our discoveries into clinical practice.

RESEARCH HIGHLIGHTS

Breast cancer

We demonstrated that families with breast cancer present a phenomenon named 'genetic anticipation' (earlier age of disease onset and shorter telomeres along generations) that is linked to mutations in *BRCA1* and *BRCA2* genes. We have initiated a prospective study in order to gain insight into the mechanisms that are associated with this fact; the study investigates, through periodic controls, how telomerase and oxidative stress speed telomere shortening and early age of disease onset. As a preliminary result, it is worth highlighting the strong association found between telomerase expression and chemotherapy.

We have established a signature of 35 miRNAs based on expression array data that differentiate tumours carrying *BRCA* mutations versus non *BRCA1/2* and sporadic tumours with 96% accuracy. We are currently validating these miRNAs in plasma by quantitative real time-PCR (QRT-PCR) analysis in a large set of hereditary and sporadic samples (around 150). These results will have important implications for clinical practice.

A similar study is being conducted in order to identify differentially expressed miRNAs in the plasma of subjects at high-risk for developing breast cancer. We have carried

out similar studies and are currently validating by QT-PCR, a set of 17 miRNAs that are only present in tumour samples.

We are participating in the Collaborative Oncological Gene-environment Study (COGS); a large-scale project funded by the European Commission. The aim of the project is to identify genetic markers underlying breast cancer susceptibility as well as genetic markers that can modify the risk of breast cancer in *BRCA1/2* mutation carriers. We have led a 'sub project' involving more than 25,000 carriers in order to investigate the role of genes involved in the Base Excision Repair pathway as genetic modifiers of cancer risk. We have identified 2 genes that modify the risk of developing breast and ovarian cancer.

Because patients carrying *BRCA1* mutations have a different response to Poly (ADP-ribose) polymerase (PARP) inhibitors, we were interested in investigating whether the type of germline mutation can influence drug response. For this purpose, we are currently conducting a study that involves 25 lymphoblastoid cell lines harbouring different *BRCA1* mutations. Our preliminary results suggest that cells harbouring missense mutations are more sensitive to treatment than those harbouring mutations that cause total loss of the BRCA1 protein; these findings support the existence of a dominant negative effect (FIGURE 1).

Finally, it is worth mentioning the identification of a new breast cancer gene: the E3 ubiquitin ligase *CUL4A*, which contributes to breast cancer development. Our Group embarked on this study three years ago with the identification of an amplified chromosomal region by array-CGH analysis. We selected 2 candidate genes and conducted different *in vitro* functional analysis. *In vivo* studies using mouse models were also carried out to examine the transforming capacity of the gene. Our results from this work suggest that *CUL4A* plays a role in breast cancer progression and aggressiveness.

Ovarian cancer

By using array-CGH, we defined a chromosomal deletion at 6q24-26 in familial and sporadic epithelial ovarian tumours that has prognostic value, independent of other known clinical variables. The increased overall survival associated with this loss was validated in 2 independent series comprising more than 500 tumours. This predictive marker could help to guide the selection of patients whose worse prognosis supports the use of new treatment regimens. Since the deletion can be analysed by Fluorescence *In Situ* Hybridisation (FISH) in paraffin sections, it may represent a marker that is suitable for routine clinical applications.

Familial cancer exome project

There are a number of families with rare or infrequent cancers with an unknown genetic basis. We have started a massive sequencing project with the aim of identifying some of these high-susceptibility genes. During 2013, we discovered a gene that is linked to a family with severe anaemia and immunodepression. The gene is related to telomere maintenance and is currently under study. A second gene responsible for type I gastric carcinoid tumours has also been discovered; the gene is a proton pump in charge of gastric acidification and its maintenance (FIGURE 2). We have generated a knock-in mouse line containing this genetic alteration in order to gain insight into the pathological changes that occur during the progression to gastric cancer. ■

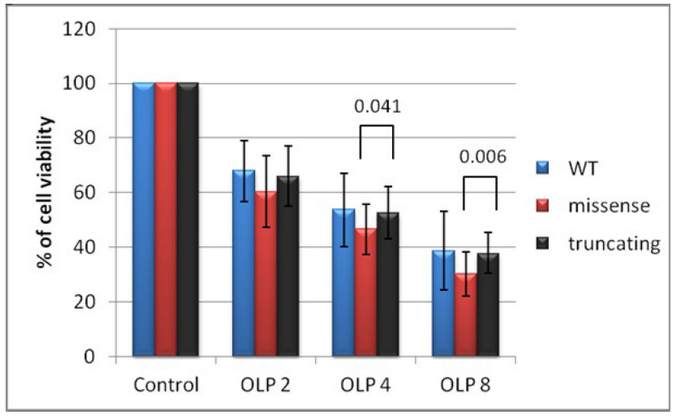

Figure 1 Cell lines carrying missense *BRCA1* mutations (red) are more sensitive to PARP inhibitors (olaparib) than those cell lines containing truncating mutations (black), suggesting the existence of a dominant negative effect.

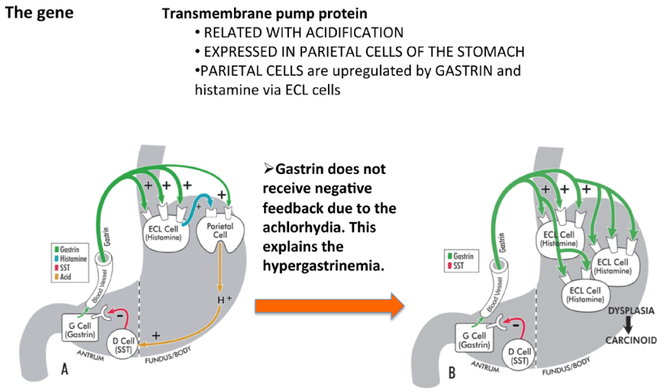

Figure 2 Gastric carcinoid type 1 is characterised by achlorhydria and hypergastrinemia. The mutation in the gene blocks the feedback between parietal cells and gastrin which results in (A) dysplasia and (B) carcinoid.

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OVERVIEW

The most recent genetic and genomic approaches, including next-generation sequencing (NGS), demonstrate that the chromosomes of tumour cells show both small changes in the DNA sequence (point mutations) as well as structural rearrangements, in the form of translocations, deletions, amplifications, and/or numerical changes (aneuploidy). Among them, we are especially interested in those rearrangements that generate chimaeric genes displaying new or altered biological activities, as well as in the molecular mechanisms by which these newly created fusion genes play a crucial role in oncogenesis.

"We identify and describe the genetic profiles that may help to explain the oncogenic features of myeloid leukaemias. These patients display mutations in specific genes that result in a profoundly aberrant epigenome and splicing machinery. Our findings open avenues for future innovative and alternative targeted therapies."

Our work is mainly based on myeloid leukaemia, a paradigm of a genetic neoplasia in which fusion genes and point mutations work together and contribute to explain the abnormal cell proliferation and the disruption of the differentiation programme of haematopoietic stem cells. Based on our ongoing collaborations with the clinical community, our research activity is being developed through:

- → The molecular characterisation of genetic, cytogenetic and epigenetic markers.
- → The design of human stem cell-based models of cell lines carrying defined chromosomal rearrangements that will allow us to study the effects of these rearrangements on the biology of the tumour, as well as the molecular pathways involved in the observed effects.
- → Very importantly, the translation of our findings to the clinical setting by providing molecular tools such as spectral human and mouse karyotypes, FISH probes, and NGS sequencing panels for both research purposes and their use as clinical reagents.

RESEARCH HIGHLIGHTS

Exome sequencing reveals novel and recurrent mutations with clinical impact in acute dendritic cell leukaemia

Acute dendritic leukaemia is a rare type of leukaemia, but one with the worst prognosis that is difficult to treat – the average patient survival rate is just 12-14 months. Also known as blastic plasmacytoid dendritic cell neoplasm, this very rare disease currently lacks genomic and genetic biomarkers to assist in its clinical management. Our Group has, for the first time, sequenced the exome – the coding, or protein-generating, regions of the genome – of three cases of this type of leukaemia. Exome sequencing analysis has revealed 37 to 99 deleterious gene mutations per exome but no common affected genes between patients. A more detailed bioinformatics study however revealed a clear overlap in terms of molecular and disease pathways (haematological and dermatological disease). Thus, we have described, for the first time in human leukaemias, mutations in four genes (*IKZF3*, *HOXB9*, *UBE2G2* and *ZEB2*) that have important cellular functions, such as gene regulation and cellular differentiation.

In an attempt to define a more complete landscape of the disease, based on these data, we designed a re-sequencing approach to identify mutations in 38 selected genes in 25 additional samples (FIGURE 1). This efficient approach

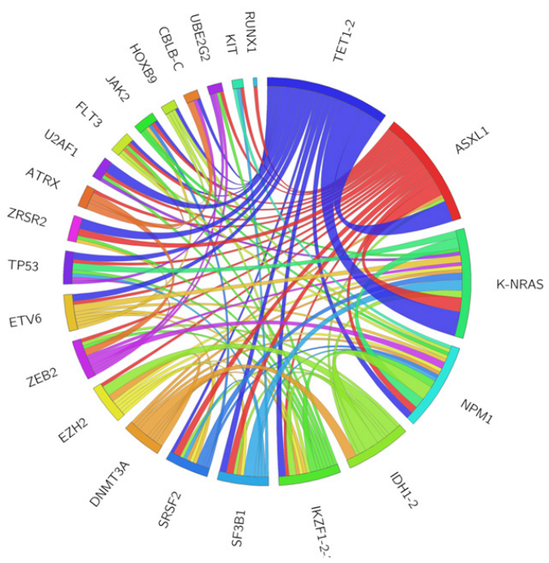

Figure 1 Recurrently mutated genes and their interaction in acute dendritic cell leukaemia. The circos diagram depicts the relative frequency and pairwise co-occurrence of mutations in the patients. The length of the arc corresponds with the frequency of mutations in the first gene, and the width of the ribbon corresponds to the percentage of patients who also had a mutation in the second gene. Pairwise co-occurrence of mutations is denoted only once, beginning with the first gene in the clockwise direction. Mutations in *TET1-2* and *IDH1-2* are mutually exclusive, as previously reported in other myeloid malignancies.

revealed recurring mutations in 29 genes, ranging in prevalence from 36% for previously known genes, such as *TET2*, to 12-16% for newly identified genes, such as *IKZF3* or *ZEB2*.

In addition to these specific genes, we have also found that more than half of the cases harboured mutations in epigenetic genes at diagnosis – these are genes that introduce chemical modifications in the DNA – something that had never been observed in this type of leukaemia. The clinical analysis revealed that patients with mutations in DNA methylation pathways had a significantly reduced overall survival (p=0.047) (FIGURE 2). Since therapies directed against these epigenetic genes already exist, these patients could potentially benefit from them. In summary, we have found that the genetic profile of acute dendritic cell leukaemia, currently treated as a lymphoid leukaemia, is similar to that of myeloid leukaemia. These results clearly suggest a change in the treatment guidelines for these patients, who were completely misplaced.

Advances in the full genomic and epigenomic characterisation of rare myeloid disorders

We have also been working on the molecular characterisation of the translocation between chromosome 1 and chromosome 19, t(1;19)(p13;p13.1); a chromosomal aberration that has rarely been reported but has recurrently been observed in myeloid neoplasms, such as acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). We have analysed three patients with this marker using several genomic approaches, including the previously mentioned target re-sequencing strategy. Once again, we failed to detect common mutations among the three patients but demonstrated that this subset of patients harboured mutations in epigenetic modifiers, thus providing a rationale for the use of drugs that target aberrant methylation in the treatment of this aggressive subtype of AML/MDS.

Finally, a complete epigenomic characterisation is ongoing in an effort to unveil the biological features in myelodisplastic syndromes that carry the cytogenetic marker known as deletion 5q. We are trying to provide a rationale for the current and successful use of certain drugs in patients who show this chromosomal aberration.

From genetic mutations to the generation of human stem cell models

Since we are committed to transferring our research activities into potential clinical applications, we are generating biological models and tools to study the role of chromosome translocations in cancer. We have developed two human haematopoietic stem cell models, based on cell lines that have been genetically engineered to carry the novel chimaeras *AML1-TMEM48* and *NUP98-HOXA*. Functional characterisation is ongoing in our laboratory in order to fully understand the molecular mechanisms that sustain the leukaemogenic properties of these proteins.

Our group also provides state-of-the-art molecular cytogenetic services. In 2013, we carried out over 2,000 assays including karyotyping of leukaemia and other tumours, design of FISH probes, spectral karyotyping, aneuploidy analysis for mouse models, and microarray-based comparative genomic hybridisation (array-CGHs) for experimental and clinically-oriented projects. As a reference laboratory in Molecular Cytogenetics, we are participating in several clinical assays, collaborative networks, and quantity performance studies both at the national and European level. ■

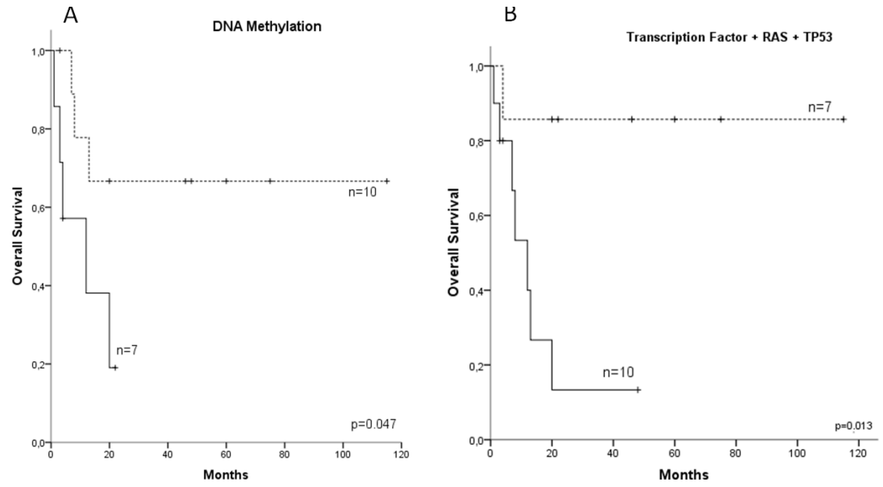

Figure 2 Overall survival (OS) curves of the patients according to the presence of gene mutations. (**A**) We observed a significant difference in the OS according to the mutational status of genes included in the DNA methylation class (P=0.047; mut: n=7 and 11 months; wt: n=10 and 79 months); (**B**) We observed a significant difference in the OS according to the mutational status of genes included in the transcription factor class */ TP53 / RAS* (P=0.013; mut: n=10 and 15 months; wt: n=7 and 99 months).

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Article in press

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AWARDS AND RECOGNITION

- Elected President (2013-2017), Human Genetics Spanish Association.
- The *"El Talento"* Prize in the Academic Talent category endowed by the Spanish business daily *Cinco Días* and the LinkedIn network, Spain.

HEREDITARY ENDOCRINE CANCER GROUP

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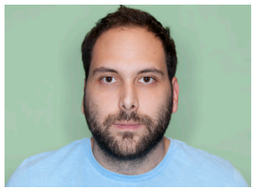
Álvaro Gómez ESP


Rocío Letón ESP


Lara Sánchez ESP

"We have added new insights into role of *EPAS1* in sporadic phaeochromocytoma (PCC), identified the *STK17B/PAX8* interaction as part of thyroid cancer heritability, showed that treatment for medullary thyroid cancer could be stratified according to tyrosine kinase inhibitor (TKI) target expression, and proposed two single nucleotide polymorphisms (SNPs) as the first markers of paclitaxel-induced neuropathy."

OVERVIEW

Our Group is interested in identifying high and low genetic risk factors involved in endocrine tumour susceptibility. To this end, we classify patients according to primary gene mutations associated with their tumour development. We are interested in revealing differences between tumour transcriptomes, mirnomes, methylomes and chromosomal gains and losses according to the different individual genetic backgrounds. We have therefore obtained a large collection of endocrine tumours from patients either with germline mutations in any of the known major susceptibility genes related to these diseases, or without mutations (sporadic cases), as well as clinical follow-up data that is regularly updated. 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. In order to do so, we are applying a candidate gene approach as well as whole genome association studies, to a large series of biological material and associated data regarding therapeutic interventions and other clinically relevant outcome variables. These efforts will collectively increase our genetic and molecular knowledge about these tumours and improve the diagnosis, prognosis and treatment of patients.

RESEARCH HIGHLIGHTS

EPAS1 is significantly involved in sporadic phaeochromocytoma development

The genetics of phaeochromocytomas (PCCs) and paragangliomas (PGLs) is extremely puzzling, with up to 40% of patients carrying a germline mutation in 1 of 10 major susceptibility genes that are known, so far, to be associated with the disease. In 2012, the endothelial PAS domain-containing protein 1 gene (*EPAS1*) was added to this growing gene list, when investigations revealed the description of patients with polycythaemia and multiple PGLs carrying *EPAS1* somatic post-zygotic mutations. Our Group was the first to find *EPAS1* mutations in sporadic PCCs and to identify a gain of 2p as an alteration that occurs exclusively in tumours harbouring *EPAS1* mutations (FIGURE 1). These tumours showed an overexpression of several hypoxia-induced genes. The discovery of somatic mutations affecting *EPAS1* in PCC/PGL not only once again links polycythaemia and cancer (i.e. *VHL, PHD2, JAK2*), but it also adds further weight to the hypothesis that the stabilisation of hypoxia inducible factor (HIF)-alpha is important in chromaffin tumour development.

Ephrin type A receptors are involved in paclitaxel-induced peripheral sensory neuropathy

Peripheral neuropathy is the dose limiting toxicity of paclitaxel, a chemotherapeutic drug that is widely used to treat solid tumours. There is great inter-individual variability in the neuropathy, with some patients being asymptomatic while others suffer from serious neuropathies necessitating dose reductions and treatment suspensions. Therefore, the identification of paclitaxel-induced neuropathy biomarkers would be of great clinical utility. Through an international collaboration and applying a whole-genome approach, our group proposed genetic variants associated with paclitaxel-induced neuropathy. Specifically, we identified *EPHA5*-rs7349683 and *XKR4*-rs4737264 as the first validated markers of this neuropathy. Meta-analysis of a previous genome-wide association study (GWAS) gave hazard ratios (HR) of 1.68 ($p=1.4\times10^{-9}$) and 1.71 ($p=3.1\times10^{-8}$), respectively. We have also proposed potential additional markers in other *EPHA* genes, and at the *LIMK2* locus. These findings suggest that genes involved in the function and repair of peripheral nerves, could substantially contribute to the genetic susceptibility to paclitaxel-induced neuropathy.

The identified markers improve the genetic prediction capability for this toxicity, and represent an important step towards individualised paclitaxel chemotherapy.

Epistasis: an answer for the hidden thyroid cancer heritability

Thyroid cancer is an example of a complex disease, with a strong genetic component, that does not follow a regular Mendelian pattern of inheritance. Susceptibility to thyroid cancer is influenced by the joint effects of multiple low-penetrance genes and environmental factors. By applying a pioneer study of epistasis to one of the largest differentiated thyroid cancer patient series described to date, we identified an interaction between variants in *STK17B* and *PAX8* genes. Furthermore, we confirmed by functional assays that the expression of these genes is inversely correlated, suggesting that these two loci indeed interact to influence susceptibility to classic papillary thyroid cancer (cPTC). Altogether, our work adds new insights into the genetic basis of thyroid cancer susceptibility, and suggests a new direction for the exploration of inherited genetic contributions to disease using association studies.

miR-183 and miR-96 contribute to PCC/PGL tumourigenesis by interfering with neuronal differentiation

It is known that Prolyl hidroxylase 3 (EGLN3) plays an essential role in mediating neuronal apoptosis during normal development and that succinate accumulation, due to an *SDHB* mutation, competitively inhibits EGLN3 activity. After identifying a robust overexpression of miR-183 and miR-96 in *SDHB*-related PCC/PGLs, we suggested that miR-183 overexpression in these tumours could further contribute to EGLN3 inhibition, by downregulating IDH2 levels and thus decreasing α-ketoglutarate availability. We measured the effect of miR-183 and/or miR-96 on neuronal differentiation in PC12 pheochromocytoma cells in the presence of low-dose nerve growth factor (NGF), concluding that the upregulation of miR-183 and miR-96 would have a negative effect on neuronal differentiation (FIGURE 2). ■

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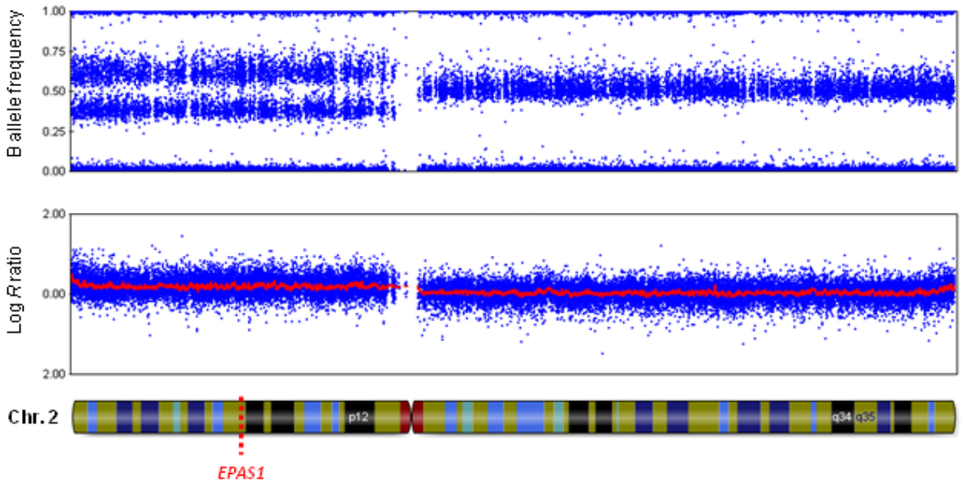
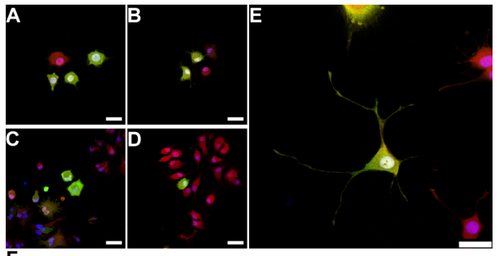
Book Chapters

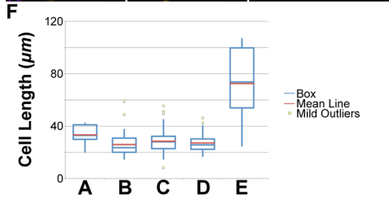
- Robledo M (2013). *Biología molecular de los TNEs e investigación traslacional aplicada. Genética de los TNE asociados a síndromes hereditarios.* In: *Módulo 2. Actualización de tumores neuroendocrinos*. http://www.aulagetne-cursonet.com.
- Robledo M (2013). *Biología molecular de los feocromocitomas y paragangliomas.* In: *Manual de diagnóstico y tratamiento de los tumores neuroendocrinos.* http://www.getne.org/%C3%81readeProfesionales/MaterialdetrabajoenTNEs.aspx.

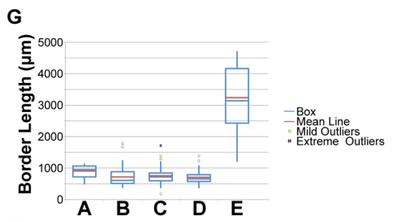
AWARDS AND RECOGNITION

- Luis J. Leandro has been awarded the *Premio Real Academia de Doctores de España* for his outstanding thesis work on the identification of predictive markers of paclitaxel toxicity and efficacy.









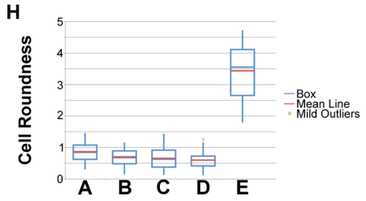


Figure 1 (up) SNP-array reveals a gain of 2p in *EPAS1*-PCC. Lower panel genomic plots indicate the presence of three alleles along the short arm. Upper panel shows that the heterozygous state splits into two clusters. Location of *EPAS1* is indicated.

Figure 2 (left) Changes in PC12 cells. (**A**) NGF-untreated cells transfected with control microRNA. NGF-treated cells transfected with: (**B**) miR-183; (**C**) miR-96; (**D**) miR-183 and miR-96; and (**E**) control miRNA. (**F**) Cell Length (mm); (**G**) Border Length (mm); and (**H**) Cell Roundness. ANOVA test was performed for each parameter. (F) $P=1.53X10^{-43}$, (G) $P=4.95X10^{-68}$, (H) $P=6.00X10^{-79}$.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

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Group Leader

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Alexandra Masson-Lecomte FRA


Antonio C. Picornell ESP


Silvia Pineda ESP


Salman M. Tajuddin ETH


Marien Castillo ESP


Esther Manso ESP


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Janire Rodríguez ESP

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Technicians
Ana Alfaro, Marien Castillo, Carlos González, Esther López, Esther Manso, Mirari Márquez, Roger L. Milne (until August), Janire Rodríguez

OVERVIEW

The scope of research carried out by the Cancer Epidemiology Group ranges from the identification of aetiological agents and mechanisms to the translation of the research findings into the clinical and public health domains, focusing on bladder, pancreatic, and breast cancer.

Epidemiology now demands the alignment and synergy of scopes, objectives, data, and tools across disciplines. By adopting an integrative research approach, we participate in large, international multidisciplinary studies requiring the development of methodological innovations in all aspects of epidemiology.

We employ a wide variety of biomarkers to better characterise exposures and cancer outcomes, as well as the genetic patterns predisposing or protecting against the disease, including variability in its clinical course. 'Omics' data provide a unique opportunity to further dissect complex exposures, genetic susceptibility, and phenotypes. The Group explores the integration of this data in epidemiologic studies.

The strategic goals of the Group are to:

- Identify environmental exposures and genetic susceptibility factors, as well as gene-environmental and gene-gene interactions involved in cancer development and progression.
- Study the differential association of germline genetic variants and environmental exposures with cancer subphenotypes characterised at the molecular/'omics' level.
- Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer and to integrate epidemiologic with 'omics' information (methylomics, genomics, and transcriptomics).
- Assess clinical and public health strategies for cancer control, using current genomic tests and data.

"Integrative statistical approaches have allowed us to identify the role of both environmental and genetic inflammatory-related factors in cancer development and progression, and to establish that, as of now, common genetic variants have a limited predictive value in cancer risk prediction."

RESEARCH HIGHLIGHTS

Urothelial bladder cancer (UBC)

During 2013, we continued exploring genetic susceptibility factors by applying more comprehensive strategies, including candidate pathway analysis and multi-single nucleotide polymorphism (multi-SNP) approaches (Bayesian LASSO and AUC-Random Forest) to detect inflammatory-gene variants' main effects. We also studied how to assess the joint effect of SNPs and copy number variations (CNVs), by using allele-specific copy numbers when testing for association in regions with common copy number variants (FIGURE 1). In addition, we participated in the identification of further variants associated with bladder cancer and in the assessment of gene-smoking interactions with already reported SNPs. In relation to our interest in epigenetics factors, we have assessed the association between LINE1 and bladder cancer. This global methylation marker is associated with blond tobacco and arsenic, while also being a risk factor by itself displaying a U-shape risk pattern not previously described. The application of 'omics' data was first explored in a pilot

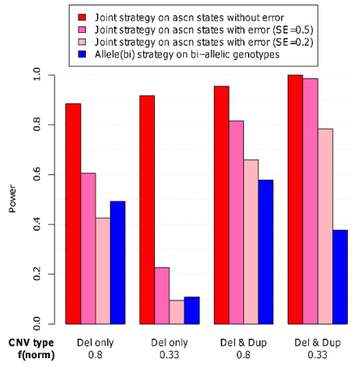

Figure 1 Statistical power of the SNP+CNV joint models when errors are introduced in the allele-specific copy number states.

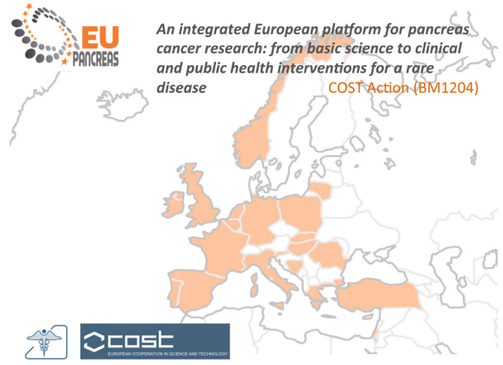

Figure 2 COST Action BM1204 EU_Pancreas. An integrated European platform for pancreatic cancer research: from basic science to clinical and public health interventions for a rare disease.

urine metabolomics study. We compared bladder cancer patients who recurred/progressed with those who did not, and several species with a strong discriminatory potential were identified. Furthermore, transcriptomics, methylomics, and genomics data from a small set of individuals are being used to identify the limitations of 'omics' data integration and to develop novel appropriate statistical approaches. Using whole-exome sequencing, in collaboration with the CNIO Epithelial Carcinogenesis Group, we have identified new genes (i.e. *STAG2* and *ARID1*) involved in bladder carcinogenesis and have explored their clinical relevance. This approach is being applied to investigate germline variants using cases and controls from the SBC/EPICURO study, in order to further dissect the genetic susceptibility of this disease.

PUBLICATIONS

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AWARDS AND RECOGNITION

- Chair of the 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".

Pancreatic cancer

Following our previous work that points to the importance of chronic inflammation in this tumour type, we have analysed inflammatory polymorphisms associated with pancreatic cancer risk and have shown, in collaboration with international groups, that some of these polymorphisms are also associated with chronic pancreatitis. To elucidate the joint effect of previously reported inflammatory-like risk factors, we are integrating data from the European case-control study (PanGen-EU) that includes >2,000 cases and 1,000 controls from 6 European countries led by our Group. The Group is exploring the role of oral and gut microbes in chronic pancreas diseases. Regarding the genetic susceptibility of pancreatic cancer, in collaboration with colleagues at the *Hospital Ramón y Cajal* in Madrid, we provide support to the Spanish Registry of Familial Pancreatic Cancer. This project includes a screening programme for high-risk relatives. As part of our participation in the PanScan3 project, led by the National Cancer Institute (NCI, USA), 11 new variants associated with pancreatic cancer risk were identified. A collaboration with the pancreatic cancer International Cancer Genome Consortium (ICGC-pancreas) will allow dissecting the genetic susceptibility to pancreatic cancer by applying whole genome and exome sequencing strategies. Importantly, we coordinate the 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", aimed at uniting groups interested in pancreatic cancer across Europe and providing a unique platform for collaboration (FIGURE 2). This initiative includes >150 multidisciplinary members from 20 European Union (EU) countries, EU governmental and non-governmental institutions, as well as private companies.

Breast cancer

We continued to participate in international consortia to further elucidate the complex genetic susceptibility to breast cancer by identifying additional genetic variants. We have undertaken a large gene-gene interaction project, assessing 72,611 candidate variants in 46,450 breast cancer cases and 42,461 controls from the European multi-consortia project (COGS); we found no evidence of two-way single nucleotide polymorphism (SNP) interactions in breast cancer susceptibility.

Public Health and Genomics (PHGEN)

We participate in the European Network assessing the implications of using genomic data and technology in populations (genetic testing, biobanks, and legal issues). ■

FAMILIAL CANCER CLINICAL UNIT

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OVERVIEW

Knowledge of the familial antecedents of cancer can help in the prevention and early detection of this disease. Familial risk interpretation requires the systematic collection and assessment of a detailed family history in the context of the genetic counselling process; the best tool to assess if a specific family has an increased susceptibility to cancer.

The aim of the Familial Cancer Clinical Unit (FCCU) is the assessment, prevention and researching of familial and hereditary forms of cancer. Our Unit offers its services to those practitioners involved in the care and management of patients with cancer, through a customised study, evaluation and counselling of families with increased cancer genetic susceptibility. The FCCU provides support to other groups in the Human Cancer Genetics Programme, by supplying biological samples as well as clinical and familial data of patients with an increased cancer susceptibility.

During 2013, the FCCU setup its consultancy at the *Hospital Universitario de Fuenlabrada*, within the Medical Oncology Services department, in order to evaluate, follow-up and provide surveillance for patients from all over Spain who have familial forms of cancer.

"Genetic counselling in cancer is a process of helping people to understand and adapt to the medical, psychological, and familial implications of the genetic contributions to cancer; this includes a clear explanation of the risk of developing cancer, along with appropriate prevention and surveillance strategies."

RESEARCH HIGHLIGHTS

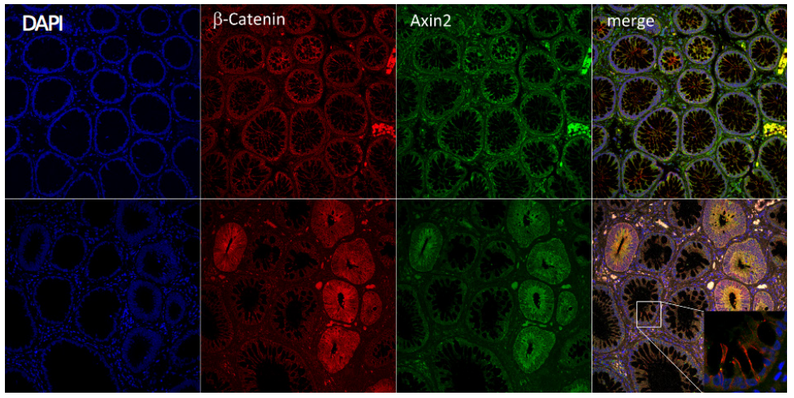

Figure AXIN2 and β-catenin overexpression in adenomatous polyp glands (lower panels) compared with paired normal colonic epithelium (upper panels). The inset shows the results for a normal gland. Lack of co-localisation of AXIN2 and β-catenin proteins in an adenomatous polyp gland (Rivera B *et al., Eur J Hum Genet*, 2013).

The Familial Cancer Clinical Unit was re-established in 2013 in order to resume its clinical activity in familial cancer, which had been previously discontinued in 2007. The goal of this new reorganisation is to promote the activity focused on the diagnosis and care of patients with increased susceptibility to cancer, to generate synergies with other institutions and professionals interested in hereditary cancer, and to provide the clinical data and necessary samples to ensure the sustainability of cancer susceptibility research.

During 2013, the FCCU evaluated and counselled 161 patients from 85 different families with an increased susceptibility to cancer. Furthermore, we have carried out 180 genetic studies in patients with colorectal cancer, and 54 additional genetic studies of other cancer genes (*CDH1, TP53, PTEN, STK11*, and others). Mutation carriers of cancer susceptibility genes were included in surveillance protocols aimed at detecting tumours at an early stage or reducing the risk of developing cancer.

We have also continued our lines of work in familial forms of colorectal cancer. We carried out a linkage analysis of 22 families with Familial Colorectal Cancer type X and a molecular characterisation – including immunohistochemistry (IHC) profiles, CpG island methylator (CIMP) phenotype study, and *KRAS/BRAF* mutation analysis – of the tumours in these families. Our results seem to point towards genetic heterogeneity being a major complicating factor in aetiological research on this syndrome.

In adenomatous polyposis families, we identified a novel variant in the *AXIN2* gene in a family with attenuated Familial Adenomatous Polyposis (FAP). This finding is part of an in-depth study of FAP families that are negative for alterations in the conventional *APC* and *MUTYH* genes.

Educational activities are another major goal of the FCCU. We have just revised 10 guidelines that contribute to improving the identification and management of families with hereditary cancers (http://www.geneticaycancer.es/doc.php?op=clinica&ap= medicinafamiliar&apx=clinica&action=guias). These guidelines are the result of a close collaboration with SEMFYC (*Sociedad Española de Medicina Familiar y Comunitaria*). ■

PUBLICATION

Kamieniak MM *et al.* (incl. Muñoz-Repeto I, Rico D, Osorio A, Urioste M, Benítez J, García MJ) (2013). DNA copy number profiling reveals extensive genomic loss in hereditary *BRCA1* and *BRCA2* ovarian carcinomas. *Br J Cancer* 108, 1732-1742.

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HUMAN GENOTYPING-*CEGEN* CORE UNIT

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OVERVIEW

The most abundant forms of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Many complex diseases in humans have a significant genetic component that is likely to be explained by differences in the patterns of these genetic changes. Association studies involving the large-scale analysis of both SNVs and CNVs can help to identify genes underlying complex diseases such cancer and drug responses. The Unit has implemented different high-throughput and cost-effective methods to measure thousands and potentially millions of SNV and CNVs. In addition, epigenetic studies are performed in the Unit using whole-genome methylation arrays. Complementarily, pharmacogenomic studies have been undertaken to identify predictive biomarkers for personalised cancer therapy.

"Our goal is to advance our understanding of why patients can have different responses to cancer therapy and to use this knowledge to improve cancer patient care."

PUBLICATIONS

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Article in press

- Milne RL *et al.* (incl. González-Neira A, Benitez J, Malats N). A large-scale assessment of two-way SNP interactions in breast cancer susceptibility using 46 450 cases and 42 461 controls from the breast cancer association consortium. *Hum Mol Genet* (in press). PMID: 24242184.

AWARDS AND RECOGNITION

- National Representative and Board Member, European Association for Predictive, Preventive and Personalised Medicine (EPMA).

RESEARCH HIGHLIGHTS

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

A two-stage genome-wide association study was carried out to identify genetic variation associated with the risk of suffering capecitabine-induced severe hand-foot syndrome; this adverse event is the leading cause of dosage reduction or therapy discontinuation for cancer patients treated with capecitabine. We identified genetic variants in the locus 20q13.33 that were associated with CiHFS and replicated the results in an independent cohort of patients. This susceptibility locus falls within the non-protein-coding portion of the genome. To better understand how genetic variation in this locus is associated with this adverse event, we performed a series of focused follow-up analyses. Fine mapping analysis of the region allowed us to define the best candidate marker in the locus associated with the development of severe CiHFS (FIGURE). Allelic imbalance analysis suggested the role of this variant in the regulation of the closest gene that is located ~90Kb downstream. Both ChipSeq and C4 cohesin experiments are being conducted in order to determine the regulatory mechanism underlying this association.

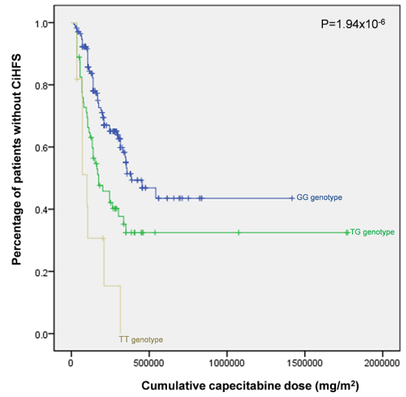

Figure Kaplan–Meier analysis of the cumulative dose of capecitabine, up to the development of grade 3 HFS, by the most associated SNP genotype in all the patients. The T variant allele is associated with an increased risk of developing CiHFS. The P value shown corresponds to Log-Rank (Mantel-Cox) test.

Novel locus associated with CiHFS by integrating patient and cell-line genomic analyses

We have performed a novel integrative enrichment approach, which combines genome-wide association study (GWAS) results from capecitabine-induced cytotoxicity in lymphoblastoid cell lines (LCLs) and CiHFS in our breast and colorectal cancer patients. We observed an enrichment of LCL cytotoxicity-associated SNPs in the HFS-associated SNPs from patients (empirical P = 0.013), thus confirming the use of the LCL model in the analysis of at least a subset of genes involved in capecitabine-induced toxicity in patients. In addition, we have identified a potential regulatory SNP associated with severe capecitabine toxicity that was replicated in a second CiHFS patient cohort (P = 0.0076). Our approach could be applied to find new genes that influence other drug-related phenotypes.

Ewing's sarcoma prognosis genes after treatment

Ewing sarcoma (ES) is the second most common bone malignancy in children and adolescents. 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 examined 384 polymorphisms in 24 genes involved in the transport/metabolism of ES chemotherapeutic agents in 495 patients from 5 European countries. Polymorphisms located in the *ABCC6* gene were related to overall survival of ES patients after treatment. ■

CLINICAL RESEARCH PROGRAMME

MANUEL HIDALGO Programme Director

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

The CRP is composed of three Clinical Research Units and two support Units. The Gastrointestinal Cancer Clinical Research Unit, led by Manuel Hidalgo, studies novel therapeutics and personalised medicine in pancreatic cancer. Miguel Quintela-Fandino leads the Breast Cancer Clinical Research Unit that concentrates on the development of kinase and angiogenesis inhibitors in breast cancer, as well as on the understanding of the molecular taxonomy of this disease. The Prostate Cancer and Genitourinary Clinical Research Unit, led by David Olmos, focuses on novel therapeutics and biomarkers in prostate cancer and has a particular interest in understanding DNA damage repair deficiency in this disease. The Molecular Diagnostic Unit, headed 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 the knowledge resulting from cancer genome studies to patient care.

"Through strong collaborations with clinical centres, we have been successful in bringing novel cancer medicines and innovative technologies to cancer patients in our community."

In 2013, the CRP's main activities were based on the expansion of its clinical trials activities through collaborations with several Hospitals in Spain. At the *Hospital de Madrid*, we consolidated our phase I clinical trials activity by launching over 20 phase I studies; more than 100 patients are projected to be treated in the coming year. We have also formalised collaborative agreements with other Hospitals in Madrid – including the *Fundación Jiménez Díaz* and *Hospital Niño Jesus* – to create an early clinical trials network. Furthermore, we have established multi-centric clinical trials in breast cancer and prostate cancer 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. We also initiated the CNIO OncoMIR Rotation Programme that aims to provide translational research training to medical oncology residents rotating through the CNIO. ■

GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

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Clinical Research Unit Head

Staff Scientist
Pedro P. López

Post-Doctoral Fellows
Lucía Fernández (since March), Lucas Moreno


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Pedro P. López ESP


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Spas Dimitrov BGR


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Graduate Students
Spas Dimitrov, Raquel Martínez, Natalia Sherina (until February)

Technicians
Natalia Baños, Camino Menéndez, Manuel Muñoz

Clinical Investigators
Carlos Gómez (until November), Victor Moreno (since October)

Clinical Research Fellow
Elena Garralda (until November)

OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development and personalised application of novel therapeutics for patients with cancers of the pancreas and colon. Our principal activity is the design, conduction, and analysis of clinical trials with novel anticancer agents. 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, improves survival in patients with pancreatic cancer.

"In 2013, *nab*-paclitaxel – a drug that we have helped to develop – was shown to improve the survival of patients with pancreatic ductal adenocarcinoma. We have also presented our first experience of integrating Avatar mouse models and next generation sequencing approaches in cancer treatment."

Key to our work is the development and characterisation of 'Avatar' mouse models for drug screening, biomarker development, and personalised medicine. Over the last few years we have developed and characterised the largest collection of these models within pancreatic cancer research. We are using the Avatar models in three critical applications. Firstly, we are conducting co-clinical trials, in which clinical trials are performed in parallel with studies using Avatar mouse models of the same cancer type, in order to elucidate mechanisms of action and biomarkers of drug response/resistance. Secondly, we are using the Avatar mouse models for personalised cancer treatment by integrating data obtained from next generation sequencing techniques. Finally, we are using these Avatar mouse models to screen new anticancer agents.

RESEARCH HIGHLIGHTS

During 2013, we continued the lines of work that were initiated in the previous year, including preclinical studies with novel anticancer agents, conduction of clinical trials in our associated hospitals, expansion of our network of collaborative centres, and the launch of several personalised medicine studies.

Avatar mouse model development and characterisation

Our group has continued its efforts to develop and characterise Avatar mouse models based on xenografts from patients with GI malignancies, as well as other tumour types, for drug screening, development of drug combinations, biomarker discovery, and personalised medicine. An example of our efforts to better understand these mouse models is illustrated in FIGURE 1. The graphs summarise studies aimed at determining the transcriptional space of Avatar models. This collection of pancreatic ductal adenocarcinoma xenografts (PDX) from pancreatic ductal adenocarcinomas (PDAC) is the largest and best characterised collection available so far, and represents an important resource for academic and industry investigators. This year, in collaboration with several EU groups, we formed a European PDX Consortia in order to join other academic efforts in this area. We have also co-authored several papers, listed below, based on collaborative work performed using our collection for drug development and biological studies.

Development of novel anticancer agents

We have significantly expanded our portfolio of early clinical trials in patients with GI cancer and other malignancies. At present, the GI Cancer Clinical Research Unit is conducting more than 20 clinical studies with novel anticancer agents, spanning a wide range of mechanisms of action such as signalling inhibitors (FGFR, RAF, MEK, HER), Notch inhibitors, conventional chemotherapy and angiogenesis inhibitors. These studies include first-in-class/

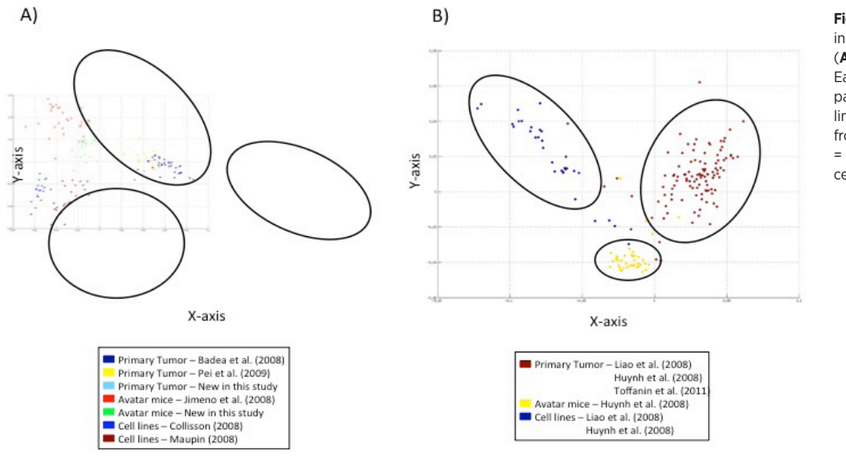

Figure 1 MCA gene expression spaces in pancreatic ductal adenocarcinoma (**A**) and hepatocellular carcinoma (**B**). Each dot represents the expression pattern from a single sample. Grey lines represent the resulting clusters from k-means clustering, for k-means = 3. Each line joins a sample with the centroid of its cluster.

first-in-human clinical trials and analysis of relevant biomarkers, as well as co-clinical studies in mouse models. An example of our contributions to drug development is the work conducted with *nab*-paclitaxel, in which a series of integrated preclinical and clinical studies in patients and mouse models of PDAC ended up in a positive phase III trial. The results from this trial were recently published; they showed an improvement in overall survival and have led to the approval of this agent. Based on the significant activity of *nab*-paclitaxel and gemcitabine in pancreatic cancer, our preclinical and clinical efforts are now geared towards developing a triple drug combination. We are currently testing a robust pipeline of new agents in Avatar mouse models and some, like the DDL4 antagonist for example, have already entered the clinic with promising results so far. In addition, we are in the process of initiating new clinical studies aimed at determining the mechanism of action of this agent in patients with PDAC.

Personalised treatment for pancreatic cancer

Our goal is to implement a step-wise protocol for personalised cancer treatment, spanning the selection of first-line treatment to the selection of the most effective experimental treatments, as well as the investigation of new targets and drugs. In keeping with this global aim, we have implemented a protocol to guide chemotherapy selection in patients with advanced pancreatic cancer and we have enrolled the first patients in this trial. We have also implemented a programme to integrate next-generation sequencing of Avatar mouse models for personalised cancer treatment. So far, we have completed over 30 individual patient genomes and have generated mouse models from 8 of these patients. Preliminary results of this innovative work provide compelling evidence that it is possible to find drug targets for many of these patients and that the Avatar mouse models are instrumental in the making of therapeutic decisions. FIGURE 2 depicts our current approach to personalised medicine. Based on these data, we have recently launched the Avatar clinical trial; a prospective randomised clinical trial that will test the impact of this strategy on patient survival. ■

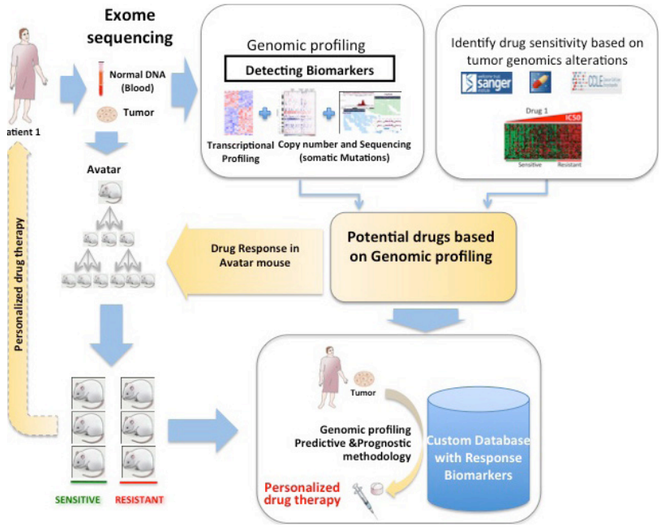

Figure 2 Schematic summary of the personalised medicine approach. Patients with cancer have their tumours profiled and analysed simultaneously, in order to identify potential therapeutic targets and treatment options that are then tested in their personalised Avatar mouse model.

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AWARDS AND RECOGNITION

- Editorial Board Member, *Molecular Oncology.*

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientists
Juan Manuel Funes (since April),
Paloma Navarro

Post-Doctoral Fellow
María José Bueno

Graduate Student
Ivana Zagorac

Technicians
Tamara Mondejar,
Jesús Sánchez

Clinical Research Fellow
Elena Hernández

Research Associate
Ramón Colomer


Miguel Quintela-Fandino ESP


Juan Manuel Funes ESP


Paloma Navarro ESP


María José Bueno ESP


Ivana Zagorac SRB


Tamara Mondejar ESP


Jesús Sánchez ESP


Ramón Colomer ESP

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 different types of 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) in breast transformation.
- → Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biologic features that also constitute potential therapeutic targets.
- → Study of the mechanisms of resistance against various targeted therapies in mouse models.
- → Conduct investigator- initiated clinical trials.

"In 2013, the BCCRU completed the first randomised, investigator-initiated clinical trial, sponsored by CNIO, in breast cancer. This trial involved the participation of 16 Spanish hospitals and 130 patients; it has helped us to understand the mechanisms underlying acquired resistance to antiangiogenics."

RESEARCH HIGHLIGHTS

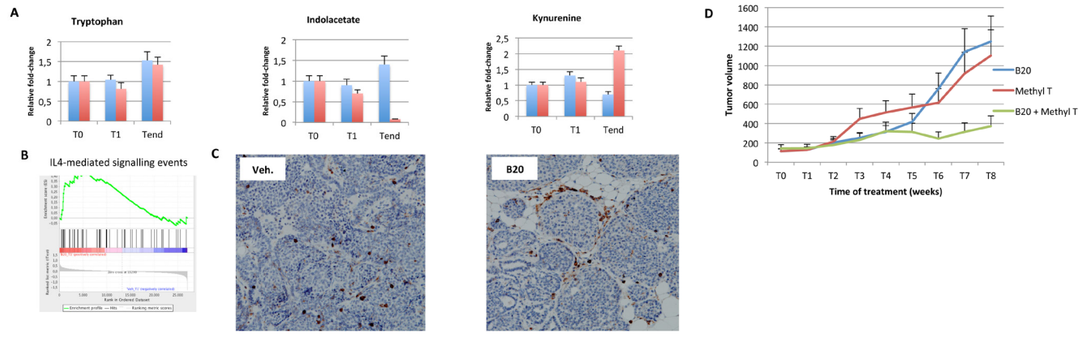

Figure PyMT animals were treated with vehicle or bevacizumab (B20). (**A**) Shift from tryptophan degradation to kynurenine (immunosuppressive) after 6 weeks of treatment. (**B**) GSEAS show evidence of increased signalling through immunosuppressive systems. (**C**) This leads to lymphocytic arrest. (**D**) All these effects, including tumour growth, were reverted by the addition of methyltriptophan, which blocks kynurenine synthesis.

New targets

We have generated a multi-transgenic mouse model with an inducible conditional knockout (KO) of the FASN allele in breast epithelium that also expresses the transforming antigen PyMT. Thus far, the KO Murine Endothelial Fibroblasts (MEFs) have proven to be impossible to transform. Our preliminary results indicate that FASN plays a key role in eliciting enhanced oncogenic signalling through MAPK and Pi3K-AKT pathways. This signalling depends on the lipid-post-translational modifications of FASN, which allow it to be anchored to the plasma membrane in order to transduce signals.

Functional taxonomy

By analysing a training set of breast cancer cases with extreme phenotypes (either very aggressive cases, or cases with more than 15 years of follow-up without relapse, paired by currently known prognostic factors), we have detected 5 genomic regions that are consistently amplified in hormone-receptor positive breast cancer. This is particularly interesting as this subtype comprises more than 2/3 of the new breast cancer cases and the next-generation sequencing maps have not detected high mutation rates compared to other subtypes. Thus, we are currently exploring how these amplicons are potential drivers of novel breast cancer entities and whether they also constitute, in a similar fashion to the HER-2 amplicon, potential targets for drug intervention.

Resistance against targeted therapies

Using a spontaneous breast cancer model, we have uncovered 2 distinct mechanisms of acquired resistance to the 2 main types of antiangiogenics: small molecules and monoclonal antibodies. Resistance against the first involves metabolic reprogramming of the tumour and downregulation of glycolysis. The second mechanism, an immunosuppressive loop caused by the deposit of antibodies in the tumour insterstitium, elicits an escape of the tumour from immunesurveillance, thus allowing tumour growth. The pharmacologic modulation of these mechanisms yields synergistic responses (FIGURE).

Clinical trials

We have recruited >200 patients for clinical trials sponsored by CNIO´s Breast Cancer Clinical Research Unit. ■

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CRIS FOUNDATION – CNIO PROSTATE CANCER AND GENITOURINARY TUMOURS JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro


David Olmos ESP


Elena Castro ESP


Nuria Romero ESP


Paz Nombela ESP


Floortje Van de Poll NLD


M. Mercedes Alonso ESP


Jesús García-Donas ESP


Enrique Grande ESP


Maria I. Pacheco ESP

"Our Unit aims to translate advances in prostate cancer research into improvements in the clinical management of patients with this disease."

OVERVIEW

Prostate cancer (PrCa) is the second most common form of cancer and the sixth leading cause of cancer-related deaths in men worldwide. Disease incidence and mortality rates, however, vary across countries due to differences in genetic backgrounds, lifestyle factors, the implementation of screening programmes, and the availability of treatment options. In most developed countries, PrCa has become the leading type of cancer among adult males; this is mainly due to life-style factors and the recent spread of prostate specific antigen (PSA) screening. Over the past 25 years, advances in PrCa diagnosis and treatment have improved the 5-year survival rates from 68.3% to almost 95% when all stages of the disease are considered. Nevertheless, over 90% of PrCas diagnosed in developed countries are organ-confined and the 5-year survival rate approaches 100% for patients undergoing conventional prostatectomy and/or radiotherapy. Since there is better survivorship now for PrCa patients, we are dedicating our efforts towards the reduction of treatment-related morbidities and the better individualisation of treatment options based on the biology of the disease.

Clinical Research Fellow
Nuria Romero

Graduate Students
Paz Nombela (since April), Floortje Van de Poll (since October)

Technician
M. Mercedes Alonso (since February)

Research Associates
Jesús García-Donas, Enrique Grande (since March), Maria I. Pacheco (since May)

RESEARCH HIGHLIGHTS

BRCA-related prostate cancer

Radical prostatectomy (RP) and external beam radiotherapy (RT) combined with adjuvant androgen deprivation (ADT) are the main standard treatment options for early stage PrCa. Over the last two decades, several predictive tools have been developed to estimate the risk of relapse following these treatment modalities. PSA at diagnosis, tumour stage and grade (Gleason score) are the usual variables incorporated by these tools. However, PrCa is a very heterogeneous disease and some patients who *a priori* have good prognosis features still relapse and succumb to the disease. This is particularly applicable to PrCa patients who harbour germline mutations in the *BRCA1* gene or, more importantly, in the *BRCA2* gene. We have recently demonstrated that *BRCA2* germline mutations are not only the genetic event that confers the highest risk of PrCa known to date (8.6-fold in men ≤65 years), but are also an independent prognostic factor for cause-specific survival (CSS) in all stages of the disease, including localised PrCa.

In a subsequent study, we investigated the effect of *BRCA* mutations on the outcome of conventional treatments for localised and locally advanced PrCa. We have showed that *BRCA* carriers have worse PrCa outcomes than non-carriers when conventionally treated with radiotherapy, seeing as they relapsed and progressed earlier to lethal metastatic disease. Pending future studies to confirm the biological role of *BRCA* genetic alterations in this setting, our results support a closer follow-up of these patients and the need for clinical trials to tailor the best radical/adjuvant treatments for their tumour type. Furthermore, there were no differences in early outcomes between carriers and non-carriers that underwent radical prostatectomy. While awaiting further data on long-term outcomes, our results suggest that clinical management for *BRCA* carriers undergoing surgical treatment may not differ from non-carriers.

Castration-resistant prostate cancer

The biology and clinical evolution of the castration-resistant prostate cancer are highly heterogeneous, restraining our ability to assess the prognosis of these patients. We are currently conducting a prospective multicentre study in order to validate the prognostic signature described by Olmos D., et al. *(Lancet Oncology*, 2012*)* in a series of metastatic castration-resistant prostate cancer (CRPC) patients conventionally treated with docetaxel. With the support of several Spanish centres, we aim to analyse the correlation between the signatures before and during treatment with the response to chemotherapy. ■

PUBLICATIONS

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Book Chapter

- Mateo J, *Castro E, Olmos D* (2013). Cancer treatment in patients with diabetes in Cancer treatments in special médical situations. Ballová V, Provencio M (eds) European Society of Medical Oncology, p72-88.

AWARDS AND RECOGNITION

- Juan Letona Award in Translational Medicine, Spain.
- Faculty Board Member, ECCO-AACR-ASCO Flims Workshop "Methods of Clinical Research", Switzerland.
- Scientific Committee Member, ECCO 17 - 38th ESMO- 32nd ESTRO European Cancer Congress, Netherlands.
- Elena Castro has been recipient of the American Association of Clinical Oncology Merit Award and the Genitourinary Cancers Symposium Merit Award.
- Nuria Romero has been awarded the SOGUG Young Investigator Award.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

Clinical Investigator
Elena García (until March)

Luis Lombardía ESP

Diana Romero ESP

OVERVIEW

The Molecular Diagnostics Unit (MDU) is entrusted to provide the Hospitals of the National Health System (NHS) with a wide range of sensitive, specific, reliable and updated assays. We routinely identify alterations in the sequence or expression levels of key genes that are involved in cancer and that could in turn be used in the diagnosis and/or prognosis of patients, the detection of minimal residual disease in patients showing clinical remission, or for monitoring response to therapy. Our Unit is also dedicated to support CNIO's Clinical Research Units by developing and implementing novel solutions for their research needs. We also form part of several international and national groups dedicated to standardising and improving molecular diagnostics in cancer. Finally, our Unit remains fully committed to promoting laboratory training and mentoring students, technicians and medical residents.

"Besides from this year´s drastic budget cuts in the National Health System, we have continued to provide Spanish Hospitals with molecular diagnostic assays that are not ordinarily available in their institutions. Also noteworthy is the fact that we have initiated our first partnership with the pharmaceutical industry in a clinical trial geared towards personalised medicine."

RESEARCH HIGHLIGHTS

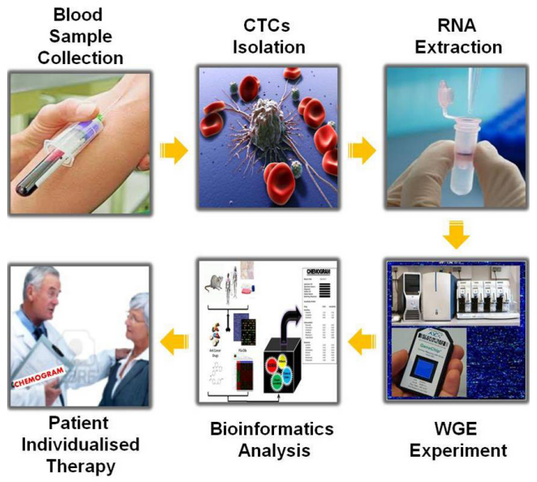

Figure Schematic workflow illustrating: from low invasive sample collection to Whole Gene Expression (WGE) analysis of Circulating Tumour Cells (CTCs), in order to guide anti-cancer therapies and, thus, the personalised treatment of patients with refractory non-small cell lung cancer.

Boosting our routine

In 2013, we added a new assay to our catalogue that allows the identification of mutations in exon 16 of the *RET* proto-oncogene; these are responsible for the rare hereditary disorder, multiple endocrine neoplasia type 2 (*MEN 2*) that is associated with the development of medullary thyroid carcinoma. Thus, this test permits the prognosis of patients at risk of developing this malignancy before any clinical signs become evident.

Expanding our activities

A promising breakthrough for the Molecular Diagnostics Unit was achieved in the summer of 2013 through the signing of our first collaborative agreement with PharmaMar (Zeltia Group) in regards to an international clinical trial geared towards personalised medicine. This study aims to establish an alternative drug therapy regime in second-line treatment of patients with non-small cell lung (NSCL) cancer. The rationale is to use the chemosensitivity profiles of total RNA extracted from Circulating Tumour Cells (CTCs) isolated from the peripheral blood samples of the patients enrolled in the trial. Data from whole gene expression experiments will be analysed using a proprietary algorithm (CellPath Therapeutics, Inc.) that is able to generate a "chemogram"; i.e. a list of potentially sensitive and resistant drugs for each patient that can be used to guide anti-cancer therapies in these patients with refractory neoplasms (FIGURE).

Cooperating and training

Another important milestone for our Unit in 2013 was the start of a new alliance with an international partner, *Labceutics*. This partnership is dedicated to building collaborative relationships between the pharmaceutical and diagnostic industries, in an effort to create pioneering diagnostics solutions and introduce them into the clinical market place. Our first project has consisted of integrating a European network of diagnostics laboratories to evaluate the reliability of using a calibrator, which could globally standardise a common diagnostics test used to detect minimal residual Chronic Myeloid Leukaemia. Moreover, in order to share our expertise, our Unit has trained a laboratory technician and hosted three rotating residents this year. ■

PUBLICATION

Martin-Sanchez E, Rodriguez-Pinilla SM, Sanchez-Beato M, Lombardía L, Domínguez-Gonzalez B, Romero D, Odqvist L, Garcia-Sanz P, Wozniak MB, Kurz G, Blanco C, Mollejo M, Alves FJ, Menarguez J, Gonzalez-Palacios F, Rodriguez-Peralto JL, Ortiz-Romero PL, Garcia JF, Bischoff JR, Piris MA (2013). Simultaneous inhibition of pan-phosphatidylinositol-3-kinases and MEK as a potential therapeutic strategy in peripheral T-cell lymphomas. *Haematologica* 98, 57-64.

TRANSLATIONAL BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Technician
Elena Piñeiro

Fátima Al-Shahrour ESP

Elena Piñeiro ESP

OVERVIEW

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

The Translational Bioinformatics Unit uses computational methodologies to perform genomic analysis 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.

"The Translational Bioinformatics Unit has established a new collaboration with the *Hospital Universitario de Sanchinarro* in order to provide its expertise in computational cancer genomics analysis as well as to guide the diagnosis and treatment decision making process for cancer patients using next-generation sequencing data."

PUBLICATIONS AT OTHER INSTITUTIONS

- Miller PG, Al-Shahrour F, Hartwell KA, Chu LP, Järås M, Puram RV, Puissant A, Callahan KP, Ashton J, McConkey ME, Poveromo LP, Cowley GS, Kharas MG, Labelle M, Shterental S, Fujisaki J, Silberstein L, Alexe G, Al-Hajj MA, Shelton CA, Armstrong SA, Root DE, Scadden DT, Hynes RO, Mukherjee S, Stegmaier K, Jordan CT, Ebert BL (2013). In Vivo RNAi screening identifies a leukemia-specific dependence on integrin beta 3 signaling. *Cancer Cell* 24, 45-58.
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- Castaño Z, Marsh T, Tadipatri R, Kuznetsov HS, Al-Shahrour F, Paktinat M, Greene-Colozzi A, Nilsson B, Richardson AL, McAllister SS. Stromal EGF and igf-I together modulate plasticity of disseminated triple-negative breast tumors (2013). *Cancer Discov*. 3, 922-35.

RESEARCH HIGHLIGHTS

The CNIO Translational Bioinformatics Unit was established in February 2012. During this second year, our major research activity was focused on the development of novel computational techniques for the integration of cancer genomic data with clinical and pathological features, and to apply these new methodologies to detect therapeutic targets and biomarkers of response to therapy.

During 2013, the Translational Bioinformatics Unit developed a new bioinformatics methodology to match patients' tumours with the existing information from these resources, in order to extrapolate drug response using gene expression signatures. With this method, we are able to extend the set of known genetic markers and to use oncogenic signatures as new predictive biomarkers that could help to guide therapeutic decisions.

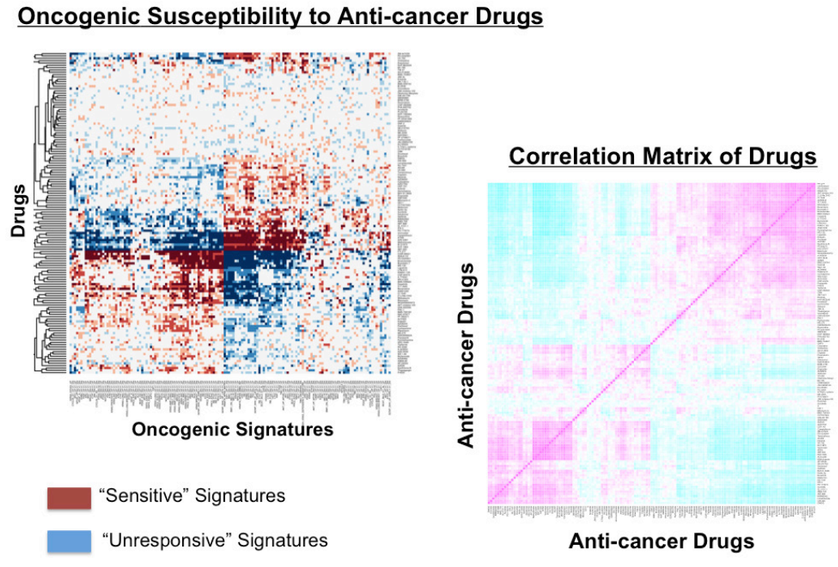
Pharmacogenomics

Two international projects – Genomics of Drug Sensitivity in Cancer, from the Wellcome Trust Sanger Institute, and Cancer Cell line Encyclopaedia, from the Broad Institute of MIT and Harvard – are underway with the aim of conducting a detailed genetic and pharmacologic characterisation of a large collection of cancer cell lines. This data can be used to better the optimal clinical application of cancer drugs, as well as the design of clinical trials of investigational compounds being developed for the clinic.

Personalised Medicine

In 2013, we established a new collaboration with the *Hospital de Madrid*. During this period, we implemented a new pipeline for the interpretation of next-generation sequencing data from patients' tumours. This computational module allows us to categorise patients' tumours and match them to effective drugs or treatments based on their genomic alterations. The output result is a ranked list of genetic variants that could serve as potential therapeutic targets and thereby also help guide treatment decisions for patients. So far we have analysed more than 50 patients and this new pipeline has facilitated the identification of actionable mutations in nearly half of those patients. ■

Figure Cluster and correlation matrix of anti-cancer drugs based on oncogenic transcriptional states associated with drug response.



BIOBANK

Manuel M. Morente
Director

Staff scientist
Lydia Sánchez

Technicians
M. Jesús Artiga, Francisco de Luna,
M. Cruz Marín


Manuel M. Morente ESP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OVERVIEW

The CNIO Biobank is a cross-service platform for CNIO researchers and the general research community oriented towards the promotion of biomedical research in cancer and related diseases. The CNIO Biobank facilitates access to human samples for research purposes, ensuring that both the acquisition and use of human samples complies with all the legal and ethical principles that protect donors' rights. CNIO's Biobank is – as defined by the Spanish Law 14/2007 on Biomedical Research, and Royal Decree RD 1716/2011 – a "Biobank for biomedical research purposes". It is therefore a public, non-profit organisation that hosts several collections of human biological samples for biomedical research.

The CNIO 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 documentation are collected in compliance with Spanish legislation and international recommendations; all this is in line with quality standards for sample collection and its subsequent management.

"The last two decades have been marked by a huge technical development in genetics that has transformed access procedures to human biological material; thereby creating a new ethical paradigm and, consequently, an extremely strict legislation regarding personal data protection that includes genetic information obtained from human samples."

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.

PUBLICATION

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Book chapters

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RESEARCH HIGHLIGHTS

Our portfolio of services includes:

Biobanking

- Collection, management, manipulation and storage 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 of collections of biological samples and/or information related to biomedical research 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 request samples must sign a Material Transfer Agreement (MTA) that specifies the terms and conditions under which the Biobank will custody samples and data.

Ethical-legal advice to 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 the creation and management of new collections that are out of the scope of the Biobank – in accordance with the present Spanish legal framework for biobanking.
- Collaborate and advice in the design and interpretation of immunohistochemical studies.
- Quality Management Programme.

CNIO's Biobank was authorised by the Health Authorities of the Community of Madrid in October, 2013. From this date onwards, we have supported 15 research projects and 35 tissue requests. The mean impact factor of the 28 publications supported by our Unit, published in 2013 was 6.2. We have also provided sample and/or documental support for the familial cancer activities of the CNIO Human Cancer Genetics Programme (25 cases).

Furthermore, our Unit actively collaborated with the ISCIII in the coordination of the *Red Temática de Biobancos Hospitalarios* (2010-2013), which involves 63 associated institutions, and has been appointed to coordinate the future *Plataforma de Biobancos* (2014-2017). 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 International Society for Biological and Environmental Repositories (ISBER), the European, Middle East and African Society of Biopreservation and Biobanking (ESBB), international think tanks such as the Marble Arch International Working Group on Clinical Biobanking, European (ESFRI) Platforms such as BBMRI-ERIC, BC-Net IARC-WHO/NCI initiative, EurocanPlatform (7th FP) and BeTheCure (IMI) projects, and the *Sociedad Española de Anatomía Patológica* (SEAP).

AWARDS AND RECOGNITION

- President of the European, Middle East & African Society of Biopreservation and Biobanking (ESBB).
- Member of the Steering Committee of the IARC-WHO/NCI BC-Net initiative (a Biobank initiative in Low- and Middle-Income Countries).
- Member of the L'AAP-BCB Evaluation Committee, *Institute Nationale du Cancer* (*INCA*), France.

Direction of Innovation

MARISOL QUINTERO
Director of Innovation
(until October)

"The Direction of Innovation enhances the value generated by CNIO's scientific activities, expanding their scope towards product and service development, and generating positive social and economic impacts."

Since its establishment in October 2011, the Direction of Innovation aims to fulfil one of the major strategic goals of the CNIO, namely, the translation of discoveries into practical solutions. The Biotechnology and Experimental Therapeutics Programmes, together with the Technology Transfer and Valorisation Office, constitute the Direction of Innovation. The Biotechnology Programme, composed of different units that act together as a technology service provider, is engaged in the development of novel technologies, such as antibodies, research tools, or the improvement of existing technological platforms. The Experimental Therapeutics Programme is dedicated to identifying novel drug targets and to validating them as potential therapeutic opportunities for the treatment of cancer. The Technology Transfer and Valorisation Office has been very active in attracting partners to commercialise or further develop CNIO's technologies.

The achievements of the Direction of Innovation were fourfold:

- During 2013 we were able to strengthen our collaborations with existing partner companies. One example is the collaboration with Roche´s Extending Innovation Network, through which we have initiated two new joint projects this year in the areas of gene therapy and drug discovery. Our partnership with another pharmaceutical company, Eli Lilly & Company, has also been reinforced with the establishment of a new CNIO-LILLY Epigenetics Section, as part of our participation in their Open Innovation Drug Discovery platform. This initiative enables us to share some of our proprietary compounds, with the goal of identifying potential new medicines that would act through novel mechanisms.
- A second key component is the spinning out of new ventures. In 2013, the first small-molecules ever developed at the CNIO have been licensed with industry for their further development and commercialisation. Two outstanding licensing agreements have been signed with the companies *Inflection Biosciences* and Merck Serono that will allow us to take full advantage of our ability to find the next generation of breakthrough therapies to fight cancer.
- Another important element is the generation of new knowledge to promote technology transfer. At the CNIO, we believe that young researchers are instrumental to the generation and diffusion of new technologies, and for that reason we promote training initiatives to foster innovation, in collaboration with *Instituto de Empresa* Business School. Thanks to the support of *Fundación Banco Santander*, a group of young researchers received training on managerial and entrepreneurial skills, which enabled them to develop their ideas into potential commercial opportunities.
- Finally, the Direction of Innovation aims to assist the CNIO in achieving an important goal: attract additional funding from private sponsors. Ongoing fundraising activities will enable the accomplishment of future strategic endeavours. ■

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 generated by the CNIO research groups, they also provide services and collaborate with groups from other institutions, both public and private.

In July 2013 the Head of the Histopathology Unit, Marta Cañamero, left the CNIO to accept a position in Roche Diagnostics GmbH in Penzberg, Germany. Under her leadership, which lasted close to 8 years, this Unit became a key partner and a highly-valued resource for the research groups at the CNIO; the Unit's contribution is reflected by the outstanding number of papers it produced. We thank Marta for the good work and wish her lots of success in her new endeavours for the years to come. A search for a new Unit Head has been initiated.

In 2013, the Programme's potential as a generator of technologies and products that can be transferred to society, and eventually be turned into sources of economic revenue for the CNIO, has been demonstrated in a number of ways. Most notably, the sales of Core Unit services to external groups in academia, hospitals and industry (including royalties generated from the sales of monoclonal antibodies) reached a historic high, representing over 600,000€ in income. Also, as an example of a technology that is in the process of being transferred, the Confocal Microscopy Unit has developed an "intelligent screening" platform that facilitates image capturing for microscopy in high-throughput mode. Discussions are ongoing with a multinational company for the deployment of this tool as an option in their advanced microscopes.

The Units' contribution to the scientific productivity of the CNIO in 2013 is reflected by the substantial number of publications (more than 30), including several papers in top journals. It is worth mentioning the contribution of five of our Units to the landmark paper published in *Nature* by the CNIO Tumour Suppression Group – under the leadership of Manuel Serrano – describing *in vivo* cell reprogramming, which has been selected as one of the most notable advancements of 2013 in the field of stem cell research by *Nature Medicine*. ■

"In 2013, the Biotechnology Programme demonstrated its competitive edge: we have succeeded in dynamically adapting our structure and portfolio of services to the evolving demands of CNIO researchers, and made essential contributions to several landmark publications, including papers reporting new advanced biotechnological tools and applications led by the Units."

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
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OVERVIEW

The genome is the complete set of genetic material that an individual transmits to the offspring. It is chemically made of DNA, but tightly packed and interpreted by a myriad of protein complexes. The genome is a linear arrangement of functional and non-functional elements: protein-coding genes constitute about 2% of the mammalian genome and novel functional non-protein-coding genes are increasingly being mapped and characterised. Our genome also features large deserts, fossils and even mobile pieces that are able to jump and integrate elsewhere. Not immutable, the genome is subject to damage and changes in the environment that can either have a neutral or positive effect, or contribute to genetic disease. Cancer is a condition derived from the accumulation of genetic mutations. The field of Genomics sheds light on this complexity; it deals with both the structure and dynamics of the genome, with its activity, and with the interactions of different genes with one another and with their environment.

"The Genomics Unit is a service provider core facility for CNIO scientists. It configures a toolbox for DNA and RNA analyses dedicated to an array of applications, either at the single locus or at a more global genomic level. The Unit actively employs its resources to help CNIO scientists to understand the molecular processes underlying cancer."

RESEARCH HIGHLIGHTS

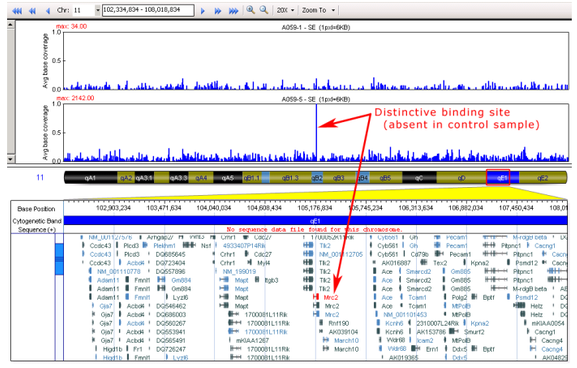

Figure Disease may cause changes in chromatin structure, which in turn influence gene activity. Vertical blue bars represent signals obtained by ChIPseq from two different samples. Red arrows point to a specific transcription factor binding site in the genome as revealed by the experimental results.

Each cancer genome is unique. Even a single tumour harbours a number of different subclonal genomes. Genomics' insights reveal basic clues for the understanding of such diversity; it helps to dissect the mechanisms of resistance to therapy or the consequences of gene modification in animal models.

Genomics employs a distinct set of powerful methodologies, with capacity to interrogate a wide number of genetic loci or even a whole genome in a single experiment. Some tools can detect modifications and differences between samples or conditions at a structural level: mutations, location of protein factors and complexes, as well as variations in chromatin folding and structure. Others are suitable to examine functional choreographies; the complex network of gene activity in response to stimuli or treatments, which may uncover potential therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of coverage. The genome-wide level is addressed by both deep-sequencing and microarray technologies. Deep-sequencing permits a variety of applications, including transcriptome analyses such as RNAseq and small RNAseq, genome-wide location of interacting protein factors on chromosomal DNA by ChIPseq, as well as whole-genome or whole-exome tumour sequencing. These applications are based on the use of the sequencing-by-synthesis technology from Illumina, which is carried out on a Genome Analyzer IIx instrument. In addition, gene expression or transcriptome and detection of chromosomal copy number aberrations are also being addressed with DNA microarrays. The platform at the CNIO is based on a DNA microarray scanner G2505C from Agilent. At the single locus level different services are available. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyser from Applied Biosystems, is being used to find mutations in candidate genes, or 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 service for the identification of transgene insertion sites in genetically engineered mouse models. Moreover, it has been asked to develop a transgenic mouse genotyping service, which has been implemented based on the use of allele-specific TaqMan probes for a quick turnaround time. ■

PUBLICATIONS

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TRANSGENIC MICE CORE UNIT

Sagrario Ortega
Core Unit Head

Technicians
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OVERVIEW

The laboratory mouse is the organism of choice for studying the genetic basis of human disease and for generating animal models of human genetic disorders. Cancer has a strong genetic component, and genetically engineered mouse models (GEMMs) are an essential tool for studies of gene function and mechanisms of this disease, as well as for drug discovery and target validation. The Transgenic Mice Unit at the CNIO offers state-of-the-art technology for the manipulation of the mouse genome and for the cryopreservation of genetically modified mouse strains. The Unit also provides support to CNIO researchers and collaborates with them in many aspects related to research with embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, as well as any type of embryo- and mouse model-based research. Finally, the Unit leads its own research projects that are aimed at the generation of mouse models for studies of tumour biology and for the screening of cancer-related genes.

"In 2013 we have generated, by gene targeting, 12 GEMMs carrying novel cancer-related alleles that will enhance our understanding of tumour biology and facilitate the development of cancer therapies. Moreover, the Unit has cryopreserved 107 mouse strains as frozen sperm and/or embryos, and has rederived 33 new strains into the CNIO Animal Facility."

RESEARCH HIGHLIGHTS

In 2013 the Transgenic Mice Core Unit has collaborated in two different research projects related to induced pluripotent stem (iPS) cell biology. In collaboration with the CNIO Tumour Suppression group, we have established that *in vivo* reprogramming is feasible in mice and results in the generation of iPS cells that exhibit totipotency features. Among other properties, these *in vivo* iPS cells are able to contribute not only to the inner cell mass and, therefore, to the embryo proper like pluripotent ES cells do, but also to the trophectoderm and the placenta. This work shows that *in vivo* reprogramming may be an alternative method to generate iPS cells for therapeutic applications. In addition, in collaboration with the CNIO Telomeres and Telomerase Group, we have generated a reporter mouse carrying a knock-in eGFP-*TRF1* fusion allele to study the role of TRF1 – a component of the sheltering complex that protects the ends of the chromosomes – in stem cell biology. We found that eGFP-TRF1 is expressed at high levels in iPS cells. Moreover, selection of iPS cells with high eGFP expression (high TRF1 levels) correlated with higher pluripotency. This work establishes that TRF1 is a stem cell marker, and plays a role in maintaining pluripotency.

In parallel, the Unit has initiated a project aimed at exploiting the potential of haploid mouse embryonic stem (ES) cells for genetic screenings in mammals. This year, for the first time, haploid ES cells have been successfully derived from mouse parthenogenetic and androgenetic embryos, thus expanding the possibilities for genetic screenings in mammals. In our Unit we are interested in applying the potential of genome-wide screenings in mouse haploid ES cells to gain insights into cancer research and, in particular, to advance preclinical drug development efforts. We have recently established our own haploid ES cell lines from wild type parthenogenetic mouse embryos (FIGURE). Moreover, at the CNIO, we have available a large collection of mouse models carrying targeted alleles of interest for cancer research, from which we plan to establish our first collection of haploid ES cells for genetic screenings in the context of cancer. These lines will be used in genetic synthetic viability/lethality screens to search for new mutations relevant to cancer biology. ■

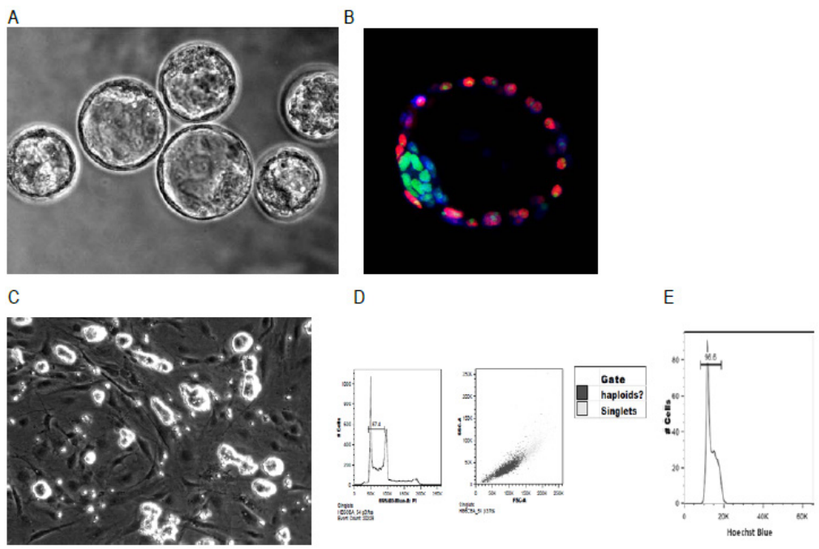

Figure Haploid ES cell lines established from parthenogenetic mouse embryos. (**A**) Blastocysts obtained by parthenogenetic activation of mouse oocytes. (**B**) Expression of Oct4 (green) in the inner cell mass and of Cdx2 (red) in the trophectoderm of parthenogenetic blastocysts. (**C**) Haploid ES cell line derived from a parthenogenetic blastocyst. (**D**) DNA content of B6.CBA haploid ES cells growing in culture. (**E**) After cell sorting for 1n DNA content, the cell population contains nearly 100% haploid ES cells.

PUBLICATIONS

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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
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Ana I. Reyes

Giovanna Roncador ITA

OVERVIEW

Our understanding of cancer biology and the diagnosis of neoplastic diseases improves with the identification of molecular markers that are selectively expressed by specific tumour subtypes. The isolation and identification of molecules involved in tumour transformation have been possible thanks to monoclonal antibodies (mAbs), which have become indispensable tools for basic and applied research.

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

"The Unit produces novel and high-quality mAbs that are used in basic research to gain novel insights into cancer biology. Being highly specialised in mAb characterisation, we provide CNIO researchers with reliable and well-validated reagents that add value to their research projects."

PUBLICATIONS

- Montes-Moreno S, Ramos-Medina R, Martínez-López A, Barrionuevo Cornejo C, Parra Cubillos A, Quintana-Truyenque S, Rodriguez Pinilla SM, Pajares R, Sanchez-Verde L, Martinez-Torrecuadrada J, Roncador G, Piris MA (2013). SPIB, a novel immunohistochemical marker for human blastic plasmacytoid dendritic cell neoplasms: characterization of its expression in major hematolymphoid neoplasms. *Blood* 121, 643-647.
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RESEARCH HIGHLIGHTS

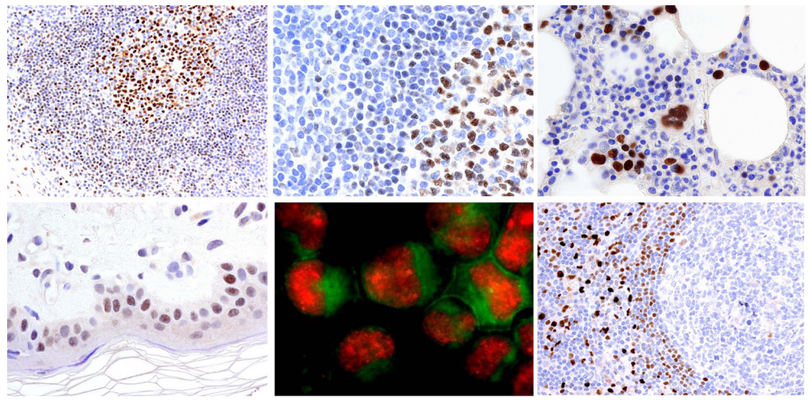

Figure Immunohistochemistry with mAbs produced by the Monoclonal Antibodies Core Unit.

During the last 13 years the Monoclonal Antibodies Unit has generated and characterised a large number of mAbs (against more than 100 different antigens), mostly targeting molecules for which mAbs are not commercially available. Many of these mAbs have been licensed to external companies, generating revenues through royalties that represent an important source of funding for the CNIO. As an example, cumulative sales of our antibody against FOXP3 since 2010 reached a milestone of 1 million Euros this year.

Each year we prepare and update a detailed catalogue of CNIO´s mAbs that contains the datasheets of more than 58 thoroughly validated, high-quality mAbs (currently available online via: http://www.cnio.es/es/servicios/anticuerpos/default.aspx).

In collaboration with Jorge Martínez-Torrecuadrada (the CNIO Proteomics Core Unit) and Luis Ángel Fernández (CNB-CSIC), we are currently developing an alternative and novel methodology for the production of mAbs that recognise a unique point mutation (PM) in proteins expressed in cancer tissues. The reliable and efficient detection of PM in tumours has become essential for progress in biomedicine, due to its wide range of potential applications in the diagnosis and prognosis of cancer. The production of these mAbs has proven to be a difficult and laborious task because they must be able to recognise a single amino acid change, and the antigens conventionally used (peptides) have low immunogenicity. Our alternative solution has been to incorporate fragments of the mutated proteins in either the capsid of the hepatitis B virus or in the surface of *Escherichia coli*. These strategies should substantially increase the likelihood of producing reliable and specific mAbs against the mutated proteins.

In 2008, in collaboration with Oxford University, we founded EuroMAbNet, the first European non-profit organisation of multidisciplinary academic laboratories specialised in mAb production. The goal of EuroMabNet is to provide a framework in which investigators working in the field can exchange knowledge, share cutting-edge methodology and materials, as well as create common strategies to standardise and improve the production and validation of mAbs. The EuroMabNet web page can be accessed by visiting: www.euromabnet.com. ■

HISTOPATHOLOGY CORE UNIT

Marta Cañamero
Core Unit Head (until June)

Technicians
Virginia Álvarez, Núria Cabrera, Elvira Gil (until April), María Gómez, Patricia González, María Lozano, Raquel Pajares, Zaira Vega


Virginia Álvarez ESP


Núria Cabrera ESP


María Gómez ESP


Patricia González ESP


María Lozano ESP


Raquel Pajares ESP


Zaira Vega ESP

OVERVIEW

Histopathology is the branch of pathology dealing with the tissue diagnosis of disease. The Histopathology Core Unit at the CNIO offers a full range of services for both human and mouse tissue samples, including processing, embedding, and cutting of paraffin tissue specimens. We also provide most standard histological stains, as well as research and diagnostic immunohistochemistry tests, antibody workup, *in situ* hybridisation (including microRNAs) and TUNEL, tissue microarray, digital slides & analysis, and laser capture microdissection services. We have increased our panel of anti-human and anti-mouse antibodies to more than 525 and 270 available antibodies, respectively. This represents a highly valuable resource for CNIO researchers, as well as for the external clinical and research community that uses the services provided by our Unit.

Our Unit is equipped with all the instruments required to automate most of the processes included in our service package; this allows us to obtain highly reproducible results. The Unit also offers specialised pathology consulting to the CNIO Research Groups and is heavily involved in training activities, thus providing students and researchers at the CNIO with the expertise in histopathology that they require for the successful execution of their research projects.

Our Unit participates in several External Quality Assessment Services/Programmes (EQAS), such as NordiQC and UK NEQAS, as well as in European projects focused on training in comparative pathology, such as the Canceropôle Network. ■

"The histopathological characterisation of mouse models, as well as its correlation with human pathology, is an essential component of the projects that are carried out by most Research Groups at the CNIO, from both Basic and Translational Research Programmes."

PUBLICATIONS

- Muñoz-Espín D, Cañamero M, Maraver A, Gómez-López G, Contreras J, Murillo-Cuesta S, Rodríguez-Baeza A, Varela-Nieto I, Ruberte J, Collado M, Serrano M (2013). Programmed Cell Senescence during Mammalian Embryonic Development. *Cell* 155, 1104-1118.
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- Eguren M, Porlan E, Manchado E, García-Higuera I, Cañamero M, Fariñas I, Malumbres M (2013). The APC/C cofactor Cdh1 prevents replicative stress and p53-dependent cell death in neural progenitors. *Nature Communications* 4, 2880.
- Pérez de Castro I, Aguirre-Portolés C, Fernández-Miranda G, Cañamero M, Cowley DO, Van Dyke T, Malumbres M (2013). Requirements for aurora-a in tissue regeneration and tumor development in adult mammals. *Cancer Res* 73, 6804-6815.
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- Rodríguez-Antona C, Muñoz-Repeto I, Inglada-Pérez L, de Cubas AA, Mancikova V, Cañamero M, Maliszewska A, Gómez A, Letón R, Leandro-García LJ, Comino-Méndez I, Sanchez L, Alvarez-Escolá C, Aller J, Cascón A, Robledo M (2013). Influence of *RET* mutations on the expression of tyrosine kinases in medullary thyroid carcinoma. *Endocr-Relat Cancer* 20, 611-619.
- Ramos-Medina R, Montes-Moreno S, Maestre L, Cañamero M, Rodríguez-Pinilla M, Martínez-Torrecuadrada JL, Piris MA, Majid A, Dyer MJ, Pulford K, Roncador G (2013). BCL7A protein expression in normal and malignant lymphoid tissues. *Br J Haematol* 160, 106-109.
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- Narlik-Grassow M, Blanco-Aparicio C, Cecilia Y, Perez M, Muñoz-Galvan S, Cañamero M, Renner O, Carnero A (2013). Conditional transgenic expression of PIM1 kinase in prostate induces inflammation-dependent neoplasia. *PLoS One* 8,e60277 .

Article in press

- Flandez M, Cendrowski J, Cañamero M, Salas A, Del Pozo N, Schoonjans K, Real FX . *Nr5a2* heterozygosity sensitises to, and cooperates with, inflammation in *KRasG12V*-driven pancreatic tumourigenesis. *Gut* (in press). PMID:23598351

MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Graduate Student
Monika Musko (until May)

Technicians
Elena Andrés, Juan A. Cámara, Silvia Sánchez, Coral Velasco


Francisca Mulero ESP


Elena Andrés ESP


Juan A. Cámara ESP


Silvia Sánchez ESP


Coral Velasco ESP

OVERVIEW

Molecular imaging is a discipline that enables the visualisation of cellular functions and the tracking of the molecular processes in intact living organisms. The multiple and numerous potential benefits offered by this field are applicable to the study and diagnosis of diseases such as cancer. Imaging techniques can thus contribute to improving the treatment of cancer by optimising the pre-clinical and clinical testing of new drugs.

Molecular imaging differs from traditional imaging in that imaging biomarkers are used as probes to facilitate the visualisation of particular targets or pathways. Biomarkers chemically interact with their environment and, in turn, alter the image according to the molecular changes occurring within the area of interest. Furthermore, molecular imaging allows for quantitative analysis, providing a higher degree of objectivity.

"The Molecular Imaging Core Unit lends wide-ranging support to CNIO researchers through an assortment of state-of-the-art equipment and high-quality techniques for the *in vivo* imaging of mice as well as patients. We provide a powerful tool for monitoring treatment response. "

RESEARCH HIGHLIGHTS

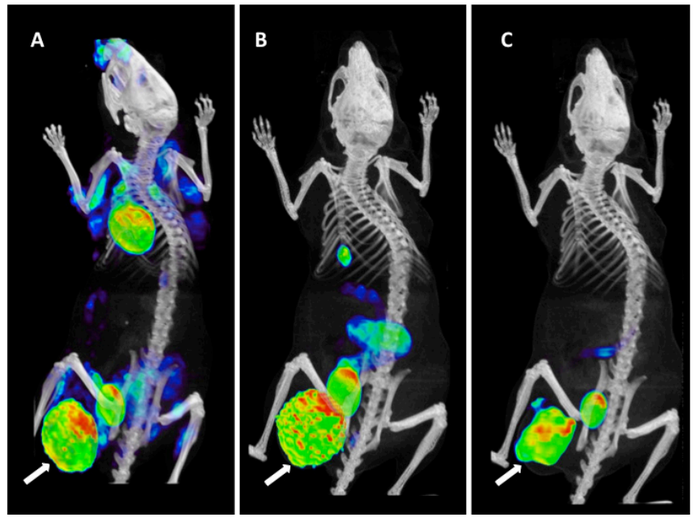

Figure PET-CT scan of an inguinal breast carcinoma. The arrows point to the tumour, which shows different uptake patterns depending on the radiolabelled compound. (**A**)^{18}F-FDG metabolic marker. (**B**) ^{18}F-MISO hypoxia marker. (**C**) ^{18}F-FLT proliferation marker.

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

We are now going a step further by also focusing on therapy monitoring and assessment. Tumour hypoxia is a key factor in predicting tumour response to treatment. Drugs that modulate vascularisation offer potential therapeutic options for many cancer types. Positron emission tomography-computed tomography (PET-CT), using ^{18}F-labelled fluorodeoxyglucose as a probe (^{18}F-FDG), has proven to be very useful for assessing tumour aggressiveness and metabolic activity in response to treatment. ^{18}F-fluoromisonidazole (^{18}F-MISO), a biomarker of hypoxic tissues, is more specific when it comes to tracing the effect of anti-angiogenic drugs. ^{18}F-MISO gets trapped in the hypoxic cell, which enables the PET scanner to image it. We have used this biomarker in different cancer types. We have also started to use the proliferation marker, ^{18}F-FLT, to monitor response to therapy in tumours that do not have a high metabolism and, therefore, lack ^{18}F-FDG uptake (FIGURE).

In 2013, we initiated a Collaborative Project with the Massachusetts Institute of Technology (MIT) – "Improved Molecular Imaging by Multi-tracer PET" – that focuses on the use of dual isotopes to simultaneously assess different biological changes. We have also actively participated, as a central imaging reader centre, in assisting the setup of a clinical PET-CT and a 3-Tesla MRI system at the *Hospital de Fuenlabrada*, which is dedicated to providing imaging support in clinical trials conducted under CNIO's Clinical Research Programme.

Furthermore, we continued our participation in both a national, CDTI-funded Advanced Molecular Imaging Techniques (AMIT) Consortium, and in international consortia –"m+visión" Madrid-MIT Consortium and Euro_BioImaging; a large_scale pan_European research infrastructure project for the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. ■

PUBLICATION

- Abarrategi A, Perez-Tavarez R, Rodriguez-Milla MA, Cubillo I, Mulero F, Alfranca A, Lopez-Lacomba JL, García-Castro J. *In Vivo* Ectopic Implantation Model to Assess Human Mesenchymal Progenitor Cell Potential (2013). *Stem Cell Rev* 9, 833-846.

AWARDS AND RECOGNITION

- Co-chair of the 2013 Selection Committee, Scientific Advisory Board Member, and Faculty of "m+visión" (Madrid-MIT Consortium), Spain.
- Project Evaluator, National Agency for Promoting Science and Technology, Ministry of Science, Technology and Productive Innovation, Argentina.

FLOW CYTOMETRY CORE UNIT

Lola Martinez
Core Unit Head

Technicians
Ultan P. Cronin, Elena Garrido,
Miguel Ángel Sánchez

Lola Martinez ESP

Ultan P. Cronin IRL

Elena Garrido ESP

Miguel Ángel Sánchez ESP

OVERVIEW

Flow Cytometry is a fast, multiparametric technique that allows 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. It is an indispensable tool in cancer research. We provide CNIO Research Groups with technical and scientific advice regarding flow cytometry technologies and assays, and collaborate with them in data analysis and interpretation. Our Core Unit is equipped with 3 benchtop analysers and 2 high-speed cell sorters, with different configurations of lasers and detectors. Analysers are available to users, after appropriate training, and cell sorters are exclusively operated by the Unit staff. Our sorters can separate up to 4 defined populations at a time, as well as perform single cell cloning. One of the sorters is installed in a biological safety cabinet, allowing us to sort human samples according to biosafety regulations.

"Our unit actively develops assays in collaboration with CNIO Research Groups. This year, as an example, we have been characterising and isolating murine erythroid progenitors from an *Mcm3* hypomorphic mouse model, in order to address the importance of Mcm in maintaining the homeostatic balance of these haematopoietic progenitors."

PUBLICATIONS

- Abad M, Mosteiro LI, Pantoja C, Cañamero M, Rayon T, Ors I, Graña O, Megías D, Domínguez O, Martinez D, Manzanares M, Ortega S, Serrano M (2013). Reprogramming in vivo produces teratomas and iPS cells with totipotency features. *Nature* 502, 340-345.
- Thomsen MK, Bakiri L, Hasenfuss SC, Hamacher R, Martinez L, Wagner EF (2013). JUNB/AP-1 controls IFN-? during inflammatory liver disease. *J Clin Invest* 123, 5258-5268.
- Fernández D1, Ortiz M, Rodríguez L, García A, Martinez D, Moreno de Alborán I (2013). The proto-oncogene c-myc regulates antibody secretion and Ig class switch recombination. *J Immunol* 190, 6135-6144.

Article in press

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RESEARCH HIGHLIGHTS

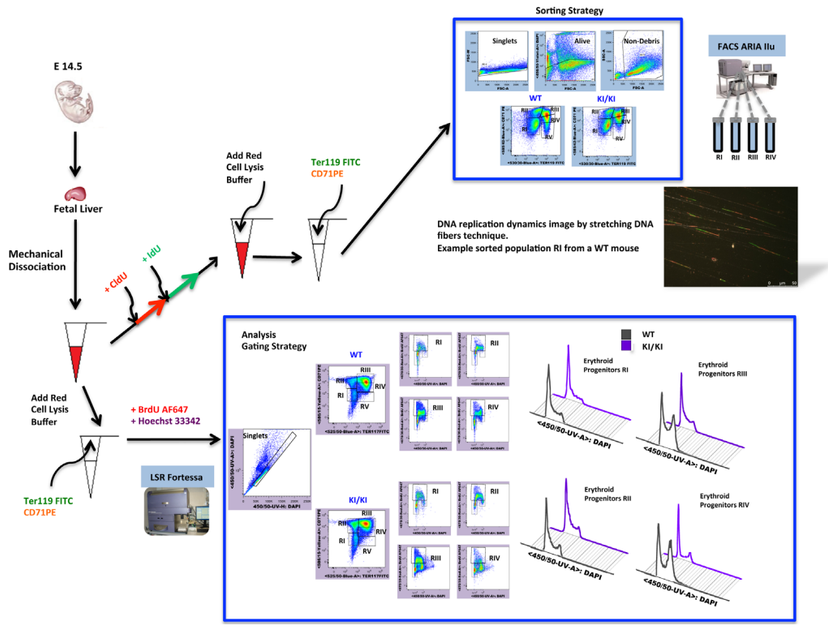

Figure Workflow of the analysis and isolation, by cell sorting, of erythroid progenitors from WT and KI/KI *Mcm3* mice. Representative plots showing the proliferation status of each subpopulation of progenitor cells using BrdU incorporation assays.

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. Flow cytometry applications developed and validated by the Unit include:

- → Cell proliferation studies (CFSE, CellTrace Violet, BrdU or EdU, DNA content, etc.).
- → Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
- → Multicolor Immunophenotyping.
- → Functional Assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric Bead Arrays for the measurement of several cytokines in cell extracts and plasma.

During 2013, we developed several new multicolour panels for the detection of different cellular progenitor subtypes, T, B and inflammatory cells derived from sources such as haematopoetic tissues, pancreas, skin, liver, etc., and combined these panels with the detection of proliferation and cell death. As part of our commitment to continually improve our services, we further assessed new fluorochromes, brilliant violet and eFluor dyes, among others, and incorporated them in certain panels with the aim of improving the resolution of subpopulations of interest. Furthermore, the 561nm excitation line acquired for our cell sorter and analyser has proved to be a good investment and is constantly being used for the detection and isolation of red fluorescent expressing cells; this is as a result of several mouse models expressing Katushka as a reporter that have recently been generated at the CNIO.

The Unit has continued to develop comprehensive training courses for different applications of flow cytometry techniques. This year, training courses benefited from the collaboration of R. Gardner (Flow Cytometry Manager, *Instituto Gulbenkian de Ciencia*, Oeiras, Portugal), and consisted of over a full week of theoretical and practical sessions that were held at the end of September. This seminar series was again a great success in terms of attendance by internal and external users, and has resulted in the successful implementation of novel protocols developed by our Core Unit. These training activities will be offered again next year. ■

CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Graduate Student
Aleksandra Amelian (until May)

Technicians
Manuel Pérez, Joaquim Soriano

Diego Megías ESP

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, including technical and scientific advice and support to the CNIO scientists. The Unit is also actively involved in developing, testing and implementing new microscopy technologies, tools and imaging applications, which can be of interest to Research Groups at the CNIO. Training activities are also an essential component of our mission.

"The Confocal Microscopy Unit is fully committed to the implementation of advanced microscopy methodologies in cancer research, with the aim of creating a benefit for society by increasing our understanding of the biology and disorders of cells that cause cancer. "

PUBLICATIONS

- Abad M, Mosteiro LI, Pantoja C, Cañamero M, Rayon T, Ors I, Graña O, Megías D, Domínguez O, Martinez D, Manzanares M, Ortega S, Serrano M (2013). Reprogramming in vivo produces teratomas and iPS cells with totipotency features. *Nature* 502, 340-345.
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- de Cubas AA et al. (incl. Comino-Méndez I, Inglada-Perez L, Pérez M, Ibarz N, Ximénez-Embún P, López-Jiménez E, León R, Maliszewska A, Gómez Graña A, Rodriguez-Antona C, Muñoz J, Megías D, Cascón A, Robledo M) (2013). Integrative analysis of miRNA and mRNA expression profiles in pheochromocytoma and paraganglioma identifies genotype-specific markers and potentially regulated pathways. *Endocr-Relat Cancer* 20, 477-493.
- Alvarez R, Musteanu M, Garcia-Garcia E, Lopez-Casas PP, Megias D, Guerra C, Muñoz M, Quijano Y, Cubillo A, Rodriguez-Pascual J, Plaza C, de Vicente E, Prados S, Tabernero S, Barbacid M, Lopez-Rios F, Hidalgo M (2013). Stromal disrupting effects of nab-paclitaxel in pancreatic cancer. *Br J Cancer* 109, 926-933.
- Rivera-Torres J1, Acín-Perez R, Cabezas-Sánchez P, Osorio FG, Gonzalez-Gómez C, Megias D, Cámara C, López-Otín C, Enríquez JA, Luque-García JL, Andrés V. (2013). Identification of mitochondrial dysfunction in Hutchinson-Gilford progeria syndrome through use of stable isotope labeling with amino acids in cell culture. *J Proteomics* 91, 466-477.

RESEARCH HIGHLIGHTS

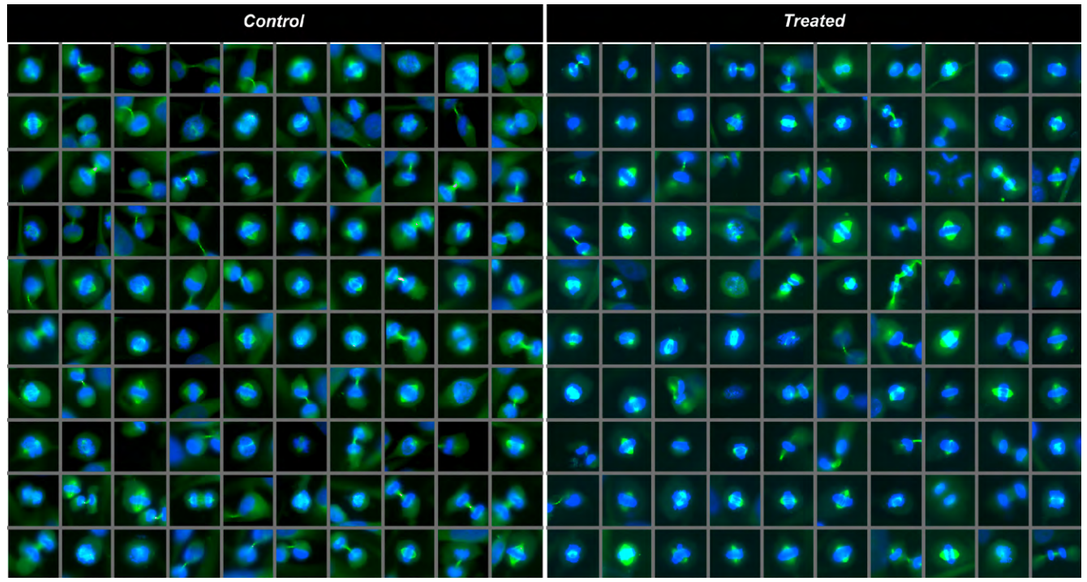

Figure Automated analysis of aberrant mitotic phenotypes.

The Confocal Microscopy Core Unit is equipped with three 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. We also have 2 wide field systems, namely, 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 applied high throughput technologies (HT) to confocal microscopy using two different systems:

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

These technological advances increase the level of information obtained from a sample and allow for the automated HT screening of cell behaviour in response to different treatments.

This year, the Confocal Microscopy Unit has expanded its portfolio of techniques to include dynamic live-cell assays in perfusion chambers. In addition, the Unit has continued to establish numerous scientific collaborations with CNIO researchers, covering several aspects of cancer studies such as the Intelligent Screening of tissues and the measurement of protein activity reporters using Fluorescence Resonance Energy Transfer (FRET) technology. Moreover, the Confocal Microscopy Unit is dedicating a significant effort towards the development and implementation of HCS technology at CNIO; for example, during this last year we helped to run screenings aimed at testing compounds that may eventually modify key aspects of tumourigenesis such as mitotic checkpoint regulation, integrity of nucleoli, DNA damage, cell survival and proliferation. ■

PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Staff Scientist
Jorge L. Martínez

Graduate Student
Natalia Miekus (until May)

Technicians
Fernando García, Rut González, Nuria Ibarz, Encarna Pucheta (until July), M. Isabel Ruppen, Pilar Ximénez de Embún


Javier Muñoz ESP


Jorge L. Martínez ESP


Fernando García ESP


Rut González ESP


Nuria Ibarz ESP


M. Isabel Ruppen ESP


Pilar Ximénez de Embún ESP

OVERVIEW

The field of proteomics aims to characterise the complete repertoire of proteins expressed by a genome (the proteome) in order to better understand how cells function. The global analysis of proteins is challenging due to the high complexity (>12,000 genes are frequently transcriptionally active) and dynamic range (10 orders of magnitude between high- and low-expressed proteins) of protein abundances. A further challenge is the post-translational modification of proteins (e.g. phosphorylation) and their interactions with one another to form complexes, both of which are highly divergent in time and space. To tackle these analytical challenges, proteomics employs a combination of techniques including sophisticated sample preparation methods, mass spectrometry (MS) and bioinformatics.

"The CNIO Proteomics Core Unit provides MS-based proteomics methodologies to CNIO Research Groups in order to better understand, at the proteome level, the underlying molecular basis of cancer. A further aspect of the Unit is to support projects involving recombinant protein production that can be deployed at multiple levels, including fee-for-service and focused hypothesis-driven research collaborations. More specifically, our core facility provides CNIO researchers and external groups with technology for medium to large-scale protein expression in *Escherichia coli*, insects and mammalian cells."

RESEARCH HIGHLIGHTS

Throughout 2013, the Unit has implemented a cost-effective stable isotope method for accurate protein quantification, termed "dimethyl labelling", which allows the comparison of up-to three samples and represents an alternative to metabolic procedures (e.g. SILAC). We have also established several collaborations with CNIO groups in order to identify substrates of certain ubiquitin ligases involved in cancer. Likewise, we have applied proteomics to better characterise and understand the protein machinery that drives DNA replication, and to study how it is regulated under conditions of replicative stress, a hallmark of cancer. Using different affinity purification strategies, we have also identified several protein partners of key genes involved in various cancer processes.

Furthermore, we have carried out the production of different Nanog protein regions to identify protein domains that are suitable for the effective generation of highly-specific antibodies against this essential transcription factor (in collaboration with the CNIO Tumour Suppression Group and the Monoclonal Antibodies Core Unit). In collaboration with the CNIO Monoclonal Antibodies Core Unit, our Unit has also been involved in the recombinant production of all members of the Myb transcription factor family – implicated in several lympho-proliferative disorders – with the aim of developing monoclonal antibodies for the differential diagnosis and classification of lymphomas. On the other hand, we have continued our work focusing on the development of recombinant antibody fragments (scFvs) against target proteins involved in angiogenesis (as part of our participation in the Angiobodies 2.0 Programme, a project funded by the Autonomous Community of Madrid). Finally, in collaboration with L. Álvarez-Vallina from the *Hospital Puerta del Hierro* (Madrid), using *in vivo* selection of human antibodies and MS analysis, we have identified the proteasome activator complex PA28 as a novel biomarker for prostate cancer. The validation of PA28 complex in primary and metastatic human prostate cancer has confirmed the potential application of this target for diagnostic and therapeutic interventions. ■

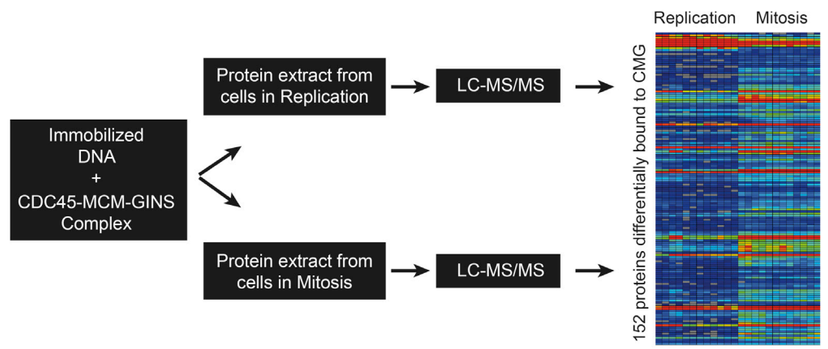

Figure Schematic workflow illustrating the quantitative proteomic approach used to identify key proteins that promote CMG complex (CDC45, MCM, GINS) formation (in collaboration with the CNIO Macromolecular Crystallography Group).

PUBLICATIONS

- Schonthaler HB, Guinea-Viniegra J, Wculek SK, Ruppen I, Ximénez-Embún P, Guío-Carrión A, Navarro R, Hogg N, Ashman K, Wagner EF (2013). S100A8/A9 (calprotectin) is a mediator in psoriasis controlling expression of complement factor C3. *Immunity* 39, 1171-1181.
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- Montes-Moreno S *et al*. (incl. Pajares R, Sanchez-Verde L, Martinez-Torrecuadrada J, Roncador G) (2013). SPIB, a novel immunohistochemical marker for human blastic plasmacytoid dendritic cell neoplasms: characterization of its expression in major hematolymphoid neoplasms. *Blood* 121, 643-647.
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- López-Contreras AJ, Ruppen I, Nieto-Soler M, Murga M, Rodriguez- Acebes S, Remeseiro S, Rodrigo-Perez S, Rojas AM, Mendez J, Muñoz J, Fernandez-Capetillo O (2013). A proteomic characterization of factors enriched at nascent DNA molecules. *Cell Rep* 3, 1-12.
- Martínez P, Gómez-López G, García F, Mercken E, Mitchell S, Flores JM, De Cabo R, Blasco MA (2013). RAP1 protects from obesity through its extratelomeric role regulating gene expression. *Cell Rep* 3, 2059-2074.

Publication at other institutions

- Altelaar AF, Munoz J, Heck AJ (2013). Next-generation proteomics: towards an integrative view of proteome dynamics. *Nat Rev Genet* 14, 35-48.

AWARDS AND RECOGNITION

- Research contract from the *Ramon y Cajal* Programme, *Ministerio de Economía y Competitividad* (*MINECO*).

ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Charles River Laboratories International, Inc.
Management

The CNIO has a state-of-the-art Animal Facility that is managed by Charles River Laboratories. The Animal Facility's primary responsibility is the supply, husbandry and quality control of laboratory animals used by CNIO's Research Programmes in their experimental protocols. The strict compliance to national, European 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 closely with 16 Research Groups from within both the Basic Research Programmes (Molecular Oncology, BBVA Foundation-CNIO Cancer Cell Biology) and the Translational Research Programmes (Molecular Pathology, Clinical Research), as well as with some Sections and Units from the Experimental Therapeutics and Biotechnology Programmes.

Our Animal Facility has the capacity to house 19,000 type IIL cages (each with an average capacity for 3.5 mice). More than 1,500 different 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.

"The Animal Facility provides the CNIO researchers with all the infrastructures and support necessary to develop their projects and procedures involving animal experimentation, thereby ensuring both the scientific and technical excellence as well as the compliance with all existing regulatory requirements."

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 Individually Ventilated Caging (IVC) units, which are integrated in the building's 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 ensure the highest productivity possible and 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.

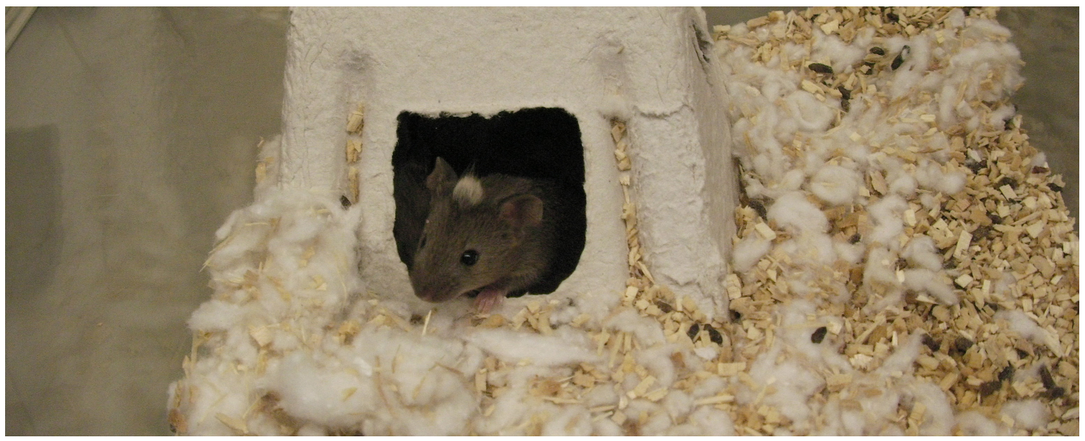
The Animal Facility currently harbours more than 1,500 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying more than 300 gene targeted alleles and close to 200 transgenic integrations. More than 100 gene targeted alleles and 50 transgenic mouse strains of cancer-related genes have been generated by the research groups at the CNIO, plus around 200 genetically modified lines 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 on the premises, including the use of gamma irradiation, exposure to UV light and volatile carcinogenic agents, as well as surgery procedures. In addition, the monitoring of the mouse models on-site, through non-invasive imaging technologies, is provided by the Molecular Imaging Core Unit that has integrated all their image acquisition instrumentation into the Animal Facility. Likewise, the work of the Transgenic Mice Core Unit is performed in a laboratory inside the SPF barrier. Finally, a necropsy laboratory, equipped with instruments for the haematological and biochemical analysis of blood and urine, complements the pathology and clinical diagnostics capabilities.

All the work carried out by the Animal Facility complies with both national and European 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 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) that has been set up to comply with the new European Directive 2010/63/UE.

The Royal Decree RD53/2013 stipulates that all animal procedures are to be carried out by qualified people in possession of the corresponding accreditation as issued by the competent authority. To abide with the former requirement, the Animal Facility offers CNIO staff an official 'Category C' qualification annual training course focused on the education and training of personnel performing work with laboratory animals. This course has been internationally accredited by FELASA (Federation of European Laboratory Animal Science Associations), being one of the only two courses awarded with this accreditation in Spain. ■

RESEARCH HIGHLIGHTS



EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

The main research areas of the Experimental Therapeutics Programme (ETP) are:

Early-Drug Discovery projects: for the advanced lead compounds, we have progressed to *in vivo proof- of- concept* (PoC) studies for the target and /or have entered into licensing agreements; i.e. phosphatidylinositol 3-kinase (PI3K α/δ), proto-oncogene serine/threonine protein kinase PIM, combined PIM/PI3K/ mammalian target of rapamycin (mTOR) inhibition, and ataxia telangiectasia and Rad3-related (ATR) inhibitors. The ATR inhibition project is the result of a successful model of collaboration between CNIO Basic Research Groups and ETP. We have also re-activated our CDK8 inhibition project, which not only focuses on cancer but also on cell reprogramming and regenerative medicine.

Exploratory projects: we have carried out High-Throughput Screening (HTS) campaigns for targets such as microtubule associated serine/threonine kinase-like (MASTL), *Kinase X, and have provided a screening collection for telomeric repeat binding factor 1 (TRF-1).

Our Programme has continued to provide support to CNIO researchers in various activities, such as detecting drug levels in biological samples or synthesising tool compounds.

Major milestones achieved in 2013:

→ PIM, PIM-PI3K-mTOR: we successfully carried out additional *in vivo* characterisation (PoC) and formulation design of selective PIM inhibitors. Regarding combined profiles, we demonstrated down-regulation of biomarkers *in vivo* using the MV4:11 tumour xenograft and KRas non-small-cell lung cancer (NSCLC) mouse models. Antitumour efficacy was achieved with the ETP-triple inhibitor. The profile of ETP-CNIO inhibitors has triggered a license agreement with *Inflection Biosciences* for further clinical development.

→ ATR: we centred our work on the development of a second series of ATR inhibitors and the exploration of structure activity and property relationships (SAR/SPR) of this series. The most interesting leads, which displayed a suitable profile in terms of potency/selectivity and drug-like properties, were selected and promoted for *in vivo PoC* studies. Compounds ETP-63/68 demonstrated down-regulation of biomarkers and antitumour effects in the allo-E-myc model. As a result of this exploration, the CNIO Genomic Instability Group (GIG) and the ETP have filed a joint patent application. The success of the ATR-CNIO project led to the establishment of a licensing agreement with Merck Serono; it contemplates fixed upfront and milestone payments associated with the advancement of the CNIO molecules.

→ PI3K α/δ inhibitors: the highly potent/selective and drug-like inhibitor ETP-444 has demonstrated interesting results as a potential agent in the prevention of obesity; it is the focus of a project carried out in collaboration with Manuel Serrano from the CNIO Tumour Suppression Group. The compound is available for licensing/development for 2 indications: cancer and obesity. ■

"The Experimental Therapeutics Programme has achieved *Proof-of-Concept* out-licensing projects. This is particularly relevant in the case of ATR: the ETP-GIG collaboration has generated great success for the CNIO; an institution that not only fosters excellence in basic cancer research but also cares about bringing novel anti-cancer therapies to patients. This is a model to be followed."

*Kinase X (confidential), in collaboration with B. Lambrecht from the VIB Inflammation Research Centre, Ghent University (Belgium). VIB (Flanders Biotechnology Institute).

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OVERVIEW

The main goal of the Experimental Therapeutics Programme (ETP), and therefore also of the Medicinal Chemistry Section, is to run Early-Drug Discovery projects in order to discover new anti-cancer drugs with good pharmacological activities, selectivity and 'drug-like' properties (Absorption-Distribution-Metabolism-Excretion and Toxicity). To this end, ETP's Medicinal Chemistry Section is responsible for the design and synthesis of novel compounds, which are then analysed biologically by ETP's Biology Section, in order to establish new hypotheses for further optimisation through an iterative process. Large amounts of chemical and biological data are generated during this process and our Section provides the required knowledge and expertise to devise and implement strategies for developing advanced lead compounds.

We are responsible for ensuring the intellectual property of the CNIO-molecules by providing protection through strong patents; thus, the molecules need to be novel and inventive, may not have been published before, and cannot be covered by claims included in any other existing patents. This process requires a deep knowledge of the chemical space that can be patented, as well as lots of creativity and technical proficiency in order to gain access to it.

Our Section is also involved in exploratory projects, including hit validation, analogue searching in specialised databases, and/or the preliminary exploration of hits. ETP's Medicinal Chemistry Section also provides support to CNIO researches in different activities, such as in the search for and synthesis of key tool compounds.

"After providing *in vivo* proof-of-concept with our advanced lead compounds in the PIM, PIM/PI3K-mTOR and ATR projects, and thereby showing the potential benefit of these molecules to treat cancer, we have licensed PIM and PIM/PI3K-mTOR CNIO molecules with *Inflection Biosciences*, and the ATR inhibitors with Merck Serono for their clinical development."

RESEARCH HIGHLIGHTS

During 2013, our Section was involved in several projects:

- → We completed the characterisation of lead compounds from our Early Drug Discovery projects: serine/threonine-protein kinase PIM, PIM-phosphatidylinositol-3 kinase (PI3K)- mammalian target of rapamycin (mTOR), and ataxia telangiectasia and Rad3-related (ATR).
- → We contributed to the development of CNIO-PI3K inhibitors for use in the treatment of obesity.
- → The cyclin-dependent protein kinase 8 (CDK8) project was re-activated.
- → We have started working on several exploratory targets, such as Kinase X (confidential) and nuclease MUS81.

PIM, PIM-PI3K-mTOR

The overall profile of these inhibitors has triggered a license agreement with *Inflection Biosciences Ltd.*; it was signed in the first quarter of 2013 for the development of these compounds in the clinic. ETP-CNIO inhibitors have been synthesised on a multi-gram scale for their *in vivo* internal/external characterisation under the agreement conditions.

ATR

ETP's Medicinal Chemistry Section has designed and synthesised over 97 novel compounds for the purpose of completing the lead optimisation process. We are focusing on the development of a second series of ATR inhibitors that afforded leads ETP-63/68. Both inhibitors have demonstrated regulation of associated biomarkers and an anti-tumour effect in the allo-Eμ-myc model after oral administration. These novel chemical series have been protected by joint patent application EP13382089.4, which has been filed by inventors from the CNIO Genomic Instability Group and the ETP.

CDK8

Based on hits from a previous HTS campaign and the CDK8 structural information available from X-Ray crystallography studies with Sorafenib, we have designed potential novel CDK8 inhibitors providing intellectual property rights for the CNIO. Docking techniques were useful to filter all virtual proposals and prioritise them for actual synthesis. In this context, 110 compounds were synthesised and 2 novel patentable series were identified. We are currently exploring the potential of these compounds on the basis of their off-target selectivity and drug-like properties. Some of these compounds have demonstrated potent biochemical and cellular activities (β-catenin), although the preliminary pharmacokinetic profiling of selected compounds has demonstrated high clearance and low bioavailability. The optimisation of these deficiencies is currently underway.

Kinase X

The Kinase X project is the result of an exploratory project carried out in collaboration with B. Lambrecht from the VIB Inflammation Research Centre, UGent (Belgium). We implemented a hit-generation strategy to complement the hit-finding High-Throughput Screening (HTS) campaign. This strategy is based on literature and database searches for molecules with off-target Kinase X activity, as a way to generate additional hits for the project. Some of these molecules, including analogues with small chemical modifications, have been synthesised for screening purposes. One of them has shown inhibitory activity in the low nanomolar range. These results provide a strong basis for the continuation of this project into the subsequent hit-to-lead (HtL) phase.

MUS81

We performed a virtual screening against the catalytic site of nuclease Mus81. The results from this screening are currently being analysed by ETP.

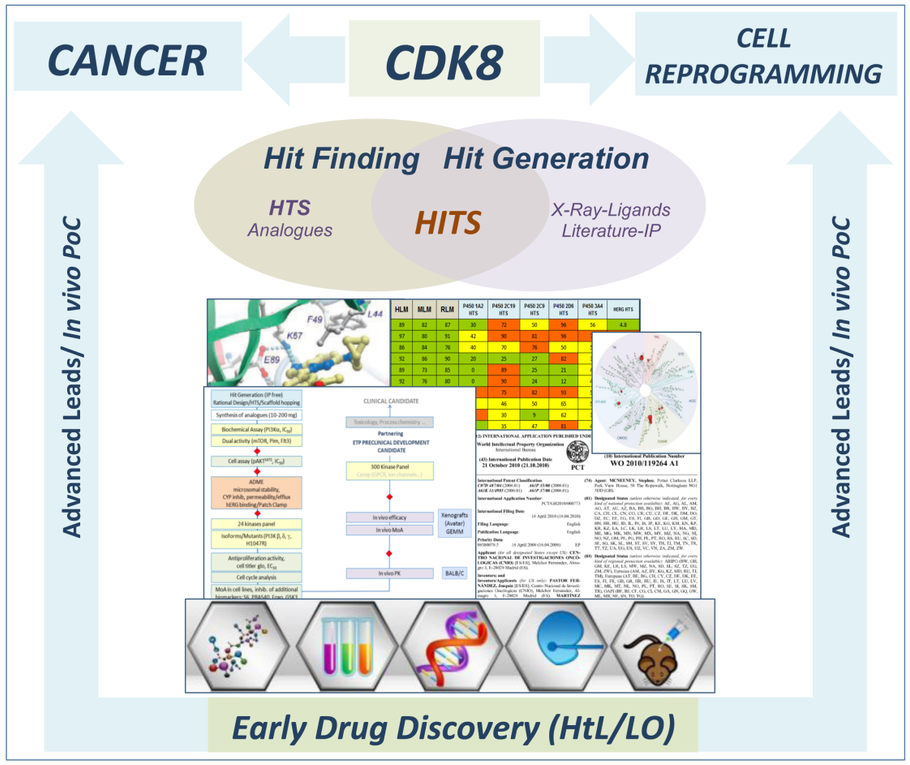
PI3K inhibitors

PI3Ki ETP-444 was previously developed as an anticancer compound. This CNIO molecule has also demonstrated excellent properties as a potential anti-obesity agent (in a joint Early-Drug Discovery project with Manuel Serrano from the CNIO Tumour Suppression Group). The ETP-Medicinal Chemistry Section continues to collaborate in the development of ETP-444 for this indication by providing the amount necessary for *in vivo* evaluation; this corresponds to the synthesis of >15 g for *in vivo* studies in mice (Tumour Suppression Group) and monkeys (NIH).

Our Section also provides support to several CNIO Groups:

- → The Tumour Suppression Group: for the selection of various CDK8 inhibitors for use in studies of pluripotency in embryonic stem cells; the synthesis of 2 CDK9 inhibitors on a milligram scale – ETP-799 has been identified as a good tool compound for such studies; and the re-synthesis of one hit obtained from nuclear disruption screening.
- → The Genes Development and Disease Group: for the multi-gram scale synthesis of Tasquinimod for *in vitro*/*in vivo* studies of psoriasis and its applications.

Figure CDK8 inhibitors are not only of interest for cancer therapy, but also for cell reprogramming and regenerative medicine applications (Manuel Serrano, CNIO Tumour Suppression Group). After a *Hit Finding* campaign, supplemented with a search for analogues, we obtained several hits. To complement this work, we designed potential CDK8 inhibitors by *Hit Generation*, taking into account structural and ligand information, as well as the intellectual property analysis. The *Hit-to-Lead* exploration of the confirmed hits is ongoing. Our objective is to progress smoothly into the *Lead Optimisation* phase and to obtain advanced CNIO molecules that will serve to demonstrate *in vivo proof-of-concept* for both applications.



- → The Brain Tumours Group: for the synthesis of several ETP-ATR inhibitors, in milligram quantities, for studies carried out in cells. We synthesised the advanced ATR inhibitor, ETP142 (1g), for *in vivo* studies in glioblastoma tumour models.
- → The Cell Division and Cancer Group: for the synthesis of a reference Haspin inhibitor.

ETP-CNIO also collaborates with external groups and institutions. In 2013, we collaborated with J.M. Rojo from the Molecular and Cellular Medicine Department (*CIB-CSIC*, Madrid), providing PI3K inhibitors for inflammation and autoimmune disease studies. ■

PUBLICATION

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PATENTS

- Pastor J, Martinez S, Martin Hernando JI, Rodriguez – Hergueta A, Rico Ferreira MR, Blanco-Aparicio C (2013). Tricyclic compounds for use as kinase inhibitors. *WO2013004984*.
- Pastor J, Fernandez- Capetillo O, Martinez S, Blanco Aparicio C, Rico Ferreira MR, Toledo LI, Rodríguez Arístegui S, Murga M, Varela C, López Contreras AJ, Renner O, Nieto M. Chemical Entities. *EP13382089.4*.

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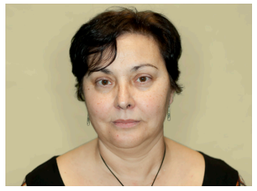
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OVERVIEW

The aim of the Biology Section is to characterise, at the biochemical, cellular, and *in vitro*/*in vivo* pharmacological levels, the compounds synthesised by the Experimental Therapeutics Programme (ETP) in order to obtain novel anticancer agents.

After completion of the target identification/validation step by CNIO's researchers, we start the early drug discovery process. Our Section is involved in the hit identification/generation phase of discovery, running screening campaigns using a High Throughput Screening (HTS) platform with diverse ETP-libraries. We develop and run biochemical and cell-based assays. Once hits have been identified and selected after multi-factorial analysis, we start the hit-to-lead (HtL) phase; in-house *in vitro* ADME (Absorption, Distribution, Metabolism, and Excretion) and off-target selectivity assays are incorporated into its flowchart.

If the compounds progress to the lead optimisation phase, they then enter the *in vivo* characterisation step of the flowchart. The molecules first undergo preclinical formulation assays in order to perform pharmacokinetic studies. Compounds with good oral bioavailability will be assessed by tolerance and Pharmacokinetic/Pharmacodynamic (PK/PD) experiments in order to determine their pharmacological effects and relate these to their mechanism(s) of action. Only the molecules that have successfully passed the previous assays are selected for the efficacy assessment. This step evaluates the antitumour activity of our compounds as single agents, or in combination with standard-of-care treatments, or with novel targeted therapies. The evaluation of our leads is carried out using appropriate tumour models for each particular case; namely, xenografts, allografts, genetically engineered mouse models (GEMMs), and Avatar mouse models.

During 2013, we focused on the development or exploration of dual Proviral Insertion site in Moloney murine leukaemia virus (PIM)/phosphatidylinositol-3 kinase (PI3K), ataxia telangiectasia and Rad3-related (ATR), cyclin-dependent protein kinase 8 (CDK8), microtubule-associated serine/threonine-protein kinase-like (MASTL), and kinase X inhibitors.

"After providing *in vivo* proof-of-concept with our advanced PIM, PIM/PI3K and ATR lead compounds, and thus demonstrating the potential benefit of these molecules to treat cancer, we have entered into license agreements with two major companies for their further clinical development —*Inflection Biosciences* for the PIM and PIM/PI3K molecules, and Merck Serono for the ATR inhibitors."

RESEARCH HIGHLIGHTS

During 2013, our Section completed the characterisation of lead compounds from our Early Drug Discovery projects (PIM, dual PIM-PI3K and ATR), the CDK8 target project was reactivated, and we started to work on other Exploratory projects (MASTL, kinase X, telomeric repeat binding factor 1 (TRF1), nucleolar disruption and gluconeogenesis).

PIM, PIM-PI3K-mammalian target of rapamycin (mTOR)

We finished the *in vivo* characterisation of lead PIM inhibitors (PIMi). Dual PIM-PI3K and triple PIM-PI3K-mTOR inhibitors were also evaluated *in vitro* (FIGURE 1) and *in vivo*, showing a strong apoptotic activity when compared with selective PIMi and PI3K inhibitors (PI3Ki).

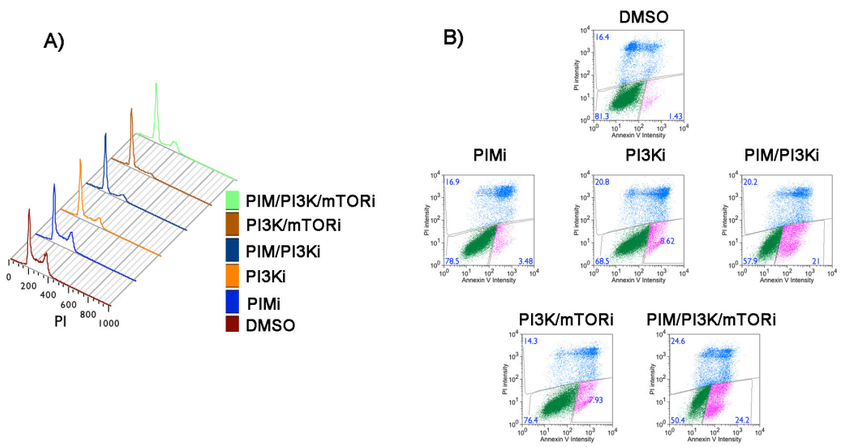

Figure 1 Characterisation of advanced ETP-PIM/PI3Ki in MV4:11 cells treated 24h at 1μM. Comparison of (**A**) cell cycle and (**B**) Annexin V staining between inhibitors with different profiles: PIM , PI3K, PI3K/mTOR selective vs. dual and triple, PIM/PI3K and PIM/PI3K/mTOR inhibitors, respectively.

ATR

Lead compounds from a second series of ATR inhibitors (ATRi) were characterised in *vitro* (FIGURE 2) and *in vivo*, in Burkitt's lymphoma cell lines and tumour models; our results demonstrated a potent apoptotic profile.

Cdk8

Cdk8 is a member of the cyclin-dependent kinase family that regulates transcription through RNA polimerase II. CDK8 was identified as an oncoprotein that promotes proliferation of colorectal cancer cells; its activity is required for the expression of β-catenin target genes. To evaluate the potential of Cdk8 inhibitors, we performed a binding assay and a β-catenin-dependent transcriptional activity assay. We tested 271 compounds in the biochemical assay, 48 of them were also tested in the cell-based assay. Fifteen compounds that confirmed both biochemical and cellular activity were further evaluated for selectivity profiles, ADME properties and preliminary pharmacokinetics. Selectivity or ADME-related issues detected at this level are currently undergoing optimisation.

Moreover, we tested an ETP-acquired collection of FDA-approved drugs from Johns Hopkins University (USA) against Cdk8. We obtained a hit rate of 0.9 in a single-point assay. Ten hits showed an IC_{50} < 300nM, but none of them had any detectable cellular activity. Nonetheless, these hits could serve as starting points for further development of cell active Cdk8 inhibitors.

MASTL

This project, similar to the ATR inhibition project, is the result of a collaborative project with the CNIO Cell Division and Cancer (CDC) Group, and Macromolecular Crystallography (MC) Group. MASTL inhibition reactivates protein phosphatase 2 (PP2A) and prevents mitosis; it is considered as an attractive therapeutic intervention point in several human tumours. We adapted a cell-based assay to identify MASTL inhibitors – developed by the CDC-Group– to a format suitable for HTS. Furthermore, we developed a custom software application to systematically analyse the data coming from the High Content Screening (HCS) assay. We screened a diverse ETP-library of 5K compounds, as a continuation of the initial screening of 640 compounds carried out by the CDC-lab. The hit rate obtained was 1.1, based on a 40% inhibition cut-off in the single-point assay. Five hits were confirmed in dose response experiments, with EC_{50} values that ranged between 5 and 0.5 μM.

To further validate these hits, the biochemical inhibition of MASTL needs to be demonstrated with the purified protein. ETP is currently collaborating with the MC-Group on the validation of these hits.

Kinase X

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 B. Lambrecht from the VIB Inflammation Research Centre, UGent (Belgium). We set up and validated the 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 medium to low nanomolar activity range; these are considered as being interesting starting points for HtL.

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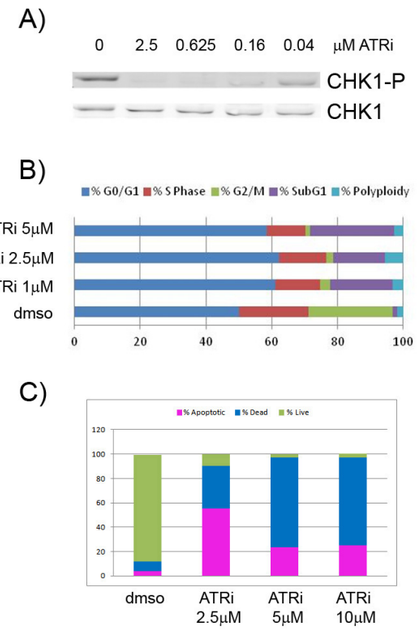
- Narlik-Grassow M, Blanco-Aparicio C, Carnero A (2013). The PIM Family of Serine/Threonine Kinases in Cancer. *Med Res Rev* 34, 136-159.
- Martin-Sanchez E, Rodriguez-Pinilla SM, Sanchez-Beato M, Lombardia L, Dominguez-Gonzalez B, Romero D, Odqvist L, Garcia-Sanz P, Wozniak MB, Kurz G, Blanco C, Mollejo M, Alves FJ, Menarguez J, Gonzalez-Palacios F, Rodriguez-Peralto JL, Ortiz-Romero PL, Garcia JF, Bischoff JR, Piris MA (2013). Simultaneous inhibition of pan-phosphatidylinositol-3-kinases and MEK as a potential therapeutic strategy in peripheral T-cell lymphomas. *Haematologica* 98, 57-64.
- Blanco-Aparicio C, Carnero A (2013). Pim kinases in cancer: diagnostic, prognostic and treatment opportunities. *Biochem Pharmacol* 85, 623-643.
- Narlik-Grassow M, Blanco-Aparicio C, Cecilia Y, Perez M, Muñoz-Galvan S, Cañamero M, Renner O, Carnero A (2013). Conditional transgenic expression of PIM1 kinase in prostate induces inflammation-dependent neoplasia. *PLoS One* 8, e60277.
- Granda TG, Cebrián D, Martínez S, Anguita PV, López EC, Link W, Merino T, Pastor J, Serelde BG, Peregrina S, Palacios I, Albarran MI, Cebriá A, Lorenzo M, Alonso P, Fominaya J, López AR, Bischoff JR (2013). Biological characterization of ETP-46321 a selective and efficacious inhibitor of phosphoinositide-3-kinases. *Invest New Drug* 31, 66-76.

Figure 2 *In vitro* characterisation of lead ETP-ATRi in Namalwa cells. (**A**) Inhibition of phosphorylation of CHK1 by ATRi, (**B**) cell cycle analysis, and (**C**) evaluation of apoptosis by Annexin V staining, after 24h treatment.



TRF1

We provided technical and scientific support to M. Méndez, from the CNIO Telomeres and Telomerase Group, to set up and run a cellular screening assay in order to search for inhibitors of TRF1 activity with a diverse ETP-library of 640 compounds. We identified several hits that are currently under characterisation.

Nucleolar disruption and Gluconeogenesis

The CNIO Tumour Suppressor Group, led by Manuel Serrano, has developed 2 assays for searching nuclear disruption agents and gluconeogenesis inhibitors. The ETP-Biology Section provided support to the Group by setting up and carrying out the screenings, as well as within the interpretation of results obtained with the diverse ETP-640 and FDA-approved drug libraries. The identified hits are under characterisation/study.

Support to other CNIO groups

We gave support to 2 Clinical Research Groups (the Gastrointestinal Cancer Clinical Research Unit and the Breast Cancer Junior Clinical Research Unit) by measuring – with liquid chromatography-tandem mass spectrometry (LC-MS/MS) – the levels of several standard-of-care drugs in tumour and host-mouse plasma samples from different mouse models of cancer. ■

PATENTS

- Pastor J, Martinez S, Martin Hernando JI, Rodriguez – Hergueta A, Rico Ferreira MR, Blanco-Aparicio C (2013). Tricyclic compounds for use as kinase inhibitors. *WO2013004984*.
- Pastor J, Fernandez- Capetillo O, Martinez S, Blanco Aparicio C, Rico Ferreira MR, Toledo LI, Rodríguez Arístegui S, Murga M, Varela C, López Contreras AJ, Renner O, Nieto M. Chemical Entities. *EP13382089.4*

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SCOPE OF THE CNIO - ELI LILLY PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer metabolism. The CNIO-Lilly Cell Signalling Therapies Section funded through a research contract with Eli Lilly focuses on the identification of small and low 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. For this purpose the Section is developing a series of biochemical and cell-based assays, exploiting advanced techniques such an NMR and metabolomics. Each drug target goes through an *in vivo* validation process that includes the use of non-invasive *in vivo* imaging technologies and animal models developed at the CNIO.

"An altered metabolic programme in tumour cells may be at the root of the malignant transformation process. We are using a combination of state-of-the-art *in vitro* and *in vivo* approaches to obtain a complete characterisation of the metabolic status of tumours."

SCIENTIFIC CONTEXT

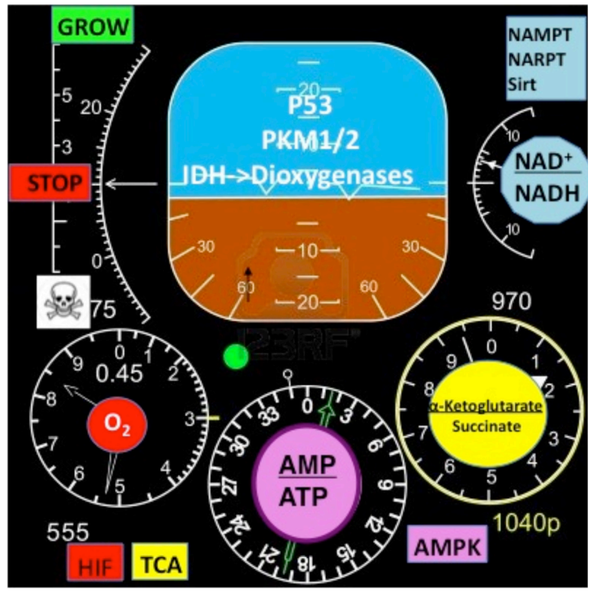

Figure The regulation of cell metabolism is a complex, fine- tuned process. The cartoon depicts some of the key metabolic regulatory switches.

The observation of an altered metabolic state in cancer cells dates back to the early 20th century when Otto Warburg observed that cancer cells preferentially utilise glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels; a phenomenon known as the "Warburg effect". Warburg, who was awarded the Nobel Prize in 1931, argued that this altered metabolic state was the underlying cause for cancer. The preferential use of glucose by tumour cells has been exploited clinically to image cancer through the utilisation of ^{18}F-fluoro-deoxyglucose-positron emission tomography (^{18}F-FDG-PET). The molecular mechanisms driving the glycolytic phenotype have only recently begun to be understood in light of the results from large-scale genome and metabolomic profiling studies. Genome sequencing studies have uncovered somatic mutations in metabolic enzymes including succinate dehydrogenase (SDH), fumarate hydratase (FH), isocitrate dehydrogenase (IDH) and phosphoglycerate dehydrogenase (PHGDH). In addition to mutations in metabolic enzymes, recent studies have shown that many oncogenes, including Myc and Ras, impart an altered metabolic phenotype in cancer cells by regulating the expression of genes involved in glycolysis, fatty acid metabolism and oxidative phosphorylation.

Cellular metabolism is a finely tuned process (FIGURE); tumours may rely heavily on specific metabolic pathways to obtain their energy while using other pathways to grow. This situation may leave tumour cells in a frail position – particularly when exposed to specific treatments or under certain circumstances – while normal cells may be able to compensate. Together, these recent insights into the mechanisms of metabolic pathways relevant to cancer, open up novel avenues to the development of therapeutics that target key enzymes in tumour metabolism, such as the pyruvate kinase isoenzyme PKM2 (this work was presented this year at the Keystone Symposia meeting on "Tumor Metabolism" in Colorado, USA) ■

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Jacinto Sarmentero ESP

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. 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 (FIGURE).

"Our goal is to identify epigenetic events that contribute to tumourigenesis and that might be susceptible to modulation by therapeutic agents. Targeting the appropriate epigenetic effectors can help to restore the proper patterns of gene expression in cancer cells and to revert the cancer phenotype"

SCIENTIFIC CONTEXT

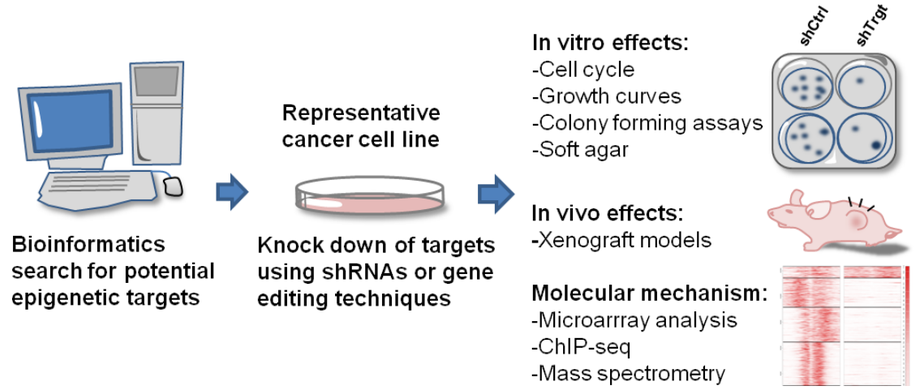


Figure *In vivo* and *in vitro* strategies for target validation.

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. DNA and histone modifications ultimately control the level of chromatin condensation, which in turn regulates the accessibility of transcription factors to the chromatin and, therefore, gene expression.

Over the last few years several studies, including our own, have suggested that 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 by targeting the appropriate epigenetic regulators. Indeed, drugs that target DNA methyltransferases and histone deacetylases have successfully demonstrated anticancer properties and are currently being used in the clinic. Therefore, identifying the molecular function of critical epigenetic regulators and their complex relationship with the cancer epigenome, in addition to the development of small molecular inhibitors of their activities, holds great promise for cancer therapy. ■

PUBLICATIONS AT OTHER INSTITUTIONS

- Barrero MJ, Izpisua Belmonte JC (2013). Polycomb complex recruitment in pluripotent stem cells. *Nat Cell Biol* 15, 348-350.
- Montero-Conde C, Ruiz-Llorente S, Dominguez JM, Knauf JA, Viale A, Sherman EJ, Ryder M, Ghossein RA, Rosen N, Fagin JA (2013). Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. *Cancer Discov* 3, 520-533.
- Marti M, Montserrat N, Pardo C, Mulero L, Miquel-Serra L, Rodrigues AM, Andrés Vaquero J, Kuebler B, Morera C, Barrero MJ, Izpisua Belmonte JC (2013). Mcad-mediated intercellular interactions activate satellite cell division. *J Cell Sci* 126, 5116-5131.
- Sesé B, Barrero MJ, Fabregat MC, Sander V, Izpisua Belmonte JC (2013). SMYD2 is induced during cell differentiation and participates in early development. *Int J Dev Biol* 57, 357-364.
- Barrero MJ, Sese B, Martí M, Izpisua Belmonte JC (2013). Macro histone variants are critical for the differentiation of human pluripotent cells. *J Biol Chem* 288, 16110-16116.
- Barrero MJ, Sese B, Kuebler B, Bilic J, Boue S, Martí M, Izpisua Belmonte JC (2013) Macrohistone variants preserve cell identity by preventing the gain of H3K4me2 during reprogramming to pluripotency. *Cell Rep* 3 , 1005-1011.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

MARISOL QUINTERO Head of Office (until October)

Anabel Sanz (since October)

In direct partnership with CNIO's Faculty, the main activity of CNIO's Technology Transfer and Valorisation Office (CNIO-TTO) is the commercial exploitation of research results obtained at the CNIO through the submission of patents, collaboration with industry and the creation of innovative companies. To this end, CNIO-TTO promotes, coordinates and manages the relationships of the researchers with the companies and other public and private stakeholders.

"The Technology Transfer and Valorisation Office aims to foster scientific progress for the benefit of society by translating the results of CNIO researchers into useful applications."

The main activities of the CNIO-TTO include:

- Evaluation of the commercial potential of inventions, research tools and software developments originating from CNIO`s research endeavours.
- Negotiation of material transfer, confidentiality, sponsored cooperative research and development agreements with other organisations.
- Licensing of intellectual property rights.
- Supporting and fostering a culture of innovation at CNIO.

The CNIO-TTO implements a rational intellectual property protection strategy, based on technological dossiers that address key issues concerning the patentability of results and their commercial viability. In 2013, in addition to several national and international patent application extensions, the CNIO-TTO has filed for patent protection of 3 novel inventions that have potential application in oncology diagnostics and therapeutics, as well as in stem cell and regenerative medicine. The office has also concluded 8 patent license agreements. Furthermore, the CNIO-TTO has been successful in consolidating strategic partnerships with industry, including the initiation of new joint research projects with leading companies in the healthcare sector such as F. Hoffmann-La Roche Ltd. and Eli Lilly. ■

PRIVATE SPONSORS

We would like to thank all our sponsors and donors for the generous support that we received from them in 2013. 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.

The Spanish Association Against Cancer *(Asociación Española Contra el Cáncer, AECC)*, through its Science Foundation, awards grants aimed at supporting cancer research. These grants provide support to scientists and clinicians who work intensively in the field of oncology. The CNIO scientist Eleonora Lapi was honoured this year with this award.

The ***Fundación BBVA*** generously supports the BBVA Foundation - CNIO Cancer Cell Biology Programme, headed by Professor Erwin Wagner since mid 2009. This Programme focuses on research into tumour processes, covering all aspects of tumour cell biology from the molecular level to the analysis of gene functions in normal and pathological conditions.

Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. *Fundación CRIS* supports the *CRIS* Foundation – CNIO Prostate Cancer and Genitourinary Tumours Clinical Research Unit, headed by David Olmos, since 2012. This group focuses on translating advances in prostate cancer research into improvements in patient care.

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.

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. This year, thanks to the support of *Fundación Banco Santander*, a group of young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.

The ***Fundación Marcelino Botín*** is committed to supporting scientific research and knowledge transfer from academia to the market through science programmes; this transfer is regarded as one of the main driving forces for Spain´s economic and social development. The *Fundación Marcelino Botín* collaborates with CNIO in this regard by supporting the research groups led by Manuel Serrano and Maria A. Blasco.

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 recipient of the *Jesús Serra* Foundation's Visiting Scientist Award in 2013 was Robert Benezra (from the Memorial Sloan–Kettering Cancer Center in New York).

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. This type of sponsorship allows long-term support to the investigator thanks to the annual interest obtained from the capital (2 million EUR) assigned to this AXA-CNIO Permanent Chair.

And last but not least, there are several private sector organisations that provide financial support for CNIO scientists to carry out their research through privately-funded projects. These include the ***Fundación Clinic, Fundación Ramón Areces, Fundación Mutua Madrileña,*** **Melanoma Research Alliance,** ***Fundación Sociedad Española de Oncología Médica (SEOM).***

OTHER SPONSORS

Our activities are also supported through individual donations – citizens who wish to contribute personally to the battle against cancer – as well as via external fundraising from the following local associations: ***Bayer Healthcare Pharmaceuticals, the Fundación Antoni Serra,*** **Fressia Group,** ***Miltenyi Biotec, Asociación Cultural Bombarra, Pfizer, Sogug, Fundación Banco Sabadell, the French Embassy, Grupo Español de Tumores Neuroendocrinos GETNE.***

We would also like to thank our anonymous benefactors who, with their decision to donate their legacy to support cancer research at the CNIO, have made a very meaningful contribution to the community for generations to come.

Communication

COMMUNICATION

JUAN J. GÓMEZ Communication Director (until December)

Nuria Noriega ESP

There is no doubt that generating knowledge implies an unbreakable commitment with society, one which includes the act of sharing. Our Centre is a source of value for the oncology field and communicating with media, the general public, cancer patients and policy makers is one of our central goals.

"Research works best when knowledge is shared."

The Communications Department has now been operating for two years. We are proud to be able to say that this year we have increased our media exposure by more than 75% compared with 2012 and by over 250% when compared to 2011.

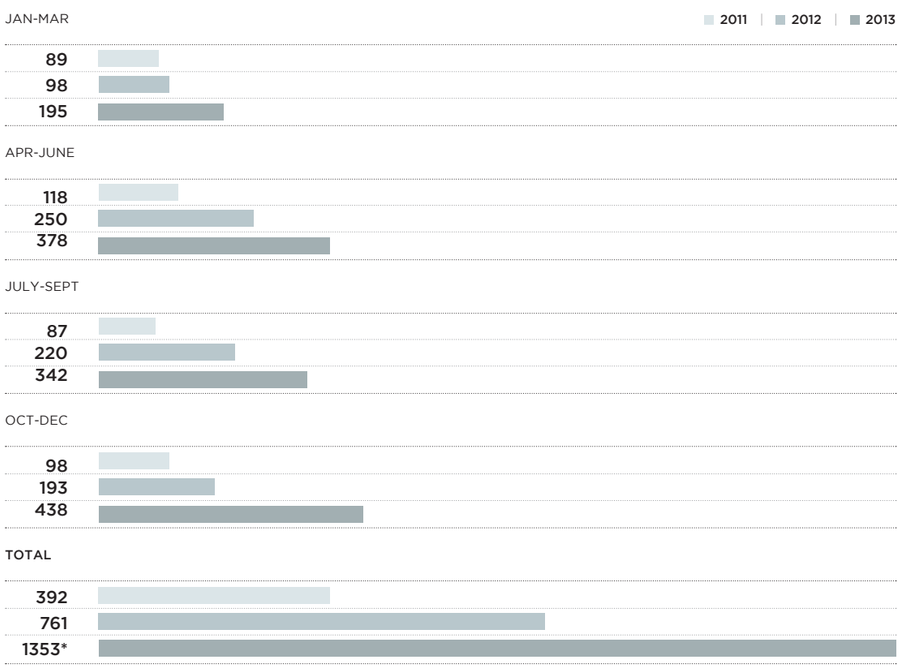
One of the top stories of the year in both the national and international media was published in a leading science journal, *Nature*, under the title "Reprogramming *in vivo* produces teratomas and IPS cells with totipotency features." This breakthrough study has appeared in over 250 digital and printed media outlets, receiving extensive radio and TV coverage and a superb impact on the international media including the BBC, The Wall Street Journal, Financial Times, Le Monde and The Telegraph, amongst many others. The story figured in all the main newspapers and TV channels from across the length and breadth of Spain. One more breakthrough was the CNIO and Merck license agreement signed at the end of the year, which was deemed a success and reflected as such by media coverage, including the prominent Wall Street Journal.

We must also bear in mind the significant increase in our visibility on social media networks, such as Twitter and YouTube, with over 4,300 Twitter followers and nearly 13,000 YouTube video views.

Moreover, during 2013 CNIO has submitted a total of 32 press releases to the global news service EurekAlert!. Along the year these stories have received over 70,000 views.

To sum up, today many more people know us, and critically, they know a lot more about us. Science research needs to be shared, which is why working hard to reach out to society is an essential part of our mission. ■

CNIO APPEARANCES IN PRINT AND DIGITAL MEDIA



PRESS CLIPPINGS

1 *La Voz de Galicia*, January 8, 2013
2 *Diario Médico*, January 31, 2013
3 *El Diario Vasco*, February 4, 2013
4 *La Razón*, February 13, 2013
5 *La Aventura del Saber*, TVE, February 25, 2013
6 *Diario Médico*, March 11, 2013
7 *Materia*, March 17, 2013
8 *ABC*, March 18, 2013
9 *Diario Médico*, April 1, 2013
10 *Diario Médico*, April 10, 2013

11 *SINC*, April 25, 2013
12 *Heraldo de Aragón*, June 4, 2013
13 *La Gaceta*, June 19, 2013
14 *El País*, June 21, 2013
15 *Diario Médico*, October 7, 2013
16 *El Mundo*, November 3, 2013
17 *El País*, November 5, 2013
18 The New York Times, November 21
19 The Wall Street Journal, December 18, 2013
20 *Cinco Días*, December 19, 2013

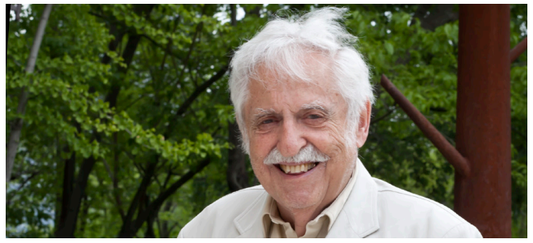
TOP STORY OF THE YEAR

1 BBC, September 11, 2013
2 *Le Monde*, September 11, 2013
3 RTVE, September 11, 2013
4 *The Thelegraph*, September 11, 2013
5 ABC, September 12, 2013
6 *El Mundo*, September 12, 2013
7 *El Periódico*, September 12, 2013
8 *La Razón*, September 12, 2013
9 *El País*, September 13, 2013
10 *Diario Médico*, December 16, 2013

INVITED GUEST SPEAKERS (Distinguished Seminar Series)

Peer Bork. January 25, 2013

Pedro Alonso. February 1, 2013

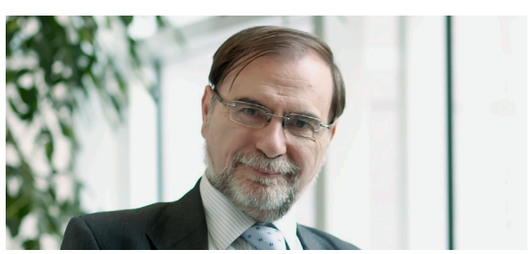


Carl Djerassi. June 7, 2013

Helen Blau. June 21, 2013

Pier Paolo Pandolfi. June 24, 2013

Juan Luis Arsuaga. September 6, 2013



José Mª Ordovás. December 13, 2013

SOCIAL EVENTS

LEFT Merck, Government and CNIO representatives during the signing of the Merck and CNIO license agreement. December 18, 2013.

BELOW CNIO participated in the 2013 World Cancer Day and organised an open-door debate about the understanding, prevention, diagnosis and treatment of cancer. February 1, 2013.

ABOVE CNIO organised, together with the renowned Nature Publishing Group, the International Symposium on Frontiers in Tumour Heterogeneity. October 27-30, 2013.

LEFT (UP) A total of 150 people outside the CNIO enjoyed Researchers' Night at the Centre. September 27, 2013.

LEFT (DOWN) The Executive Committee of the Foro Mujeres *Amistad Hispano-Francesa Diálogo* visited the CNIO to learn about breakthroughs in cancer research. September 26, 2013.

CNIO Offices

DEAN´S OFFICE

MARIA S. SOENGAS Dean for Academic Affairs

The CNIO is recognised by the relevance of its Scientific Programmes as well as by their international projection. Key to our mission is to nurture and foster the development of our scientists-in-training, in order to maximize their chances of success. Since its inception, the CNIO has dedicated particular attention to establishing agreements with various institutions that have generously funded highly competitive PhD and Postdoctoral fellowships. Also very successful are our undergraduate summer internships, as well as diverse exchange and visitor programmes, which further help to bridge the gap between basic and clinical research in oncology.

"At the CNIO we aim high: to perform 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."

Importantly, our trainees are active participants in the activities of the Dean's Office. In fact, the CNIO Student Association (CNIOSA) and Postdoc Association (CNIOPDA) are the driving forces behind an inspiring series of seminars and workshops that we hold during the year. In this context, scientific reasoning, grant writing, manuscript organisation and CV preparation are just some of the many topics covered in our curriculum. Likewise, we acknowledge that career options extend beyond the bench and therefore pay special attention to the areas of public communication, management of intellectual property, and the creation of *start ups* or *spin offs*. These activities are performed in concert with CNIO´s Training Programmes, 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 will help 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 place particular emphasis on knowledge dissemination. Members of CNIOSA and CNIOPDA have participated in various awareness-raising campaigns such as *MoviLab*, *AULA 2013* and the annual meeting of the Spanish Group of Patients with Cancer (*GEPAC*). Open doors activities such as *Researchers´Night* were also highly attended, with over 150 participants involved in hands-on experiments. We are also glad to see a growing interest in our video "CNIO for Kids"; a film that portrays the daily life at the centre in an accessible style for primary schools. Furthermore, we take pride in *EscueLab*. This is an initiative launched by CNIO´s graduate students and postdocs – that has also attracted external participants – with the aim of bringing research into middle school classrooms. In 2013, *EscueLab* received a *Think Big* award granted by the *Fundación Telefónica*, and won a "Making More Health" challenge sponsored by the *Ashoka*-Boehringer Ingelheim programme. Their scientific short film "a day in the life of an investigator" was runner-up at the Iberoamerican contest "Science in Action", and will be shown at various international events where they have been invited to present.

Finally, I would like to congratulate the winners of the "1st CNIO Award for publications by PhD students": Cristina Balbás-Martínez, Miguel Foronda and Eva Briso, first authors of papers in *Nature Medicine*, *Nature Genetics* and *Genes & Development*, respectively; and Sebastian Hassenfuss, author of two scientific articles in *Cell Metabolism* and *Hepatology*. These are just some examples of the outstanding work carried out at the CNIO by a vibrant community of young and active investigators, mentored by a committed faculty on the frontline of cancer research. ■

WOMEN IN SCIENCE OFFICE

MIRNA PÉREZ-MORENO Coordinator **MARISOL QUINTERO** Coordinator (until October)

MIrna Pérez-Moreno

Lola Martínez

Francisca Mulero

Águeda Tejera

REFERENCES

- Report on women and men in leadership positions and Gender equality strategy mid-term review. European Commission - MEMO/13/882 14/10/2013.
- She figures 2012. Gender in Research and Innovation. Statistics and Indicators. European Commission. doi 10.2777/38520.

MEMBERS OF THE CNIO-WISE WORKING GROUPS

- *Work and Life Balance*: Pablo Fernández, Diego Megías.
- *Networking and Seminars*: Maria Blasco, Nuria Noriega, Fernando Peláez, Anabel Sanz.
- *Mentorship Ad hoc Members*: Manuel Serrano, Marisol Soengas, Francisco Real, Erwin Wagner.
- Former Members: Peter Klatt (until May), Giovanna Roncador (until Sep), Nuria Malats (until Sep), Sara Alvarez (until Dec).

Working Group Coordinators
Lola Martínez, Francisca Mulero,
Águeda Tejera

Gender diversity in science is key to generate new ideas to advance scientific knowledge and it provides a greater opportunity for innovation, quality and competitiveness, with clear benefits to society. Despite many educational initiatives that have promoted equal recruitment, fostering, and retention of women, the statistics provided by the European Commission indicate that women are still under-represented at senior and decision-making positions across Europe (1,2).

Although 63% of the CNIO staff are women, most of them are either technicians or in the early stages of their professional careers, and the percentage of women drops dramatically at tenure-track and senior position levels. Hence, at the end of 2012, the CNIO created the Women in Science Office (WISE) to promote an institutional transformation wherein, after a qualitative and quantitative examination, several actions have already been taken to achieve a gender-neutral career development in science. This includes clear policies to ensure a professional environment free of unintended differences that contributes to the equal development of scientists at our institute.

“The major goal of the CNIO-WISE office is to develop effective and sustainable efforts to achieve a gender-neutral advancement in the professional ladder at our institute. This has the support of different working groups focusing on several relevant areas, including work/life balance, networking and seminars, as well as mentorship issues.”

Our specific objectives are to:

- Monitor and report on the position of male and female staff at the CNIO.
- Foster good policy practices to promote the transparency of recruitment processes and promotions, in coordination with the CNIO-Scientific Committee.
- Stimulate institutional gender awareness and networking through seminars and events, in order to create a more gender-balanced sense of community and an innovative working environment.
- Promote mentoring to mainstream gender disparities and encourage the equal advancement of individuals to senior and decision-making positions.
- Encourage the improvement of work-life balance within the CNIO, proposing initiatives and measures to address these issues.

In 2013, the CNIO-WISE office established several organisational approaches with an inclusive vision aiming to contribute to a diverse and productive scientific community in which all individuals are recognised equally for their contributions.

These activities include the following:

- An in-house CNIO survey analysis.
- The encouragement of the implementation of good policy practices in coordination with the CNIO-Scientific Committee.
- The launch of the CNIO-WISE seminar series, with the purpose of stimulating institutional gender awareness and networking. In 2013, Inés Sanchez de Madariaga – Head of the Women and Science Unit at the cabinet of the Secretary of State for Research, Development and Innovation, the Ministry of Economy and Competitiveness – inaugurated these series. In addition, the CNIO was also honoured with the visit of the Executive Committee of the *Foro Mujeres Diálogo*, from the Spanish-French Association *Diálogo*.

We believe that the implementation of these activities will have a potential impact on the scientific development of the institute and society as a whole.

Facts & Figures

COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from Spanish, European and international public funding agencies, as well as from private entities. In 2013, researchers at the CNIO were involved in 140 projects that received extramural funding. Of these 31 were international collaborative projects – 4 of which are coordinated by the CNIO – and 27 were collaborative projects with other groups in Spain. The international collaborative projects were funded by institutions, such as the European Commission through the 7th Framework Programme, The Melanoma Research Alliance 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 2013, 23 of these projects received international funding and 59 received national funding. Individual research projects are also funded by the European Research Council (ERC) advanced and starting grants, the NIH, the European Commission ("People" Programme), the Association for International Cancer Research (AICR), US National Institutes of Health (NIH), the European Science Foundation (ESF), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

AXA RESEARCH FUND

PRINCIPAL INVESTIGATORS	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

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 TITLES
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 INVESTIGATORS	PROJECT TITLES
Blasco, Maria A.	MARK-AGE: European study to establish biomarkers of human ageing
Benítez, Javier	COGS: Collaborative oncological gene-environment study
Heeschen, Christopher	MULTIFUN: Multifunctional nanotechnology for selective detection and treatment of cancer
Malumbres, Marcos	MitoSys : Systems biology of mitosis
Valencia, Alfonso	A BLUEPRINT of haematopoietic epigenomes
Valencia, Alfonso	ASSET: Analysing and striking the sensitivities of embrional tumours
Valencia, Alfonso	MICROME: The microme project: a knowledge-based bioinformatics framework for microbial pathways genomics
Valencia, Alfonso	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Fernández-Capetillo, Óscar	ITN aDDRess: Joint training and research network on chromatin dynamics and the DNA damage response
Losada, Ana	ITN Nucleosome4D: Nucleosome structure & function across biological scales and biological function

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 INVESTIGATORS	PROJECT TITLES
Barbacid, Mariano	LUNGTARGET: New approaches for the targeted therapy of non-small cell lung cancer
Blasco, Maria A.	EuroBATS : Identifying biomarkers of ageing using whole transcriptomic sequencing
Heeschen, Christopher Real, Francisco X.	EPC-TM-Net: Targeting the tumour microenvironment to improve pancreatic cancer prognosis
Heeschen, Christopher	CAM-PaC: Integrative analysis of gene functions in cellular and animal models of pancreatic cancer
Malats, Núria Real, Francisco X. (coordinator)	CANCERALIA: Development of novel diagnostic and therapeutic approaches to improve patient outcome in lung and pancreatic tumours
Malats, Núria	TransBioBC: Translation of novel biomarkers for bladder cancer for clinical outcome prediction
Malats, Núria	UROMOL : Prediction of bladder cancer disease course using risk scores that combine molecular and clinical risk factors
Robledo, Mercedes	ENS@T- CANCER: European network for the study of adrenal tumours-structuring clinical research on adrenal cancers in adults

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (MIT)

M+VISION

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Hidalgo, Manuel	Team Albumin: Monitoring of stroma-targeting pancreatic cancer treatments with MRI
Mulero, Francisca	Team PET: Improved molecular imaging by multi-tracer PET
Olmos, David	Team Cell: Development and testing of a biological use case of technology-rare cell detection
Soengas, María S.	Team TxResponse: Early assessment of treatment response in advanced melanoma patients

MELANOMA RESEARCH ALLIANCE (MRA)

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

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Malats, Núria	Bladder cancer risk and genomic alterations
Valencia, Alfonso	GENCODE: Integrated human genome annotation: generation of a reference gene set

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH (AICR)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Djouder, Nabil	Defining the oncogenicity of URI in hepatocellular carcinoma (HCC) development
Fernández-Capetillo, Óscar	Exploiting oncogene-induced replicative stress for the selective killing of cancer cells
Malats, Núria	Bladder cancer risk: The role of trace metals and oxidative stress
Pérez-Moreno, Mirna	Role of p120 catenin in inflammatory skin cancer development
Soengas, María S.	Biosensor and response indicators in dsRNA-based anti-melanoma therapy
Wagner, Erwin F.	Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis

BAYER HC

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	Programme: "Grants for Targets": Inhibiting mitosis and restoring PP2A by targeting Mastl, a new kinase for cancer therapy

EUROPEAN COMMISSION FRAMEWORK PROGRAMMES

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Al-Shahrour, Fátima	PERSMEDOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy
Fernández-Capetillo, Óscar	HISTONEDDR: Role of histone modifications in DNA damage response in mammals
Gervasio, Francesco L.	DmoNickaseDesign: Safer gene repair and targeting based on the monomeric meganuclease I-DmoI by design of homologous-recombination-inducing nickase activity
Malumbres, Marcos	Mastl CDC: Role of the protein kinase Mastl in cell division and cancer
Montoya, Guillermo	SMARTBREAKER: Rational designing of new meganucleases as molecular scissors for genomic tailoring
Real, Francisco X.	PANCLON: Clonal analysis in pancreatic development, differentiation and carcinogenesis

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Barbacid, Mariano	ERC Advanced Grant RAS AHEAD: Ras genes in health and disease
Blasco, Maria A.	ERC Advanced Grant TEL STEM CELL: From telomere chromatin to stem cell biology
Fernández-Capetillo, Óscar	ERC Starting grant CHROMOREPAIR: Genome maintenance in the context of chromatin
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

EUROPEAN FOUNDATION FOR THE STUDY OF DIABETES (EFSD)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Role of URI in obesity/type 2 diabetes-mediated hepatic metabolic dysfunctions

HOWARD HUGHES MEDICAL INSTITUTE (HHMI)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	Exploring the role of replicative stress in cancer and ageing

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Hidalgo, Manuel	Tailoring new drugs in pancreatic cancer
Soengas, María S.	The unfolded protein response in melanoma progression and chemoresistance

NATIONAL GRANTS COLLABORATIVE PROJECTS

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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 Gervasio, Francesco L. 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: Interactomica 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)*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Benítez, Javier	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Cigudosa, Juan C.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Malats, Núria	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Morente, Manuel M. (coordinator)	*RETIC Biobancos*
Real, Francisco X.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Valencia, Alfonso	*Red Temática de Investigación Cooperativa en Biomedicina Computacional (COMBIOMED)*

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Gómez, Carlos Jesús	Chemosensitivity profiles for the personalized therapy of advanced colorectal cancer
Hidalgo, Manuel	Personalized treatment for pancreatic cancer patients

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Benítez, Javier	*Proyecto INNPRONTA LIFE: Desafío integral al cáncer de mama*
Cigudosa, Juan C.	*Proyecto INNPACTO PROCARDIO: Desarrollo de tecnologías avanzadas de producción y validación de un producto celular alogénico para el tratamiento de la enfermedad cardiovascular*
Heeschen, Christopher	*Proyecto FCCI: Nanopartículas multifuncionales para tratamiento dirigido e imagen in vivo de células troncales tumorales*
Hidalgo, Manuel	*Proyecto INNPACTO ORALBEADS: Desarrollo de dispersiones sólidas micro/nanoestructuradas para administración oral de compuestos marinos*
Liébanes, María Dolores	*Programa EUROCIENCIA: Plan Estratégico de participación en el 7º Programa Marco*
Losada, Ana Méndez, Juan	*Programa CONSOLIDER INESGEN: Inestabilidad genómica*
Soengas, María S.	*Programa CONSOLIDER RNAREG: Una aproximación integrada a la regulación post-transcripcional de la expresión génica y su papel en enfermedad*
Soengas, María S.	*Proyecto INNPACTO: Nanocomplejos de ARN sintético como nueva terapia contra cánceres agresivos para los que no se dispone de tratamiento efectivo*
Valencia, Alfonso	*Programa CONSOLIDER SYEC: Supercomputación y eCiencia*

CENTRE FOR INDUSTRIAL TECHNOLOGICAL DEVELOPMENT / *CENTRO PARA EL DESARROLLO TECNOLÓGICO INDUSTRIAL (CDTI)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Mulero, Francisca	*Programa Cenit (AMIT): Tecnologías de imagen molecular avanzada*

NATIONAL GRANTS INDIVIDUAL PROJECTS

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	*Premio Miguel Catalán 2008 de la Comunidad de Madrid. Modalidad: "Jóvenes investigadores menores de 40 años"*

SPANISH GROUP ON 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 masivow*

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

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Álvarez, Sara	Identification of biomarkers that predicts the clinical response to DNA hypomethylating therapies on myelodisplastic syndromes
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
Benítez, Javier	Identification and analysis of risk factors in breast and ovarian cancer families (INTRASALUD)
Cascón, Alberto	Exome sequencing of trios, mother-father-proband, in pediatric patients with multiple pheochromocytomas/paragangliomas
Cigudosa, Juan C.	Human stem cell models with inducible chromosome translocations: Integraded genomic and biological study of the t(7;11)(p15;p15), its fusion gen *NUP98-HOXA9* and its leukeamic effects (INTRASALUD)
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
Colomer, Ramón	Structural destabilisation for breast cancer oncogenes addition due to the fatty acid synthase action
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
Guerra, Carmen	Preventative and therapeutic strategies in noonan, costello and cardio facio cutaneous syndromes
Heeschen, Christopher	Development of novel therapeutic strategies for the targeted elimination of metastatic cancer stem cells
Heeschen, Christopher	Molecular characterization of cancer (Stem) cells for the development of novel targeted treatments modalities
Hidalgo, Manuel	Targeting Pancreatic Cancer Stroma
Malats, Núria	Aetiology of pancreas cancer: Application of “omics” technologies in the assessment of risk factors
Milne, Roger L.	FGF Receptors and breast cancer susceptibility: an analysis of main effects, gene-gene and gene environment interactions at an international level
Quintela-Fandino, Miguel	Development of an integrated translational platform for the study of predictive factors and resistance mechanism for antiangiogenic drugs in early breast cancer
Robledo, Mercedes	Use of massive analysis platforms on endocrine tumours studies: from OMICS to patients
Urioste, Miguel	Research of the tumorigenic pathways involved in familial colorectal cancer type X. Analysis of: 1) specific genes; 2) methylation patterns; 3) telomeric dysfunction and 4) expression of proteins with a relevant role in colorectal tumorigenesis

LILLY FOUNDATION / *FUNDACIÓN LILLY*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	Lilly Foundation Award for Preclinical Research

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

COMPLEMENTARY ACTIONS / *ACCIONES COMPLEMENTARIAS*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Real, Francisco X.	*Genómica del Cáncer de vejiga urinaria: validación a gran escala de nuevos candidatos*

NATIONAL PROGRAMME FOR BASIC RESEARCH PROJECTS / *PROGRAMA NACIONAL DE PROYECTOS DE INVESTIGACIÓN FUNDAMENTAL*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Barbacid, Mariano	Inhibition of oncogenic K-Ras signaling in cancer
Blasco, Maria A.	Mammalian telomeres and telomerase: from chromatin structure to stem cell biology
Djouder, Nabil	Decoding the URI role in the hepatocellular carcinoma (HCC) development
Fernández-Capetillo, Óscar	Exploring the role of replication stress in cancer and ageing
Gervasio, Francesco L.	An experimentally validated computational approach to study protein conformational plasticity and its alteration
Guinea-Viniegra, Juan	Investigating the role of microRNA21/TIMP-3/TACE in psoriasis - evaluating the potential therapeutic implications
Heeschen, Christopher	Vascular regeneration in the 21st century – definition of the optimal cell therapy protocol for patients with cardiovascular diseases
Lietha, Daniel	From the molecular study of growth signaling and cellular adhesion to the drug discovery
Losada, Ana	Animal models for the study of cohesin functions
Malumbres, Marcos	Physiological and therapeutic relevance of mitotic kinases and phosphatases
Méndez, Juan	MCM complex functions in the DNA replication and the genetic stability
Montoya, Guillermo	Structural biology of macromolecular machines involved in chromosome dynamics
Ortega, Sagrario	New murine genetic models for the angiogenesis and lynphangiogenesis in tumours
Osorio, Ana	Implications of the type of germline mutation in the prognosis and treatment of patients with hereditary breast cancer carrying mutations in the *BRCA1* gene
Pérez de Castro, Ignacio	Aurora A, *in vivo* essential functions, anti-tumoral target validation and identification of new regulatory mechanisms
Pérez-Moreno, Mirna	Intercellular crosstalk in skin physiology and disease

Ramón-Campos, Santiago	Structural determination of the architecture of CAD, an antitumoral target that controls the biosynthesis of pyrimidines
Real, Francisco X.	Pancreatic adenocarcinoma: Role of the acinar and ductal components and development of animal models
Rodríguez, Cristina	Identification of markers predictive of paclitaxel severe neurotoxicity using genome-wide platforms
Serrano, Manuel	New mouse models for the study of cancer and aging
Soengas, María S.	Cellular stress in melanoma progression and chemoresistance
Valencia, Alfonso	Gene group functions
Valencia, Alfonso	Development of biocomputing systems and subjacent computational methods for the analysis of oncologic personalised therapies
Wagner, Erwin F.	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"*

SUB-PROGRAMME OF SUPPORT TO TECHNOLOGY TRANSFER CAPACITIES IN RESEARCH CENTRES/ *SUBPROGRAMA DE APOYO A LA FUNCIÓN TRANSFERENCIA EN CENTROS DE INVESTIGACIÓN (INNCIDE)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Quintero, Marisol	*Proyecto INNCIDE-CNIO para favorecer la creación de valor económico de los conocimientos derivados de los descubrimientos científicos y de los resultados de investigación y desarrollo del CNIO*

MUTUA MADRILEÑA FOUNDATION / *FUNDACIÓN MUTUA MADRILEÑA*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Rodríguez, Cristina	*Estudio de microRNAs como predictores de respuesta a Sunitinib en pacientes con carcinoma renal*
Sánchez-Carbayo, Marta	*Perfiles epigenéticos como marcadores diagnósticos y pronóstico en cáncer de vejiga*
Guinea-Viniegra, Juan	*JunB/AP-1, supresor tumoral en la piel. Mecanismos moleculares e interacción funcional con p53*

RAMON ARECES FOUNDATION / *FUNDACIÓN RAMÓN ARECES*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
Guerra, Carmen	*Implicación de los oncogenes RAS en el desarrollo de los síndromes Costello y Noonan*
Malumbres, Marcos	*Base genética y celular del síndrome de microdeleción 16p11.2-p12.2 y de los trastornos neurales relacionados*
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 ASSOCIATION AGAINST CANCER / *ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González-Neira, Anna	*Farmacogenética en tumores infantiles*

SPANISH ONCOLOGY GENITOURINARY GROUP / *GRUPO ESPAÑOL DE ONCOLOGÍA GENITOURINARIA (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 SOCIETY OF MEDICAL ONCOLOGY / *SOCIEDAD ESPAÑOLA DE ONCOLOGÍA MÉDICA (SEOM)*

PRINCIPAL INVESTIGATORS	PROJECT TITLES
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*
Quintela-Fandino, Miguel	*Validación de una estrategia novedosa en la identificación de genes implicados en la evolución de cáncer de mama hormonopositivo*

EDUCATION & TRAINING PROGRAMMES

The CNIO is highly dedicated to providing an excellent training environment for the next generation of researchers and professionals in the health sector at all career levels. To achieve this goal, the CNIO collaborates with public and private academic institutions all over the country. In 2013, the CNIO has established new collaborations with academia through new collaborative agreements with 3 Spanish Universities: *Universidad Autónoma de Madrid, Universidad de Alicante* and *Universidad Rey Juan Carlos*. Other collaborative agreements currently exist with 6 Spanish Universities: *Universidad de Alcalá de Henares, Universidad CEU San-Pablo, Universidad Complutense de Madrid, Universidad de Lleida, Universidad Politécnica de Madrid* and *Universidad Pompeu Fabra*.

TRAINING PROGRAMMES

	2008	2009	2010	2011	2012	2013
Training of PhD students	144	133	132	123	121	116
Post-doctoral training	69	75	95	83	81	67
Training for MDs	18	24	25	20	16	21
Laboratory training for MSc/BSc students	31	39	54	46	42	36
Laboratory training for technicians	27	22	29	26	26	19
Master's Degree in Molecular Oncology	35	36	26	37	37	37

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 2 years of study in the biomedical field. The programme encompasses 8 weeks of full-time laboratory training (312 hours). During this time the students actively participate in research projects in one of the CNIO groups. During 2013, 8 students from 5 countries participated in this programme.

→ Students can also apply for laboratory training throughout the academic year by directly contacting the heads of CNIO individual Research Groups or Units. This year, 36 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, 13 students obtained their PhD degrees in 2013 and 13 more joined the CNIO in that same year. Almost half of the 116 students working at the CNIO in 2013 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

In 2008, the *Fundación "la Caixa"* initiated an innovative programme that 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 graduate students from all over the world who have an interest in pursuing an ambitious PhD project. Until 2013, the 4-year programme supported 10 fellows per year; they were selected after an international call and a competitive selection process.

The 2013 call was very successful, attracting over 200 eligible applications of undergraduates from 42 different countries.

The distribution of students across the CNIO's Research Programmes in 2013 was as follows: 36.3% of students worked in the Molecular Oncology Programme, 19.8% in the Molecular Pathology Programme, 17.2% in the Human Cancer Genetics Programme, 15.5% in the Structural Biology and Biocomputing Programme, 7.8% in the BBVA Foundation- CNIO Cancer Cell Biology Programme and 3.4% in the Clinical Research Programme.

FUNDING OF PHD TRAINING	NO.
SPANISH ENTITIES	86
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad (MINECO) (I+D Projects)*	6
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad (MINECO) (FPI)*	14
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte (MECD) (FPU)*	10
Health Research Fund / *Fondo de Investigaciones Sanitarias (FIS) (I+D Projects)*	3
Health Research Fund / *Fondo de Investigaciones Sanitarias (FIS) (FPI)*	3
Community of Madrid / *Comunidad Autónoma de Madrid (CAM) (Projects)*	1
Community of Madrid / *Comunidad Autónoma de Madrid (CAM) (FPI)*	1
Cellectis Agreement	1
Centre for Industrial Technological Development / *Centro para el Desarrollo Tecnológico Industrial (CDTI) (I+D Projects)*	1
CIBERER	1
Ferrer Group / *Grupo Ferrer*	1
"la Caixa" Foundation / *Fundación "la Caixa"*	41
Botín Foundation / *Fundación Botín*	1
CNIO	2
INTERNATIONAL ENTITIES	30
European Commission Framework Programme / *Programa Marco de la Comisión Europea*	13
European Research Council (ERC)	8
University of Leiden Clinical Trials Agreement	1
European Urology Association (EUA)	1
Melanoma Research Alliance (MRA)	2
Howard Hughes Medical Institute (Prize)	2
Pfizer	1
Fullbright España	2
TOTAL	**116**

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 2013, 67 postdoctoral fellows worked at the CNIO. Notably 58% of these fellows were from outside of Spain, many coming from very prestigious international institutions.

The CNIO launched the CNIO-*Caja Navarra* International Postdoctoral Programme in 2008. During 2013, this 2-year programme supported 3 postdoctoral fellows at the CNIO.

In 2013, the *Fundación Banco Santander* funded a highly competitive fellowship programme aimed to support outstanding young scientists who have been trained in the UK and wish to start or continue their postdoctoral training at the CNIO. Two young scientists from the University of Leicester and the London Research Institute - Cancer Research UK were awarded a Santander Foundation - CNIO Fellowship.

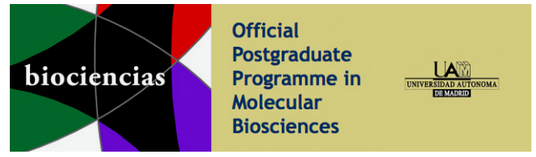
FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ENTITIES	40
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad (MINECO) (I+D Projects)*	4
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad (MINECO)*	5
Health Research Fund / *Fondo de Investigaciones Sanitarias (FIS) (I+D Projects)*	2
Health Research Fund / *Fondo de Investigaciones Sanitarias (FIS)*	8
Community of Madrid / *Comunidad Autónoma de Madrid (CAM)*	2
Spanish Association Against Cancer / *Fundación Científica de la Asociación Española Contra el Cancer (AECC)*	7
Banco Santander Foundation / *Fundación Banco Santander*	2
CIBERER	2
Caja Navarra Foundation / *Fundación Caja Navarra*	3
CNIO	5
INTERNATIONAL ENTITIES	27
European Commission Framework Programme / *Programa Marco de la Comisión Europea*	10
European Research Council (ERC)	6
German Research Foundation (DFG)	1
Association for International Cancer Research (AICR)	4
Daiichi Sankyo Agreement	1
European Association for the Study of Diabetes (EASD)	1
F. Hoffmann - La Roche	1
Clinical trials Boehringer / *Ensayo clínico Boehringer*	1
Federation of the Societies of Biochemistry and Molecular Biology (FEBS)	1
Pfizer	1
TOTAL	**67**

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 the Faculty of Sciences at the Autonomous University of Madrid (*UAM*) through 4 Official Postgraduate Programmes, namely the Doctorate in Biosciences, Masters in Molecular and Cell Biology, Masters in Molecular Biomedicine, and Masters in Biotechnology.



Master's Degree in Biocomputing and Computational Biology

The *Master en 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)*, the Madrid Science Park Foundation (*Fundación Parque Científico de Madrid, FPCM*), and the Spanish Society of Biotechnology (*Sociedad Española de Biotecnología, SEBiot*), through a collaborative agreement.

Official Master's Degree in Clinical and Applied Cancer Research

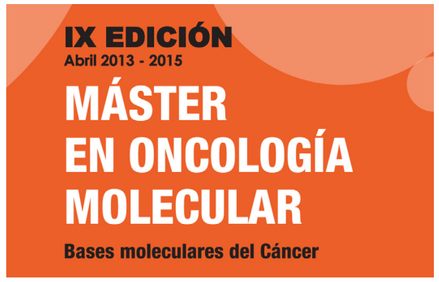
Manuel Hidalgo, CNIO's Vice Director of Translational Research, codirects – in collaboration with the CEU-San Pablo University in Madrid (*USP-CEU*) – a Postgraduate Training Programme in Clinical and Applied Cancer Research; the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.

Official Master's Degree in Therapeutic Targets, Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department of 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 500 hours of training, a certificate for a Master's degree in Molecular Oncology – recognised by the European School of Oncology (ESO) – is awarded.



LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology and is organised through agreements with 9 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 19 weeks (710 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student in Medical Archiving and Recording for 13 weeks (440 hours). Of the 19 students who participated in this programme in 2013, 2 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 to MDs and other health care professionals through 3 different programmes: the Immersion Programme in Molecular Pathology for MIR, BIR and FIR; the Familial Cancer Programme for MIR, BIR and FIR; and the CNIO Onco-MIR Rotation Programme. This initiative is a collaborative effort with the Spanish Ministry of Health (*Ministerio de Sanidad, Servicios Sociales e Igualdad*). Training usually consists of a 3-month period during residency. During 2013, 21 medical residents from 17 different hospitals enjoyed the benefits of rotations within the different groups and units of the CNIO's Molecular Pathology, Human Cancer Genetics and Clinical Research Programmes.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2013, 9 scientists were supported by the *Ramón y Cajal* Programme. This initiative – established in 2001 by the former Spanish Ministry of Science and Technology (now *Ministerio de Economía y Competitividad*) – aims to encourage scientists who work abroad to complete their scientific training in Spanish research institutions. 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.

In addition, 28 other scientists were financially supported by similar programmes, including the *Miguel Servet* (5 contracts), *Sara Borrell* (4 contracts) and *Río Hortega* (4 contracts) programmes, funded by the *Fondo de Investigaciones Sanitarias* del *Instituto de Salud Carlos III*; the *Juan de la Cierva* programme (5 contracts), funded by the Spanish Ministry of Economy and Competitiveness; and the *Ayudas para Investigadores en Oncología* (10 contracts) funded by the *Asociación Española Contra el Cáncer*.

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 and to promote scientific collaborations. During 2013, Robert Benezra from the Memorial Sloan-Kettering Cancer Center in New York was beneficiary of the *Jesús Serra* Foundation's Visiting Researcher Programme.

Fundación Jesús Serra
Catalana Occidente

SCIENTIFIC EVENTS

NATURE-CNIO CANCER SYMPOSIUM: FRONTIERS IN TUMOUR HETEROGENEITY AND PLASTICITY OCTOBER 27-30, 2013

Nature and CNIO held a conference focusing on the Frontiers in Tumour Heterogeneity and Plasticity. Tumour heterogeneity and plasticity have substantial implications for fundamental and clinical cancer research. To cover some of the most relevant and recent advances in this area, the symposium brought together world-leading experts to give presentations about the cancer cell of origin, the clonal evolution of cancer, microenvironment and heterogeneity, tumour cell plasticity and tumour heterogeneity in the clinic. The main lectures were complemented by 10 short talks selected from the submitted abstracts and a large number of posters.

The exciting 2.5 day programme of invited talks, included keynote lectures by Kornelia Polyak and Jose Baselga, poster sessions and short talks, aimed at setting the scene for the exchange of novel ideas and discussions on emerging molecular mechanisms of tumour heterogeneity and its clinical implications. The conference venue was the *Mutua Madrileña* Foundation Auditorium located at the core of the business and shopping areas in Madrid. This international event was organised by Mirna Pérez-Moreno, Scott Lowe and Erwin Wagner.

SCIENTIFIC ORGANISERS

- **Mirna Pérez-Moreno**, CNIO, Spain
- **Scott Lowe**, Memorial Sloan-Kettering Cancer Center (MSKCC), USA
- **Erwin Wagner**, CNIO, Spain

CO-ORGANISERS

- **Barbara Marte**, Nature, UK
- **Nicola McCarthy**, Nature Reviews Cancer, UK
- **Alexia-Ileana Zaromytidou**, Nature Cell Biology, USA

SPEAKERS

- **José Baselga,** Memorial Sloan-Kettering Cancer Center, USA
- **Eduard Batlle,** IRB, Spain
- **Cedric Blanpain,** Université Libre de Bruxelles, Belgium
- **Gail Eckhardt,** University of Colorado School of Medicine, USA
- **Jeff Engelman,** Massachusetts General Hospital Cancer Center, USA
- **Mike Hemann,** Institute for Integrative Cancer Research-MIT, USA
- **Christoph Klein,** University Hospital Regensburg, Germany
- **Ross Levine,** Memorial Sloan-Kettering Cancer Center, USA
- **Scott Lowe,** Memorial Sloan-Kettering Cancer Center, USA
- **Hiroyuki Mano,** Graduate School of Medicine, The University of Tokyo, Japan
- **Alberto Mantovani,** Humanitas Clinical and Research Center, Italy
- **Elaine Mardis,** The Genome Institute, Washington University School of Medicine, USA
- **Sean Morrison,** UT Southwestern Medical Center, USA
- **Ángela Nieto,** Institute of Neuroscience, CSIC-UMH, Spain
- **Luis Parada,** University of Texas Southwestern Medical Center, USA
- **Dana Pe'er,** Columbia University, USA
- **Kornelia Polyak,** Dana-Farber Cancer Institute, USA
- **Victoria Seewaldt,** Duke University School of Medicine, USA
- **Lillian Siu,** Princess Margaret Hospital, Canada
- **Charles Swanton,** Cancer Research UK, London Research Institute, UK
- **Snorri Thorgeirsson,** National Institutes of Health (NIH)/CCR/NCI/LEC, USA
- **Frank Winkler**, DKFZ, Germany

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.

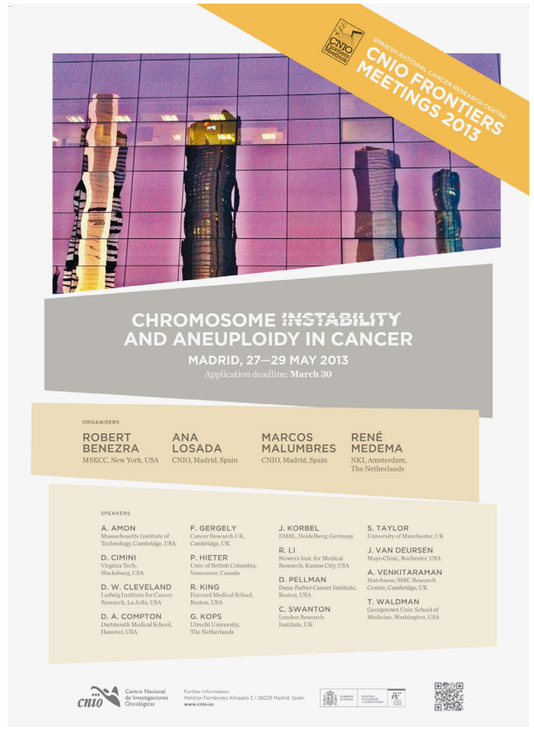
CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER
27-29 MAY, 2013

ORGANISERS

- **Robert Benezra**, Memorial Sloan-Kettering Cancer Center (MSKCC), USA
- **Ana Losada**, CNIO, Spain
- **Marcos Malumbres**, CNIO, Spain
- **René Medema**, NKI, The Netherlands

SESSIONS

- Mechanisms of aneuploidy generation
- Modeling aneuploidy in mouse
- The effects of aneuploidy. A therapeutic opportunity?
- CIN genes in human cancer



SPEAKERS

- **Angelika Amon**, Koch Institute for Integrative Cancer Research, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, USA
- **Robert Benezra**, Memorial Sloan-Kettering Cancer Center, New York, USA
- **Daniela Cimini,** Virginia Tech, Blacksburg, USA
- **Don Cleveland**, Ludwig Institute for Cancer Research, University of California, San Diego, USA
- **Duane Compton**, Geisel School of Medicine at Dartmouth, Hanover, USA
- **Fanni Gergely**, Cancer Research UK Cambridge Research Institute, UK
- **Philip Hieter**, University of British Columbia, Vancouver, Canada
- **Randall King**, Harvard Medical School, Boston, USA
- **Geert Kops,** University Medical Center Utrecht, The Netherlands
- **Jan Korbel**, EMBL, Heidelberg, Germany
- **Ana Losada**, CNIO, Madrid, Spain
- **Rong Li**, Stowers Institute for Medical Research, Kansas City, USA
- **Marcos Malumbres**, CNIO, Madrid, Spain
- **René Medema**, The Netherlands Cancer Institute, Amsterdam, The Netherlands
- **Charles Swanton**, Cancer Research UK, London Research Institute and UCL Hospitals/Cancer Institute, UK
- **Stephen Taylor,** University of Manchester, UK
- **Jan Van Deursen,** Mayo Clinic, Rochester, USA
- **Ashok Venkitaraman,** University of Cambridge and the Medical Research Council Cancer Cell Unit, UK
- **Todd Waldman**, Georgetown University School of Medicine, Lombardi Comprehensive Cancer Center, Washington, USA

In addition, 13 short talks were selected among participants' contributions and 32 posters were presented.

OTHER MEETINGS & CONFERENCES

In addition to the CNIO Frontiers Meetings, the CNIO annually participates in various meetings and conferences. Within this category, the 3 events of 2013 focused on recent advances in the areas of pancreatic cancer, breast and ovarian cancer, as well as on the challenges and opportunities for entrepreneurship in Spain.

COGS MEETING (COLLABORATIVE ONCOLOGICAL GENE ENVIRONMENTAL STUDY) 20-29 APRIL, 2013

ORGANISER

- **Javier Benítez**, CNIO, Madrid, Spain

PARTICIPANTS

- **ENIGMA Consortium**
- **Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA)**
- **Breast Cancer Association Consortium (BCAC)**
- **Ovarian Cancer Association Consortium Meeting**

CNIO-SEBBM MINISYMPOSIUM ON ENTREPRENEURSHIP 3 SEPTEMBER, 2013

ORGANISERS

- **María S. Soengas**, CNIO, Madrid, Spain
- **Damiá Tormo**, Bioncotech Therapeutics, Valencia, Spain
- **Juan L Ramos Martín**, SEBBM, Madrid, Spain

SPEAKERS

- **María José Alonso**, University of Santiago Compostela, Spain
- **Joan Ballesteros**, ViviaBiotech, Madrid, Spain
- **Claire Bastien**, IE Business School, Madrid, Spain
- **Luis Blanco**, CBMSO, CSO, X Pol/Sygnis, Madrid, Spain
- **Javier Fernández**, BioCapital Advisors, Madrid, Spain
- **David Horna**, Aglaris Cell, Madrid, Spain
- **Julio Mayol**, MIT-Madrid M+Vision, Madrid, Spain
- **Federico Mayor Jr.,** CBMSO-UAM, SEBBM, Madrid, Spain
- **Marisol Quintero,** CNIO, Madrid, Spain
- **Angel Sánchez**, iDeals Everis, Madrid, Spain

- **María S. Soengas,** CNIO, Madrid, Spain
- **Xavier Testar**, University of Barcelona (AEECT), Barcelona, Spain
- **Damia Tormo,** Bioncotech Therapeutics, Valencia, Spain

PANCREATIC CANCER FORUM 2013 *CENTRO INTEGRAL ONCOLÓGICO CLARA CAMPAL (CIOCC)* 29-30 NOVEMBER, 2013

ORGANISERS

- **Manuel Hidalgo**, CIOCC; CNIO; Madrid, Spain
- **Alfredo Carrato**, Ramón y Cajal University Hospital, Madrid, Spain

SPEAKERS

- **Maria Antonietta Bali**, Erasme Hospital, Université Libre de Bruxelles, Belgium
- **Mariano Barbacid**, CNIO, Madrid, Spain
- **Stefano Cascinu**, *Universita Politecnica delle Marche*, Ancona, Italy
- **Pippa Corrie**, Cambridge University Hospitals NHS Foundation Trust, UK
- **Antonio Cubillo**, *HM Hospitales,* Madrid, Spain
- **Eric Van Cutsem**, University Hospitals Leuven, Belgium
- **Jeff Evans**, University of Glasgow, UK
- **Mario F Fraga**, Spanish National Centre of Biotechnology (CNB), Madrid, Spain
- **Christopher Heeschen**, CNIO, Madrid, Spain
- **Volker Heinemann**, Ludwig-Maximilians-University of Munich, Germany
- **Christophe Louvet**, *Institut Mutualiste Montsouris,* Paris, France
- **Núria Malats**, CNIO, Madrid, Spain
- **John D. McPherson**, Ontario Institute for Cancer Research, Canada
- **Malcolm Moore**, Princess Margaret Hospital, Toronto, Canada
- **Rienk Offringa**, University of Heidelberg, Germany
- **Michele Reni**, *Ospedale San Raffaele*, Milan, Italy
- **Aldo Scarpa**, Verona University, Italy
- **Werner Scheithauer**, Medical University of Vienna, Austria
- **Roland Schmid**, Technical University of Munich, Germany
- **Josep Tabernero**, Vall D'Hebron Institute of Oncology, Barcelona, 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 professionals 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.

COURSE OF LABORATORY ANIMAL SCIENCE FOR RESEARCHERS CATEGORY C
FEBRUARY 25 - MARCH 15, 2013

ORGANISERS
- **Isabel Blanco**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Ignacio Álvarez**, Complutense University of Madrid, Spain
- **José M. Orellana**, University of Alcalá, Spain

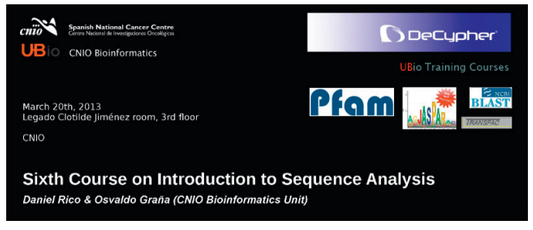
SPEAKERS
- **Ignacio Álvarez,** Complutense University of Madrid, Spain
- **Javier Benito**, Complutense University of Madrid, Spain
- **Isabel Blanco**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Argelia Castaño**, Carlos III Institute, Madrid, Spain
- **Ernesto de la Cueva**, Charles River, Madrid, Spain;
- **Colin Dunn**, Charles River, UK / Laboratory Animals Ltd.
- **Ricardo Feinstein**, National Veterinary Department, Sweden
- **Michael Festing**, Animal Procedures Committee, UK
- **Javier Guillén**, AAALAC International
- **Bryan Howard**, University of Sheffield, UK
- **Marcos Malumbres**, Spanish National Cancer Research Centre (CNIO) Madrid, Spain
- **Antonio Martinez**, GSK, Madrid, Spain
- **Jesús Martínez**, CIEMAT, Madrid, Spain
- **José M. S. Morgado**, Spanish National Cardiovascular Research Center (CNIC), Madrid, Spain

- **David Morton**, University of Birmingham, UK
- **Francisca Mulero**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **José Mª Orellana**, University of Alcalá, Spain
- **Sagrario Ortega**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Belén Pintado**, Spanish National Biotechnology Center (CNB), Madrid, Spain
- **Sergio Salazar**, Charles River, Paris, France
- **Salvador Nieves**, Cajal Neuroscience Institute, Madrid, Spain
- **Graham Tobin**, Animal Welfare Consultant, UK
- **Patri Vergara**, Autónoma University of Barcelona, Spain

SIXTH COURSE ON INTRODUCTION TO SEQUENCE ANALYSIS MARCH 20, 2013

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

SPEAKERS
- **Osvaldo Graña**, CNIO, Madrid, Spain
- **Daniel Rico**, CNIO, Madrid, Spain



URO-ONCOLOGICAL PATHOLOGY TUTORIAL: A 2-DAY "MEET THE EXPERT" APRIL 24-25, 2013

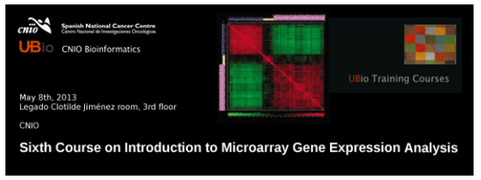
ORGANISERS
- **Ferran Algaba**, Puigvert Foundation, Barcelona, Spain
- **Yves Allory**, Henri Mondor Hospital, Paris, France
- **Núria Malats**, CNIO, Madrid, Spain
- **Francisco X. Real**, CNIO, Madrid, Spain

SPEAKERS
- **Yves Allory**, Henri Mondor Hospital, Paris, France
- **Ferran Algaba**, Puigvert Foundation, Barcelona, Spain

SIXTH COURSE ON INTRODUCTION TO MICROARRAY GENE EXPRESSION ANALYSIS MAY 8, 2013

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

SPEAKERS
- **Gonzalo Gómez,** CNIO, Madrid, Spain
- **Daniel Rico,** CNIO, Madrid, Spain
- **Orlando Dominguez,** CNIO, Madrid, Spain

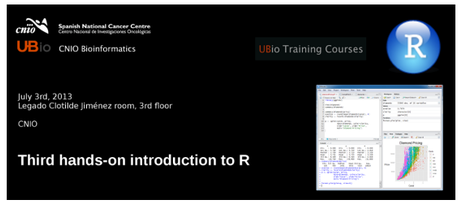


I WORKSHOP OF BLADDER CANCER: "CHANGING THE PARADIGMS OF RESEARCH IN BLADDER CANCER" JUNE 10, 2013

ORGANISER
- **Albert Font**, ICO Badalona, Spain

SPEAKERS
- **Daniel Castellano,** 12 de Octubre Hospital, Madrid, Spain
- **Francisco X. Real,** CNIO, Madrid, Spain
- **Albert Font,** ICO Badalona, Spain
- **Yves Allory,** CNIO, Madrid, Spain
- **Jesús Paramio,** CIEMAT, Madrid, Spain
- **Miquel Taron,** ICO Badalona, Spain
- **Ignacio Melero,** Clinica Universitaria de Navarra, Pamplona, Spain
- **Jose Luis Perez-Gracia,** Clinical University of Navarra, Spain
- **LM. Antón-Aparicio**, C.H.U., La Coruña, Spain

THIRD HANDS-ON INTRODUCTION TO R JULY 3, 2013



ORGANISERS

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

SPEAKER

- **Ramón Díaz Uriarte**, "Alberto Sols" Biomedical Research Institute, UAM-CSIC, Madrid, Spain.

MINI -COURSE: ONCOLOGY: FROM BENCH TO BEDSIDE JULY 16, 2013

ORGANISERS

- **Jacob Hooker**, Massachusetts General Hospital, Boston, USA
- **Timothy Padera**, Harvard Medical School, Boston, USA
- **Francisca Mulero**, CNIO, Madrid, Spain

SPEAKERS

- **Jacob Hooker**, Harvard Medical School /PET Core at Masssachusetts General Hospital/Athinoula A. Martinos Center Department of Radiology, MG, USA
- **Timothy P. Padera,** Harvard Medical School, Boston, USA
- **Francisca Mulero**, CNIO, Madrid, Spain
- **David Olmos**, CNIO, Madrid, Spain
- **Maria A. Blasco**, CNIO, Madrid, Spain
- **Marisol Quintero**, CNIO, Madrid, Spain
- **Enrique Grande Pulido**, Ramón y Cajal University Hospital, Madrid, Spain
- **Maria Die Trill**, Hospital Universitario Gregorio Marañón/Universidad Complutense Madrid, Spain
- **Tatiana Massarrah Sánchez,** Hospital Universitario Gregorio Marañón, Madrid, Spain
- **Iria-Flavia Heredero,** Breast Cancer Patient

ACCESS TO GENES AND GENOMES WITH ENSEMBL 2013 SEPTEMBER 18, 2013

ORGANISERS

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

SPEAKER

- **Denise Carvalho-Silva**, European Bionformatics Institute, UK

BIOINFORMATICS CORNER. BIOZENTRUM U BASEL-CNIO WORKSHOP OCTOBER 1, 2013

ORGANISERS

- **Alfonso Valencia**, CNIO, Madrid, Spain
- **Erik van Nimwegen** and **Mihaela Zavolan,** Biozentrum University of Basel, Switzerland

SPEAKERS

- **Federico Abascal; Daniel Rico; Milana Morgenstern**, CNIO, Madrid, Spain

DISEÑO, ANÁLISIS DE DATOS DE GENOTIPADO E INTERPRETACIÓN DE RESULTADOS ESTADÍSTICOS NOVEMBER 25-26, 2013

ORGANISERS

- **CEGEN-CNIO**

SPEAKERS

- **Javier Benítez,** CeGen-CNIO, Madrid, Spain
- **Anna González-Neira,** CeGen-CNIO, Madrid, Spain
- **Pablo Fernández,** *Centro Nacional de Epidemiología*, ISCIII/ CIBERESP, Madrid, Spain
- **Raquel Cruz,** CIBERER/ *Universidade de Santiago de Compostela*, Spain
- **David González Pisano**, CNIO, Madrid, Spain
- **Osvaldo Graña,** CNIO, Madrid, Spain
- **Guillermo Pita,** CNIO, Madrid, Spain.

SEVENTH COURSE ON FUNCTIONAL ANALYSIS OF GENE EXPRESSION EXPERIMENTS 2013 NOVEMBER 27, 2013

ORGANISERS

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

SPEAKERS

- **Gonzalo Gómez**, CNIO, Madrid, Spain
- **Daniel Rico**, CNIO, Madrid, Spain

HOW TO ACCESS ENCODE DATA THROUGH THE UCSC GENOME BROWSER NOVEMBER 26, 2013

ORGANISERS

- Cegen, CNIO, USC, ISCIII

SPEAKERS

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

CNIO DISTINGUISHED SEMINARS

The purpose of the Distinguished Seminars 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 usually held on Friday noon in the CNIO Auditorium throughout the year, with the exception of holidays and the July to September summer break. Each Distinguished Seminar series includes world-leading scientists who address topics that are of general interest to the CNIO faculty. In total, the CNIO hosted 28 distinguished speakers in 2013.

This year, the Distinguished Seminars Series included speakers who address topics that are not necessarily related to cancer research, but that provide novel perspectives and ideas that contribute to the CNIO's intellectually challenging trans-disciplinary environment. These out-of-the-box seminars were financed by the *Banco Sabadell* Foundation.

DATE	SPEAKER	ORGANISATION
JANUARY		
11/01/2013	**Simon Boulton**	London Research Institute, London, UK
18/01/2013	**Paul Flicek**	EMBL Outstation - Hinxton, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK
25/01/2013	**Peer Bork**	EMBL, Heidelberg, Germany
FEBRUARY		
01/02/2013	**Pedro Alonso**	Institute for Global Health, Barcelona, Spain
04/02/2013	**Thomas Jenuwein**	Max Planck Institute of Immunobiology and Epigenetic, Freiburg, Germany
08/02/2013	**René Medema**	The Netherlands Cancer Institute, Amsterdam, The Netherlands
15/02/2013	**James Lupski**	Baylor College of Medicine, Houston, USA
MARCH		
08/03/2013	**Allan Balmain**	University of California, San Francisco, USA
15/03/2013	**Richard Marais**	CRUK, Paterson Cancer Center in Manchester, UK
22/03/2013	**Jan Löwe**	MRC Laboratory of Molecular Biology, Cambridge, UK
APRIL		
05/04/2013	**Elías Campo**	Clinic Hospital, Barcelona, Spain
12/04/2013	**Gideon Schreiber**	Weizmann Institute of Science, Rehovot, Israel
29/04/2013	**Nic Jones**	Paterson Institute for Cancer Research, Manchester, UK
MAY		
17/05/2013	**Roel Nusse**	Howard Hughes Medical Institute, Stanford, US
24/05/2013	**Bruno Amati**	Center for Genomic Science of IIT@SEMM; *Fondazione Istituto Italiano di Tecnologia* (IIT); European Institute of Oncology (IEO); Milan, Italy
JUNE		
07/06/2013	**Carl Djerassi**	Stanford University, Stanford, USA
14/06/2013	**Victor Velculescu**	The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, USA
21/06/2013	**Helen Blau**	Stanford University School of Medicine, USA
24/06/2013	**Pier Paolo Pandolfi**	Beth Israel Deaconess Medical Center, Boston, USA
28/06/2013	**Luis Paz Ares**	Virgen del Rocío Hospital, Sevilla, Spain

SEPTEMBER		
06/09/2013	**Juan Luis Arsuaga**	UCM-ISCIII, Madrid, Spain
13/09/2013	**Jorge Ripoll**	Carlos III University & Gregorio Marañón Hospital, Madrid, Spain
OCTOBER		
04/10/2013	**Ian Tomlinson**	Wellcome Trust Centre for Genetics , Oxford, UK
11/10/2013	**Miguel Martin**	General University Hospital Gregorio Marañon, Madrid, Spain
NOVEMBER		
08/11/2013	**Jiri Lukas**	NovoNordisk Foundation, Center for Protein Research, Hellerup, Denmark
22/11/2013	**Alan Ashworth**	The Institute of Cancer Research, London, UK
DECEMBER		
09/12/2013	**John Blenis**	Harvard Medical School, Boston, USA
13/12/2013	**José María Ordovas**	Tufts University, Boston, USA

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 44 *ad-hoc* seminars were organised by CNIO researchers in 2013.

DATE	SPEAKER	ORGANISATION
JANUARY		
22/01/2013	**Klaus Rajewsky**	Max-Delbrück-Center for Molecular Medicine, Berlin, Germany
25/01/2013	**Cédric Cortijo**	EPFL, Lausanne, Switzerland
31/01/2013	**Laufey Thora Amundadottir**	National Cancer Institute, National Institutes of Health Advanced Technology Center, Bethesda, USA
FEBRUARY		
07/02/2013	**Jan Nedergaard**	The Wenner-Gren Institute Stockholm University, Stockholm, Sweden
14/02/2013	**Eytan Ruppin**	School of Computer Sciences & School of Medicine, Tel Aviv University, Tel Aviv, Israel
14/02/2013	**Jaime Huerta-Cepas**	CRG-Centre for Genomic Regulation, Barcelona, Spain
MARCH		
04/03/2013	**Roderic Guigó**	CRG-UPF, Barcelona, Spain
07/03/2013	**Ivan Plaza-Menacho**	Structural Biology Laboratory, London Research Institute, Cancer Research UK, London, UK
APRIL		
15/04/2013	**Andre Nussenzweig**	Genomic Integrity Branch, National Cancer Institute/NIH, Bethesda, USA
19/04/2013	**Nicola Ternette**	University of Oxford, UK
26/04/2013	**Miguel López**	Research Center of Molecular Medicine and Chronic Diseases (CIMUS); Santiago de Compostela University (USC), Spain
29/04/2013	**Harold Brooks**	Eli Lilly and Company, Indianapolis, USA
MAY		
07/05/2013	**Juan Iovanna**	French National Institute of Health and Medical Research (Inserm) & Cancer Research Center of Marseille (CRCM), France
13/05/2013	**Toru Hirota**	Cancer Institute of the Japanese Foundation for Cancer Research (JFCR), Tokyo, Japan
13/05/2013	**Elissaveta Petrova**	PhD student at Weill Cornell Graduate School of Medical Sciences in the Department of Pharmacology, in Dr. Marilyn Resh's laboratory at Memorial Sloan-Kettering Cancer Center, NY, USA
14/05/2013	**Ron Smits**	Erasmus University Medical Center Rotterdam (Erasmus MC), The Netherlands

JUNE		
11/06/2013	**Hiroshi Takayanagi**	Faculty of Medicine, The University of Tokyo, Hongo Bunkyo-ku, Tokyo, Japan
13/06/2013	**Raquel Pérez**	The Royal Marsden, London, UK
13/06/2013	**Joaquín Mateo**	The Royal Marsden, London, UK
14/06/2013	**Barbara Witham**	The Jackson Laboratory, Bar Harbor, USA
20/06/2013	**Marta Rava**	INSERM, CESP, Centre for Research in Epidemiology and Population Health, Vellejuif, France
26/06/2013	**Kum Kum Khanna**	The Queensland Institute of Medical Research, Queensland, Australia
JULY		
01/07/2013	**Carien Niessen**	Department of Dermatology, Center for Molecular Medicine, University of Cologne, Germany
SEPTEMBER		
16/09/2013	**Masanori Hatakeyama**	The University of Tokyo, Japan
17/09/2013	**Amaia Lujambio**	Memorial Sloan-Kettering Cancer Center, New York, USA
23/09/2013	**Rui Gardner & Lola Martinez**	*Instituto Gulbenkian Ciência & CNIO*
24/09/2013	**Lola Martinez & Rui Gardner**	*CNIO & Instituto Gulbenkian Ciência*
25/09/2013	**Rui Gardner & Lola Martinez**	*Instituto Gulbenkian Ciência & CNIO*
27/09/2013	**Rui Gardner & Lola Martinez**	*Instituto Gulbenkian Ciência & CNIO*

OCTOBER		
08/10/2013	**Deborah Rathjen**	BSc (Hons). Bionomics, Thebarton, Australia
24/10/2013	**Mark Dessing**	Sony Biotechnology Europe, UK
24/10/2013	**Laura Schneider**	Cell Signaling Technology Europe
28/10/2013	**Israel Sanchez**	Ramakrishnan-Lab, MRC Laboratory of Molecular Biology, Cambridge, UK
NOVEMBER		
07/11/2013	**Zili Lei**	Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Sweden
07/11/2013	**Bruce A. Littlefield**	Eisai Inc, Andover, Massachusetts, USA
11/11/2013	**George Daley**	Boston Children's Hospital, Harvard Medical School, Boston, USA
11/11/2013	**Meritxell Huch**	Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht - The Netherlands/ Gurdon Institute, Cambridge- UK
12/11/2013	**Raghu Kalluri**	University of Texas. MD Anderson Cancer Center, Houston, USA
21/11/2013	**Ignacio Varela**	*Instituto de Biomedicina y Biotecnología de Cantabria* (IBBTEC), Santander, Spain
27/11/2013	**Victor Moreno**	Catalan Institute of Oncology, Barcelona, Spain
DECEMBER		
05/12/2013	**Stefan Walter**	University of California San Francisco, USA
11/12/2013	**Nathalie Mouraret**	*Paris-Est-Créteil* University (UPEC), France
12/12/2013	**Josep Rizo**	UT Southwestern Medical Center, Dallas, USA
18/12/2013	**Alba de Martino Rodríguez**	Campus Vienna Biocenter, Vienna, Austria

SCIENTIFIC DIVULGATION EVENTS

SCIENCE WEEK

Open Doors Day: Investigating to Disarm Cancer

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 *(XIII Semana de la Ciencia,* November 4 –17, 2013). In 2013, approximately 240 people participated in guided visits to the Centre's research facilities over the course of 4 days.

AULA

This year, the CNIO supported *AULA* (February 11, 2013) and joined other "Severo Ochoa" Centres of Excellence, Public Research Organisations, the FECYT and the National Museum of Science and Technology (MUNCYT), to hold an information stand providing visitors with direct insights into scientific research and development, technology and innovation. An interactive area was set up at the stand in which a variety of visitor activities took place, including talks about CNIO's research activities and training programmes, a microscope workshop to teach the youngest visitors about some of the tools that scientists at the Centre use to study cancer, or the showing of a video titled "A Day in the Life of the Spanish National Cancer Research Centre", performed by CNIO pre-doctoral (CNIOSA) and post-doctoral (CNIO-PDA) researchers. *AULA* is the most important annual educational and training event of the year in Spain as well as the only one promoted by the Ministry of Education, Culture and Sport.

RESEARCHERS´ NIGHT

This year, the CNIO participated in *Researchers' Night*, an activity aimed at bringing researchers closer to the general public and families and learning about what researchers do for society. Each year, more than 300 European cities participate all at once 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 150 people came to the Spanish National Cancer Research Centre (CNIO) to attend *Researchers' Night* (September 27, 2013) and learn about cancer research. The activities were organised entirely 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.

MOVILAB

In line with the institution's commitment to science education, the CNIO boarded the laboratory on wheels *MOVILAB* (May 8, 2013) to raise awareness about cancer research among young people. This activity, aimed at primary school students, represents the first collaboration between CNIO and *MOVILAB*. The activity included the talk titled *Cuando las células se convierten en supervillanos* – given by Ángela Monasor, from the Genomic Instability Group, and Cristina Balbas, from the Epithelial Carcinogenesis Group – and the showing of the video *Un día en el Centro Nacional de Investigaciones Oncológicas*, in which CNIO pre-doctoral (CNIOSA) and post-doctoral (CNIO-PDA) researchers talk about their day-to-day efforts and how they work to produce new discoveries. *MOVILAB* is an initiative of the Spanish Foundation for Science and Technology (FECYT), the Spanish National Research Council (CSIC) and the *Padrosa* Foundation.

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 2013, more than 262 people participated in such guided visits; most of them were *ESO* and *Bachillerato* student groups, but also professionals in the health sector.

ADMINISTRATION

BOARD OF TRUSTEES

→ **Honorary President**

· **Luis de Guindos Jurado**
Minister of Economy and Competitiveness
Ministro de Economía y Competitividad

→ **President**

· **Carmen Vela Olmo**
Secretary of State for Research, Development and Innovation
Secretaria de Estado de Investigación, Desarrollo e Innovación

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· **María Fernández Pérez** (until September)
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Presidente del Grupo PRISA

· **Pío Díaz de Tuesta Vázquez**
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Director de la Fundación Caja Madrid

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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 219,513 Euros. Of this amount, 209,513 Euros were received as base salary, seniority and small bonuses, and 10,000 Euros as supplemental salary from research projects.
- Members of the CNIO Board of Trustees are not remunerated.

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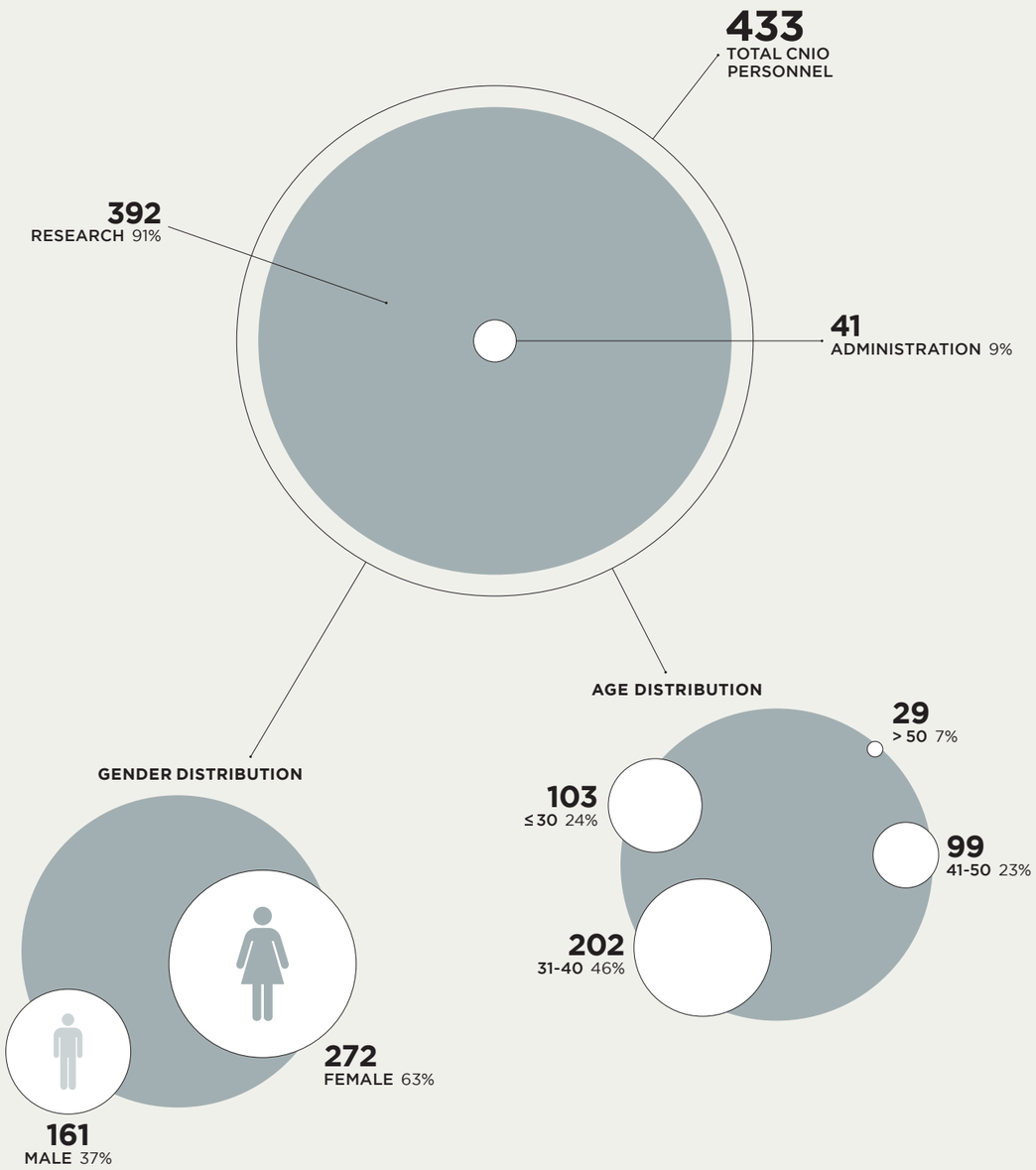
	INFORMATION TECHNOLOGIES	**Fernández, José Luis** Head **de Miguel, Marcos**	
EXTRAMURAL CLINICAL RESEARCH	**López, Antonio** Director		

CNIO PERSONNEL 2013



SCIENTIFIC PERSONNEL 2013

TOTAL SCIENTIFIC PERSONNEL 100% **392**

DISTRIBUTION BY PROGRAMMES

STRUCTURAL BIOLOGY AND BIOCOMPUTING 14%	54
BIOTECHNOLOGY 11%	43
BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY 7%	29
HUMAN CANCER GENETICS 15%	59
CLINICAL RESEARCH 6%	25
MOLECULAR ONCOLOGY 24%	92
MOLECULAR PATHOLOGY 14%	54
EXPERIMENTAL THERAPEUTICS 9%	36

DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 13%	49
GRADUATE STUDENTS 22%	87
STAFF SCIENTISTS 20%	78
PRINCIPAL INVESTIGATORS 11%	45
TECHNICIANS 34%	133

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS	FEMALE	49%	**24**
	MALE	51%	**25**
GRADUATE STUDENTS	FEMALE	68%	**59**
	MALE	32%	**28**
STAFF SCIENTISTS	FEMALE	68%	**53**
	MALE	32%	**25**
PRINCIPAL INVESTIGATORS	FEMALE	36%	**16**
	MALE	64%	**29**
TECHNICIANS	FEMALE	72%	**96**
	MALE	28%	**37**
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**248**
	MALE		**144**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL 100% **392**

POST-DOCTORAL FELLOWS 17%	31
GRADUATE STUDENTS 30%	52
STAFF SCIENTISTS 23%	40
PRINCIPAL INVESTIGATORS 10%	17
TECHNICIANS 20%	35
TOTAL 100%	175

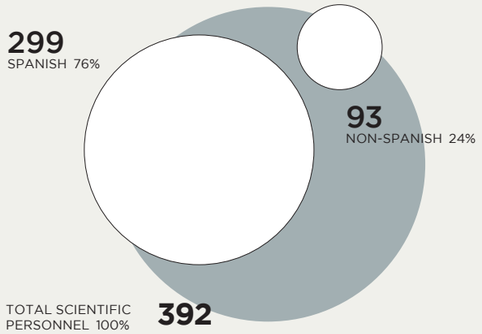
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

POST-DOCTORAL FELLOWS 12%	17
GRADUATE STUDENTS 25%	35
STAFF SCIENTISTS 16%	22
PRINCIPAL INVESTIGATORS 11%	15
TECHNICIANS 36%	49
TOTAL 100%	138

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

POST-DOCTORAL FELLOWS 1%	1
STAFF SCIENTISTS 22%	16
PRINCIPAL INVESTIGATORS 15%	13
TECHNICIANS 62%	49
TOTAL 100%	79

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

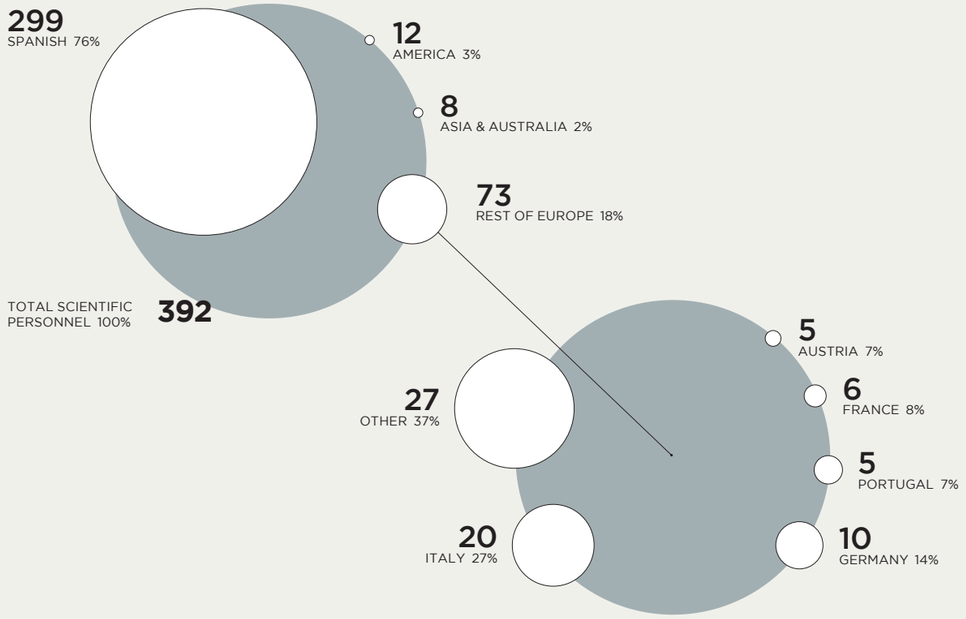


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 47%	23
GRADUATE STUDENTS 39%	34
STAFF SCIENTISTS 23%	18
PRINCIPAL INVESTIGATORS 13%	6
TECHNICIANS 9%	12

Total foreign scientific personnel 93
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



*For details please see sections in the report within their respective Research Group/Unit/Section (names of countries are represented by 3-letter country codes according to the ISO3166-1 alpha 3 standard).

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 the partnership of two freelance designers with 10 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. Underbau's total-design approach puts the emphasis on efficiency and coherency. To achieve that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product.

The working team for the 2013 Scientific Report is formed by Carlos del Barrio, Javier Pividal, Pablo Suárez, and Juanjo Justicia.

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Coordination **Sonia Cerdá**
Edition **Sonia Cerdá and An Devriese**
Publication database **Victoria López**
Coordination of Photography **Ana Merino**
Photography **Amparo Garrido**
Design **Underbau**

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